Maulin P. Shah *Editor*

Industrial Wastewater Reuse

Applications, Prospects and Challenges

Springer

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Constructed Wetlands and Vermifiltration Two Successful Alternatives of Wastewater Reuse: A Commentary on Development of These Alternate Strategies of Wastewater Treatment

Aditi Nag, Sonia Sethi, and Tejaswini Kumawat

1 Introduction

Water that has undergone physical, chemical, or biological changes as a consequence of the incorporation of pollutants, rendering it inappropriate for particular uses like drinking, is referred to as wastewater. Because water is so important to man's daily activities, "trash" is dumped into the water. It is common knowledge that a significant portion of the supplied water is wasted, necessitating its treatment. In order to safeguard the environment and the general public's health, wastewater treatment is the technique and procedure for removing the bulk of toxins present in wastewater. Therefore, wastewater management comprises managing wastewater in a way that promotes environmental protection, public health, and political, social, and economic stability.

2 History of Wastewater Treatment

Although drainage systems were developed well before the eighteenth century, wastewater treatment is a relatively modern activity. This was delivered to rural communities and spread throughout agricultural grounds. The introduction of flush toilets in the nineteenth century resulted in an increase in the volume of waste generated on these agricultural grounds. Due to this transportation difficulty, towns began to employ drainage and storm sewers to carry wastewater into water bodies, notwithstanding Edwin Chadwick's proposal in 1842 that "rain should go to the river and sewage should go to the land." Waste discharged into waterways resulted

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in severe pollution and health issues for downstream consumers. The use of centralized wastewater systems was planned and promoted. Communities that discharge into the facility bear the expense of wastewater treatment.

Today, there have been significant advancements in the production of portable water from wastewater. In recent years, regardless of the receiving stream's capacity, a minimum treatment level has been needed before discharge licenses have been given. Currently, the focus is turning away from centralized systems and toward more sustainable decentralized wastewater treatment (DEWATS), particularly in developing nations like Ghana, where wastewater infrastructure is lacking and traditional methods are difficult to maintain.

3 Objectives of Wastewater Treatment

Wastewater treatment is necessary for:

a. Decrease in the amount of biodegradable organic compounds in the environment.
b. A reduction in nutrient concentrations in the environment and water bodies, making them eutrophic, allowing algae and other aquatic plants to proliferate.
c. Pathogens secreted in vast amounts in the feces of diseased animals and people are eliminated.
d. Water recycling and reuse for a growing population, putting further strain on already restricted water supplies.

Groundwater supplies are being depleted, making the distribution of water both temporally and spatially a considerable concern (National Academy 2005).

4 Characteristics of Wastewater

Wastewater has different qualities depending on where it comes from. Industrial wastewater with municipal or household wastewater characteristics might be discharged simultaneously. Because the qualities of wastewater change by industry, different treatment techniques are required. Physical, chemical, and biological pollutants are the three types of contaminants found in wastewater. The following are some of the indicators used to identify these pollutants:

A. **Physical**

- Total dissolved solids (TDS) are made up of inorganic salts and trace amounts of organic matter dissolved in water.
- Electrical conductivity (EC) measures the salt concentration.
- Suspended solids (SS) are solid particles that are suspended in water but are not dissolved.

B. **Chemical**

- Dissolved oxygen (DO) indicates the amount of oxygen in water.
- Biochemical oxygen demand (BOD) indicates the amount of oxygen required by aerobic bacteria to break down the organic material in a water sample in a predetermined amount of time.
- Chemical oxygen demand (COD) measures how much organic matter in a sample is capable of being oxidized by a potent chemical oxidant.
- Organic compound total (TOC).
- Dissolved nitrogen is shown by NH4-N and NO3-N (ammonium and nitrate, respectively).
- Total Kjeldahl nitrogen is a measurement of ammonia nitrogen that is bonded to organic matter.
- Total-P measures the total amount of phosphorus present in a sample.

C. **Biological**

- Total coliforms (TC), a broad indicator of potential water contamination, includes fecal coliforms as well as typical soil microorganisms.
- Fecal coliforms (FC) are a sign of fecal matter contamination in the water. The bacteria *Escherichia coli*, sometimes known as *E. coli*, is a typical lead indicator.
- Finding worm eggs in the water is the goal of helminth analysis.

5 Levels of Wastewater Treatment

There are three broad levels of treatment: primary, secondary, and tertiary. Sometimes, preliminary treatment precedes primary treatment.

A. Preliminary treatment eliminates grits and coarse suspended matter. This makes it easier to operate and maintain successive treatment units. Organic and inorganic particles, organic nitrogen, organic phosphorus, and heavy metals are all removed during primary treatment. Primary effluent is the wastewater that comes from primary sedimentation units (FAO 2006).
B. Secondary treatment is the process of removing residual organics and suspended particles from initial effluent. Aerobic biological treatment techniques also remove biodegradable dissolved and colloidal organic waste, nitrogenous and phosphorous chemicals, and pathogenic bacteria. Mechanical treatment, such as trickling filters, activated sludge techniques, and rotating biological contactors (RBC), and non-mechanical treatment, such as anaerobic treatment, oxidation ditches, and stabilization ponds, are examples.
C. Tertiary treatment, also known as advance treatment, is used when certain wastewater elements cannot be eliminated by secondary treatment. Significant quantities of nitrogen, phosphate, heavy metals, biodegradable organics, bacteria, and viruses are removed during advanced treatment.

6 Methods of Wastewater Treatment

There are conventional and non-conventional wastewater treatment methods which have been proven and found to be efficient in the treatment of wastewater.

6.1 Conventional Methods

Activated sludge, trickling filters, and rotating biological contactors are examples of traditional wastewater treatment processes. Temperature-sensitive trickling filters and rotating biological contactors remove less BOD, and trickling filters are more expensive to construct than activated sludge systems. Because energy is required to power pumps and blowers, activated sludge systems are substantially more expensive to operate (National Programme on Technology Enhanced Learning [NPTEL] 2010).

6.1.1 Activated Sludge

Biological treatment systems that employ a suspended growth of organisms to remove BOD and suspended particles are referred to as activated sludge. It works on the idea that vigorous wastewater aeration creates bacteria flocs (activated sludge), which decompose organic materials and may be separated by sedimentation. A portion of the activated sludge is recycled to maintain the concentration of active bacteria in the tank. Primary effluent (or plant influent) is combined with return activated sludge to create mixed liquor, which is then aerated for a set period of time. Activated sludge organisms consume the available organic matter as food when the system is aerated, resulting in stable solids and additional organisms. The suspended particles generated by the process, as well as any extra organisms, are incorporated into the activated sludge (NPTEL).

6.1.2 Trickling Filter

It is a growing method in which therapeutic microorganisms are coupled to an inert packaging material. It consists of a circular tank containing a carrier substance (volcanic rock, gravel, or synthetic material). Organic material in the wastewater is adsorbed by a population of microorganisms (aerobic, anaerobic, and facultative bacteria; fungi; algae; and protozoa) attached to the medium as a biological film or slime layer. Wastewater is supplied from above and trickles through filter media, allowing organic material in the wastewater to be adsorbed by a population of microorganisms (aerobic, anaerobic, and facultative bacteria (approximately 0.1 to 0.2 mm thick).

Organic material is degraded by aerobic bacteria in the slime layer's outermost layer. Because oxygen cannot reach the medium face when the layer thickens due to microbial development, anaerobic organisms emerge. The biological film thickens to the point that microorganisms near the surface can no longer adhere to the medium, and a section of the slime layer slips off the filter.

6.1.3 Rotating Biological Contactors

Plastic media are stacked vertically on a horizontal rotating shaft in rotating biological contactors (RBCs). The polymers have a diameter of 2 to 4 m and a maximum thickness of 10 mm. The shaft gently rotates at 1–1.5 rpm with about 40% of the biomass-coated media submerged, exposing the media alternately to wastewater and ambient oxygen. Although RBC systems appeared to be best suited to treat municipal wastewater, they have been placed in numerous petroleum plants due to their capacity to quickly rebound from unfavorable circumstances.

6.1.4 Membrane Bioreactors

Unique processes known as membrane bioreactor (MBR) systems combine anoxic and aerobic biological treatment with an integrated membrane system that may be used with nearly any suspended growth, biological wastewater treatment system. Sewage is filtered before it goes into the biological treatment tank. Aeration maintains solids suspended while supplying oxygen for biological respiration within the aerobic-reactor zone. Active biomass is kept alive throughout the process by MBR using submerged membranes. This makes it possible for the biological process to continue operating for longer periods of time (about 20–100 days for an MBR) and to increase mixed-liquor, suspended solids (MLSS) concentrations (around 8,000–15,000 mg/l) for more effective pollutant removal (TEC 2010).

6.2 Non-conventional Methods

These are low-cost, low-tech biological wastewater treatment systems that are also simpler to operate and maintain. These systems take more space than conventional high-rate biological processes, but if they are properly constructed and are not overloaded, they are typically more effective at eliminating infections (FAO 2006). Some of the unconventional methods include soil aquifer treatment, stabilization ponds, artificial wetlands, oxidation ditches, and more.

6.2.1 Waste Stabilization Ponds

Anaerobic, facultative, or maturation ponds can be found in single or multiple series within shallow, artificial basins known as stabilization of waste ponds. This low-tech treatment technique makes use of four or five ponds with different depths and levels of biological activity. During treatment, the components of wastewater are either transformed by biological and chemical processes or removed via sedimentation (National Academy 2005). The main purposes of the anaerobic ponds are the settling and elimination of suspended particles as well as the decomposition of some organic compounds (BOD5). In facultative ponds, organic matter is further broken down to carbon dioxide, nitrogen, and phosphorus by using oxygen produced by the pond's algae.

6.2.2 Constructed Wetlands

Constructed wetlands (CWs) are structures that have been fully planned out, engineered, and built to use wetland foliage to help treat wastewater within a more supervised condition that exists in natural wetlands. They are a good and environmental substitute for secondary and tertiary remediation of industrial and municipal wastewater. They work well for removing pathogens, toxic substances, hazardous contaminants, nutrients, suspended particles, and organic compounds. They are not suitable for the pre-treatment of industrial wastewater or the treatment of raw sewage in order to preserve the biological balance of the wetland ecosystem. Free water surface (FWS) and subsurface flow (SSF) systems are two different forms of CWs. As the name implies, with FWS, plants are rooted in the sediment layer under the water column while water flows above the earth. With SSF, water passes through a porous medium, like gravels, through which the roots of the plants are embedded.

Wetland plants or macrophytes utilized in CWs including *Cattails (Typha latifolia sp)*, *Scirpus (Bulrus)*, *Lemna (duckweed)*, *Eichornia crassipes (water hyacinth)*, *Pistia stratiotes (water lettuce)*, *Hydrocotyle spp. (pennywort)*, and *Phragmites (reed)* have been known and used in constructed wetlands.

6.2.3 Oxidation Ditches

A customized activated sludge biological treatment method known as a "aeration tank" removes biodegradable organics by using hydraulic retention times of 24 to 48 h and sludge ages of 12 to 20 days. Although they can be altered, oxidation ditches are normally complete mix systems. The typical oxidation ditch treatment system consists of a ring- or oval-shaped design with one or more channels. Aerators that are positioned horizontally or vertically give the ditch circulation, oxygen transmission, and aeration. Return sludge from a secondary clarifier is combined with aerated flow to the oxidation ditch. In order to promote microbial development, the mixing process introduces oxygen into the mixed liquor, and the motive velocity guarantees that microorganisms come into touch with the influent. Aeration raises the concentration of dissolved oxygen, but it falls when the biomass absorbs it.

6.2.4 Upflow Anaerobic Sludge Blanket (UASB)

A covering of microorganisms is used in an anaerobic technique known as upflow anaerobic sludge blanket to absorb polluted burden. It is an anaerobic digester type that creates a layer of granular sludge that floats in the tank. The anaerobic bacteria in the blanket digest (degrade) the wastewater as it moves upward through it. With the help of flocculants, the upward flow and gravity's settling action combine to suspend the blanket.

6.2.5 Vermifiltration

Vermifiltration is another non-conventional wastewater treatment process which is considered to be a sustainable solution of wastewater treatment for developing and underdeveloped countries. The setup consists of various types of gravel layers as well as a layer which has earthworms added to it. The worms interact with the local microflora and decompose the toxic organic waste to less harmful products thereby improving the quality of water by many parameters. More details on this approach are discussed in the subsequent sections.

7 Constructed Wetlands

Wetlands are a type of transitional habitat. In terms of location, they can be found along the coast between dry ground and open sea, surrounding inland lakes and rivers, or as mires stretched across the terrain. Wetlands are an ecological transition zone between terrestrial and aquatic habitats. Most wetlands will either become dry ground as a consequence of falling water tables, sedimentation, or plant succession, or be submerged as a result of rising water tables associated with relative sea-level rise or climate change. Wetlands are generally part of a vast continuum of community types, making it difficult to draw clear lines between them.

Contrary to physicochemical or biological procedures, built wetlands (CWs) technology has been widely employed to treat municipal and industrial wastewater in recent years as a cost-effective and long-term solution (Wang et al. 2020). Wetlands are places inundated with shallow water or soil that has been soaked with water for long enough to form hydric soils that sustain specialist macrophytes suited to anaerobic conditions. However, it was pointed out that, while the concepts of shallow water or saturated conditions, unique wetland soils, and vegetation adapted to wet conditions are relatively simple, combining these three factors to obtain a precise definition is difficult due to a number of characteristics that distinguish wetlands from other ecosystems while also making them similar to them.

The primary premise of CWs is to treat wastewater with gravel, sand, wetland plants, and microbial activity. On a field and laboratory scale, the potential of CWs for the treatment of polluted wastewater has been assessed. These studies discussed either the detoxification of contaminants or their removal from various types of wastewaters and polluted aqueous solutions (Sultana et al. 2015). CWs have already been shown to be effective at removing Cr from wastewater in several experiments (Liang et al. 2017).

Constructed wetlands (CWs) are an environmentally friendly, sustainable wastewater treatment option that can be used in place of or in addition to traditional WWTPs. They filter and remediate water contaminants using natural processes involving substrates, plants, soils, and bacteria. New research on CWs has received attention because of their capacity to remove drugs from wastewater from hospitals and municipalities. In addition to organic contaminants and heavy metals that have also been theorized to select for antibiotic resistance, there is evidence to show that CWs can be a useful mitigation technique for antibiotic medications, ARBs, and ARGs (Abou-Kandil et al. 2020).

CWs have been reported to demonstrate a 2 log (Karimi et al. 2014) to 4 log decrease of pathogens with varying combinations and designs leading in differing removal efficiencies. They may be employed alone or after primary treatment. When used as secondary/tertiary units in conjunction with main therapy in a WWTP, CWs help to improve the WWTP's performance (Chen et al. 2014). Vertical flow (VFCW), horizontal subsurface flow (HSSF-CW), surface flow (SFCW), and hybrid systems, sometimes known as integrated flow CWs, are the four types of CWs based on route flow/hydrology (IFCW).

The usefulness of CWs for the reduction of antibiotic medications (Bôto et al. 2016; Bayati et al. 2020), ARBs (Santos et al. 2019), and ARGs (Ávila et al. 2020) is gaining popularity. Recent research has focused on the use of CWs to polish effluents from conventional treatment facilities, with the goal of reducing antibiotic residues and coliforms (Ilyas and Van Hullebusch 2020; Engida et al. 2020). When compared to traditional WWTP solutions, the costs of construction, operation, and maintenance are much cheaper, making CW a cost-effective and viable choice, particularly for developing/low-income nations. Another benefit is that CWs may be utilized as a decentralized solution in both rural and urban locations, resulting in visually beautiful landscapes that encourage biodiversity and provide animal habitats with minimal daily maintenance. As a result, CWs help to achieve a variety of UN sustainable development objectives, including clean water and sanitation, excellent health and well-being, industrial innovation and infrastructure, sustainable cities and communities, life on land, and life below water (Rahman et al. 2020).

Given the urgent need to address antibiotic resistance sustainably, the potential for wastewater treatments as an antibiotic resistance critical control point, and encouraging data gathered to date on the potential of CWs for mitigating antibiotic drugs, ARBs, and ARGs in the waste stream, the current study was conducted with the specific goal of reviewing and exploring the performance efficiency of CWs toward multiple measures of antibiotic resistance, identifying the major antibiotic resistance control points, and identifying the major antibiotic resistance control points. The current research will serve as a foundation for better understanding and analyzing the issues that developing/low-income nations confront in managing and disposing of their massive volumes of wastewater, with a particular focus on sanitation.

7.1 Types of Constructed Wetlands

7.1.1 Horizontal Subsurface Flow

Constructed wetlands (HSSF-CW) seem to have attracted interest for their efficiency in removing organics, nutrients, and pathogens in addition to being widely regarded as being cost-effective, particularly for small towns (Jorsaraei et al. 2014). These treatment units have been used to treat many different types of wastewater, including municipal wastewater, industrial effluents, agricultural and storm water runoff, grey-water, and landfill leachate, due to its many benefits, including low energy consumption, ease of operation and maintenance, minimal sludge generation, and the creation of an aesthetically pleasing landscape (Merino-Solis et al. 2015; Ewemoje et al. 2015). HRT is one of the crucial factors being taken into account (Sa et al. 2019).

7.1.2 Surface Flow Constructed Wetlands

If rooted macrophytes are available, surface flow built wetlands often include shallow basins or channels with soil or other suitable mediums to support macrophyte growth. SF wetlands are also known as aerobic wetlands or free water surface wetlands. Above the substrate, the water flows gently through the marsh. While the deeper waters and substrate are often anaerobic, the near-surface layer of water is aerobic. Typically, cattails, reeds, sedges, and rushes make up the vegetation. These SF systems' plants are capable of withstanding perpetually saturated soil conditions and the associated anaerobic soils.

In wastewater containing harmful metals, SF wetlands are frequently employed to collect water, provide aeration, and retain the water for long enough for metals in the water to precipitate out. The vast water surface and slow flow in SF wetlands promote metal oxidation and hydrolysis. In the marshes, the chemicals condense and are trapped. Dissolved metal concentrations, dissolved oxygen levels, pH, the length of time the water stays in the wetland, and other factors all affect how much metal can be removed. SF wetlands work best with water that has net alkalinity to balance metal acidity and allow metals precipitation to happen.

7.1.3 CWs with Free-Floating Macrophytes

Free-floating macrophytes range in size and habitat from large plants with large leaves and roots, such as *Eichhornia crassipes (water hyacinth) or Pistia stratiotes (water lettuce*), to very small plants with tiny roots, such as *Lemnaceae (duckweeds*, e.g., *Lemna spp.*, *Sprodelapolyrhiza*, or *Wolffia spp.*). Free-floating plants are extremely prolific and among the world's fastest growing plants. Frost-sensitive *E. crassipes and P. stratiotes* are only found in the tropics and subtropics, and cannot thrive in moderate or cold climates. Lemnaceae, on the other hand, have a significantly broader geographic distribution since they can endure moderate frost.

7.1.4 CW with Floating-Leaved Macrophytes

Plant species that are rooted in the substrate and have leaves on long peduncles that float on the water's surface are known as floating-leaved macrophytes. *Water lilies (Nymphaea spp.*), *spatterdock (Nuphar lutea)*, and *Indian lotus* are examples of this kind of macrophyte (Nelumbo nucifera). This group of plants has huge rhizomes and leaves that float on the water's surface and are attached to the rhizomes by long peduncles.

7.1.5 CW with Submerged Macrophytes

The roots of submerged macrophytes are embedded in the substrate, and the entire plant is immersed in water. Plants that are submerged get nutrients from the sediments; nevertheless, it has been shown that some plants may absorb nutrients straight from the water column. Submerged macrophytes can only be used in well-oxygenated environments with low suspended solids concentrations. High suspended solids concentrations can obstruct the penetration of PHAR (photosynthetically active radiation), which is required for complete photosynthesis. As a result, it has been suggested that submerged macrophytes be used in artificial wetlands for tertiary treatment. There are several species that can be utilized in created wetlands and have been employed in laboratory or small-scale systems; however, Myriophyllum spicatum (watermilfoil) has been used in full-scale constructed wetlands (Vymazal 2013).

7.1.6 CWs with Emergent Macrophytes

A typical surface flow CW with emergent macrophytes comprises of a shallow basin or series of basins with 20–30 cm of rooting soil, 10–60 cm of water depth, and a thick stand of macrophytes. *Phragmites australis (common reed)*, *Typha spp. (cattails)*, and *Scirpus/Schoenoplectus spp.* (bulrushes) are the most popular plants used in this form of CW. The shallow water depth, low flow velocity, and presence of plant stalks and litter restrict water flow and assure plug-flow conditions, especially in long, narrow channels (Vymazal 2013).

Because of air diffusion and the creation of oxygen by the photosynthetic activities of algae and cyanobacteria, most surface flow-created wetlands include aerated zones, especially near the water surface. Anoxic and even anaerobic conditions can develop at the bottom, particularly in the decomposing plant material layer.

7.1.7 CWs with Floating Mats of Emergent Macrophytes

Even though their individual plants are not capable of generating floating mats, some emergent macrophytes may produce them. Floating islands of emergent macrophytes can arise spontaneously in field situations as a result of bottom disturbance (Vymazal 2012; Lynch et al. 2015). Floating mats are a widespread occurrence in both temperate and (sub)tropical wetlands across the world. Pallis originally documented the floating wetlands (known as "plavs") in the Danube delta in Romania in 1915.

8 Performance of CW for Elimination of Antibiotics, Antibiotic-Resistant Bacteria and Genes

The importance of biological therapy techniques that use CWs is becoming more well recognized. CWs have a long history of being used to remove pollutants, and research has shown that they may reduce organic pollutants, nutrients, heavy metals, antibiotics, and emerging contaminants like ARG/ARB significantly. They may be constructed in a variety of ways and modified to fit specific requirements (Ezzat and Moustafa 2020).

8.1 Efficiency of CWs for Removal of Antibiotics

Antibiotic medication residues are discharged into the environment by WWTP, posing a threat to the microbial populations that are critical to CW operation. Antibiotics impede bacterial growth in some cases, have an effect on denitrifying bacteria (Chen et al. 2021), and may affect denitrification rates. Biological denitrification is used to treat wastewater with a high nitrate content, and CW plays an important part in this process (Fan et al. 2020). The removal of organic carbon (the soluble labile forms present in primary treated residential wastewater), the removal of contaminants such as heavy metals, and the removal of total nitrogen are all key CW activities that rely on microbial processes.

Sulfate reduction is also crucial in metal removal; hence, sulfide oxidation is a key step in CWs. When antibiotics are introduced to CWs, for example, the presence of ciprofloxacin in wastewater influent increases resistance to other antibiotic classes such as penicillins, tetracyclines, sulfonamides, and cephalosporins, which include ciprofloxacin. Studies by Yang et al. (2019) evaluated the high concentration of erythromycin and sulfamethoxazole in the influent of CW and removal of these medicines from wastewater was 13–99%.

Li et al. (2020) examined tetracycline removal in an integrated VFCW with three days of hydraulic retention time (HRT) and continuous flow of residential wastewater, reporting up to 75% removal efficiency. Tetracycline and quinolone antibiotics are less soluble and have a higher tendency to become adsorbed to substrates, where they are photodegraded by sunlight. Sulfamethazine, sulfadiazine, and sulfamethoxazole have also been found to be reduced in wastewater by VFCWs, with removal efficiencies ranging from 26.42 to 84.05% (Chen et al. 2020).

The intermittent flow permits nitrification to occur in the unsaturated zone, boosting antimicrobial mediated microbial breakdown in a way that is superior than HSSF-CW, which stays saturated throughout (Nivala, et al. 2018). As a result of the unsaturated periods between pulses, the nitrification capacity for the removal of NH4-N and organic matter is increased (Kahl et al. 2017). As Reports by Fernandes et al. 2015 microbial degradation was responsible for up to a 94% drop in tetracycline and enrofloxacin concentrations. The partial saturation of the media (gravel or other media) in the bottom zone produced redox gradients across the filter beds (Pelissari, et al. 2017).

When compared to ordinary WWTPs, VSSFCWs remove more pollutants, notably emerging contaminants like antibiotics (sulfamethoxazole, erythromycin, trimethoprim, etc.) and ARGs. Liu et al. (2019) have evaluated 106 CW and found that they removed 39 drug classes as well as antibiotic resistance genes. VSSFCWs were shown to be the most effective of all the designs, with an average removal of 70% of the antibiotics. In a VFCW system, a research found that ciprofloxacin was removed in the range of 83–93% while sulfamethazine was removed in the range of 56–69%. In a VSSF-CW, Liu et al. (2019) demonstrated an 81% decrease in macrolide concentration.

Sochachi et al. (2018) found that VFCWs planted with reed canary grass “Picta” (Phalaris arundinacea L. var. picta L) for the treatment of urban wastewater removed sulfamethoxazole in the range of 52.8–91.2%. Antibiotics or medicines, as well as their transformation products, were shown to have a direct effect on the floating plants, causing them to deteriorate and decay, indicating phytotoxic activity. It was also shown that, despite high antibiotic/pharmaceutical concentrations, emergent macrophytic species in subsurface flow built wetlands (SSFCW), such as Giant Miscanthus, showed no physical changes, suggesting the usage of hybrid systems for SSFCW and SFCW. Tetracycline removal percentages were from 89 to 100%, whereas ciprofloxacin removal percentages ranged from 60 to 94%, with an inverse association between nutrient content and antibiotic removal. The importance of plant species linked with microbial communities in effectively eliminating antibiotics has been highlighted in lab-based investigations.

In a 2016 study by Choi et al., it was discovered that Pharagmites australis and Triarrhena sacchariflora were capable to effectively removing the drugs sulfadiazine, sulfamethoxazole, sulfamethazine, and trimethoprim with a 48-h retention time. Additionally, in the presence of a significant nutrient content, Chrysopogon zizanioides has been utilized to extract drugs, particularly tetracycline and ciprofloxacin, from secondary wastewater effluent (specifically N and P). Tetracycline removal ranged from 89 to 100%, while ciprofloxacin removal ranged from 60 to 94%. The concentration of nutrients and antibiotic removal was inversely correlated. Studies conducted in laboratories have shown the importance of plant species connected to microbial ecosystems in effectively eliminating antibiotics.

Kurade et al. (2019), chose *Ipomea aquatica* for its ability to remediate 100% sulfamethoxazole in 30 h. Biodegradation was shown to be the major route of sulfamethoxazole elimination, accounting for 82% of the reported decrease. Ipomea transformed sulfamethoxazole to simple molecules such 4-aminophenol as its end product, according to the findings. Plant roots aided in bio-adsorption but only removed 0.77% of the sulfamethoxazole, while leaves bio-accumulated up to 16.94% of the medication.

After 48 h of exposure to sulfamethoxazole, there was an 8% rise in chlorophyll and a 9% drop in carotenoids, but there was no harmful effect on *Ipomea's* photosynthetic activity. It possesses a toxicant and abiotic stress defense system that is triggered upon the first exposure to sulfamethoxazole. In another study, the efficiency of CW planted with *Juncus acutus* was used to remove ciprofloxacin at up to 93.9% removal rates, but no substantial removal of sulfamethoxazole was detected (Christofilopoulos et al. 2016).

Juncus acutus is a halophyte that can withstand a variety of stresses, including organic xenobiotics, heavy metals, hormone-disrupting compounds, medications, and personal care items. This makes it an excellent candidate for CW. Only 2% of plant species (out of all known plant species) are halophytes with excellent phytoremediation capacity (Alhaddad et al. 2021). Phytostabilization, in addition to metal buildup, has been identified as another remediation process in the root structure of J. acutus.

8.2 Efficiency of CWs for Removal of ARBs and ARGs

CWs have been studied for their capacity to lower antibiotic drug concentrations in wastewater systems, as well as for their ability to ameliorate ARBs and ARGs. In this context, a vast body of research investigating the efficacy of CWs for reducing fecal bacteria, particularly fecal pathogens, contributes to our understanding of CWs' potential for remediating antibiotic-resistant bacteria from both feces and the environment. Previous studies have already reported the removal of pathogenic and fecal indicator bacteria in a variety of CW systems (Martin et al. 2012).

Pathogenic and fecal indicator bacteria have been removed from a number of CW systems in previous research. Also, positive relationships between microbiological parameters and NH4, total nitrogen (TN), have been found for CW, as well as a negative association between *E. coli* and total suspended solids (TSS), TP, COD, and biochemical oxygen demand (BOD) (Russo et al. 2018). Lamori et al. (2019) highlights that the *E. coli* concentration in a CW's effluent fell by up to 50% when compared to the influent. The presence of E. *coli*, *Enterococci*, *Bacteroidales* (HF183), and ARG (erm(F) and int1) in the influent and treated effluent was compared in the research. It was shown that erm(F) and int were lowered by 13% and 67.2%, respectively.

The study also discovered that there was no link between turbidity and ARG, despite the fact that sediments serve as a reservoir for ARGs. Water temperature, sedimentation rate, pollution source, and overall bacterial growth all influenced *E. coli* growth. At higher temperatures (particularly during the summer), *E. coli* (which was found in 100% of the water samples) was reduced by 31 to 70%, and *Enterococci* was reduced by 70 to 99%. Temperature influenced microbial composition in treatment plants, which influenced bacterial clearance rates. The microorganisms thrive and live in the pH range of 5.5–7.5, and they are pH-sensitive. In CWs, higher pH was linked to more bacterial/pathogen clearance.

Pathogen clearance is also influenced by physicochemical characteristics of wastewater and the source of treated effluent. Overall bacteria levels appear to be decreasing, but they are still numerous in the final effluent of a typical treatment facility. Because of vegetation, warmth, sunshine, and a long retention duration, CWs lower bacteria concentrations even further. Furthermore, as reported by Fang et al. (2017), the clearance rate of ARGs is significantly higher in the winter than in the summer, most likely due to the lower temperature.

Hien et al. (2017) used *Pharagmites australis* to test the elimination of five tetracycline-resistant genes (tet C, M, O, Q, and W) and reported full removal by a CW with 1 day of retention duration. The CW showed a decrease in ARGs of up to 3 logs, which increased after a 2–3 day HRT. Sulfonamide- and tetracycline-resistant genes were found in the influent and effluent of WWTPs with and without a CW, according to a study published recently. ARG removal capability was assessed at two WWTPs: one utilizing a traditional activated sludge (AS) system and the other coupling a CW with AS. When compared to AS, the CW was found to be more effective for reducing ARG.

The int1 gene showed a substantial positive connection with ARGs, indicating that MGE influences ARG dispersion in CW. The HSSF-CW was used to assess the fate of ARGs sulI, sul2 (sulfadiazine), tetM, tetO, tetQ, tetW (from tetracycline), and 16S rDNA genes. The HSSF-CW was proven to outperform a typical treatment plant with aerated filters or UV disinfection. The drop in ARG following treatment was on the range of 1–3 log units (Fang et al. 2017).

Miller et al. (2016) has reported on the effectiveness of CW for the elimination of ARGs, especially when the HLR and HRT are taken into account. High infiltration and HLR in the VSSF-CW aid to filter out germs and bind extracellular DNA to soil particles. HRT over a lengthy period of time causes the bacteria to breakdown the antibiotics, enabling the process to take place in the CW bed.

9 Pros and Cons of Using Constructed Wetlands

Generally, the overall treatment process (i.e., what other (primary) treatment measures are being implemented) and the amount of available space and land must be taken into consideration when making the ultimate decision on whether to apply and use wetland technology. Construction of wetlands necessitates careful management that improves the wetland's capacity to treat water (Table 1).

To meet out and resolve the challenges posed by the conventional methods, a natural decomposer of the organic waste was introduced to develop a new treatment technique. Vermifiltration is an innovative, decentralized and emerging sustainable technology, where earthworms inside a biofilter help to remove the organic pollutants and pathogens with the help of indigenous microbes. Vermifiltration is a bio-oxidative process in which earthworms interact intensively with microorganisms within the decomposer community, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties. The muscular action of the foregut of earthworm is important for the digestion and decomposition of pollutants and waste. Cast excreted by earthworm was found to have high nutritional value as it usually consists of minerals like Ca, N, P, K, Na, Mg, Cu, Fe, Zn, etc.

Table 1 Pros and cons of constructed wetlands

Advantages	Disadvantages
Very low operational costs	Capital cost is ranging from medium to high
Very low energy and materials inputs	Require large area and lands
Different treatment processes are available which can stand a wide range of pollutants and toxicants	May accumulate toxic substances in the sediments and may contaminate the site
Considered a natural, sustainable, and suitable practice for polluted and wastewater treatment	They are mimicking natural ecosystems and usually have seasonal activity patterns which may result in seasonal variations in performance
Constructed wetlands for wastewater treatment may act as useful wildlife habitats and preserve nature	Attract some wildlife to wetlands constructed for wastewater treatment performance through secondary contamination of water or through physical damage of wetland vegetation by wildlife Depending on the actual site, the use of high trees and shrubs can interject flight routes and sight lines and reduce the systems' habitat value for certain species
	Treatment efficiency for BOD, large land requirements, and little flexibility in operating conditions. Systems with pre-sedimentation require good maintenance of stage

It has also been reported that earthworms create aerobic conditions in the waste materials by their burrowing actions; inhibiting the action of anaerobic microorganisms and the enhanced performance of the vermifiltration process for wastewater is due to better aerobic conditions from the burrowing action of earthworms, the greater adsorption effect of the earthworm casts, and higher levels of microbial activity stimulated earthworm feeding (Zhao et al. 2010). The end product of vermifiltration is a nutritionally rich content that serves as excellent fertilizer to improve soil fertility and is known as the "vermicompost." Microbial activity in vermicompost is higher than the soil and organic matter from which the compost was formed. It is rich in beneficial microflora such as fixers, phosphorus solubilizers, and cellulose decomposing microflora and the enzymes such as phosphatase and cellulase released enrich the soil microbes.

Vermicompost contains earthworm cocoons and increases the population and activity of earthworm in the soil. It enhances the decomposition of organic matter in soil. Vermifiltration has an advantage over conventional treatments as the end product is free from pathogens, toxic elements, weed seeds, etc. It prevents nutrient losses and increases the use efficiency of chemical fertilizers. This vermifiltration can be very well called an efficient technology with higher treatment potential, low sludge production, and comparatively lower operating and capital cost.

10 History of Development of Vermifiltration

The first recorded application of earthworms to manage landfill and waste by process of vermicomposting is by Holland in 1970. Later, other countries like the UK and Canada followed by the US, Italy, Philippines, and other orient countries, Russia, etc.

In India, the vermicomposting program was launched in the 1990s pioneered by Bhawalkar Earthworm Research Institute (BERI) in Pune and Tata Energy Research Institute (TERI) in Delhi. In recent years, vermicomposting has mostly grown as a part of a "sustainable non-chemical agriculture" program combined with the government's "poverty eradication" program targeting farmers, who are using vermicompost on large scale in agriculture.

Although very useful in increasing soil fertility of agricultural soil is one of the popular uses in India, vermicomposting's salient feature of breaking down organic waste has also made it quite successful in wastewater and aerobic sludge treatment. The worms not only ingest the organic matter but also any fungi, protozoa, algae, nematodes, or bacteria present in it. These then pass through the worm's digestive tract. Although the majority of the bacteria and organic matter is passed through undigested (although the organic matter has been ground into smaller particles), it has been reported that the overall pathogenicity of wastewater reduces after being treated with vermifiltration method. Eastman et al. showed that all the indicator pathogens like *E. coli* and *Salmonellae* reduced during the formations compared to the controls (Arora et al. 2014a,b). Additionally, it has now been demonstrated that vermifilter was successful in removal of antibiotic-resistant bacteria from the wastewater (Arora et al. 2021).

The vermicomposting process may not remove toxic substances, but it can in some instances change the chemical makeup of the sewage. Since vermicomposting was found to be successfully improving the sewage, one Australian group tried to use vermicomposting in the sludge. Their large-scale operation converted sewage sludge into an end product that could be safely used for agricultural purposes. Soon after the biofilter action of earthworms was used to treat wastewater by improving parameters like BOD, TSS, etc. (Sinha et al. 2010), various innovations have since been done to improve the technology and outcomes as described during the applications section ahead.

11 Mechanism of Vermifiltration

Vermifiltration is the process used for the treatment of wastewater with the help of earthworms and microbes present in filter beds of vermifilter (VF). The body of earthworm acts as a biofilter as they utilize the organic waste products present in the wastewater absorbed by filter beds. VF filter beds play a very important role in the process of filtration. These beds are made up of soil mixture, compost, and artificial matrix. Filter beds help in the absorption of homogenous organic matter produced from the digestion of insoluble organic matter by earthworms. It also enriches the growth of beneficial microorganisms which further helps in the decomposition of waste matter and increases earthworm biomass. On the other side, the filter bed produces a sieving effect for the filtration of wastewater and sludge during treatment in VF. Thus, the use of appropriate filter material for the construction of filter bed in VF is critical for enhancing the working efficiency of vermifiltration.

Various filter beds have been used to test the effectiveness of each filter material in vermifiltration. In 2010, Wang et al. utilized converter slag–coal cinder as a filter bed to improve the efficiency of VF for absorption of inorganic waste like phosphorus, ammonium nitrogen from waste matter. Xing et al. (2010), constructed the two different filter beds made up of Ceramit and quartz sand and compared its effectiveness in reducing and stabilizing municipal sewage sludge, and concluded that for earthworm filtration Ceramit earthworm filter bed was better of the two (Xing et al. 2010). Kumar et al. (2014), have illustrated the potential of river bed material as filter bed and compared its performance with geofilter (without earthworms) by applying wastewater at different HLR in VF (Kumar et al. 2014).

In another study, Kumar et al. (2015), evaluated the vermifiltration process by using different natural ingredients as filter bed in the river, or other bed material like wood coal, glass balls, and mud balls (Kumar et al. 2015). Arora et al. (2014), compared four different media materials for the removal of pathogens during vermifiltration. These studies show different filter materials have different absorption capacity and hydraulic permeability for wastewater in the vermifilter (Jiang et al. 2016). Therefore, the selection of better filter media to treat types of pollutants plays an important role in the efficiency of vermifiltration. The earthworms burrow inside the filter bed and thus increase the porosity of the bed which helps to absorb the waste particles present in the filter bed.

Further, earthworm grinds the waste matter present in bed with gizzard and thus reduces the percentage of organic, inorganic, and suspended solids. The excreted product of earthworms called vermicast contains different beneficial microbes and is rich in minerals and further can be used in agricultural lands. These beneficial microbes and earthworms work symbiotically to degrade the waste and thus enhance the system's working capacity (Arora et al. 2014a,c). The whole process of vermifiltration takes place in aerobic conditions. Mucus and coelomic fluid secreted by earthworm mixes with the waste matter in bed and enhances the biochemical activity of the process (Rajneesh Singh et al. 2017) (Fig. 1).

The addition of earthworms in filter beds is also shown to reduce the emission of odorous gases like NH_3, N_2O, and CH_4.

12 Role of Earthworm-Microbe Interaction in Vermifiltration

The main concept which comes into play in the process of vermifiltration is earthworm–microbes interaction. The process of this interaction starts with the earthworm where it transforms the insoluble organic matter with the grinding action of gizzard and muscular action of its foregut into homogenous, soluble form, thus giving the finer particles with the increased surface area. These fine particles are readily available to the microbes for further biodegradation of the waste matter particles. As a result, the amount of organic matter in the waste is reduced and its degree of mineralization increases. The earthworm activity, i.e., burrowing, grinding, digesting and casting in the filter bed, alters the microbial biomass as well as the microbial community structure and their functional activity.

The burrowing action of the earthworm improves the aerobic condition in the VF and promotes the growth of aerobic microbes. This leads to a greater diversity and range of bacteria from top to bottom in the worm filter bed in VF (Zhao et al. 2010). Hence, the presence, distribution, and activity of earthworms play an important role in regulating microbial activity in VF (Liu et al. 2012), and therefore, research is being conducted to decipher the complex symbiotic relationship between earthworms and microorganisms and their role in wastewater treatment (Arora et al. 2020).

It has been shown that even the Cast and Mucus secreted and released by earthworms have stimulatory effects on the growth of microbes in VF (Zhao et al. 2010). The study showed that Mucus could accelerate the mineralization and humification rate of organic matter (Huang et al. 2018) which significantly stimulates microbial activity and bacterial abundance during the treatment of waste material. The Cast excreted by the earthworm is due to the action of enzymes and gut-associated microbes which convert organic matter to a more exchangeable and available form.

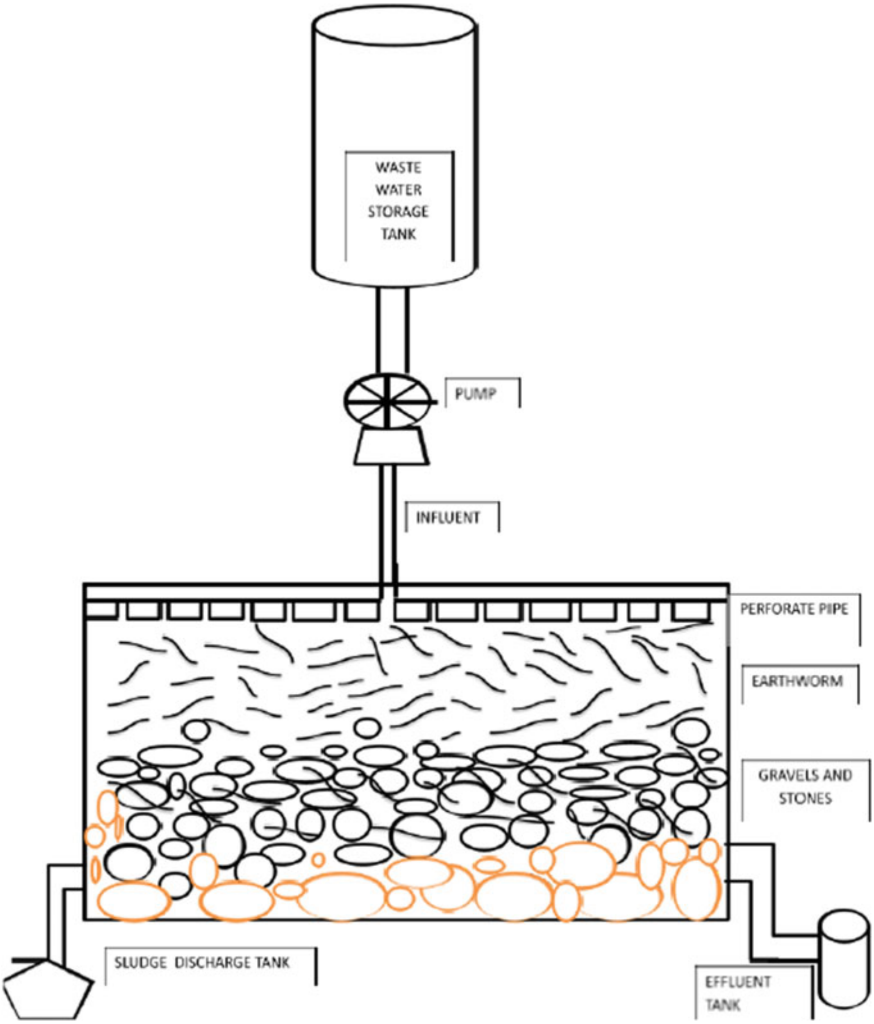

Fig. 1 Schematic diagram of a typical sewage treatment process as compared to a vermifiltration treatment process. Adopted from Arora et al. (2021)

Thus, the Cast alters the physicochemical properties of organic matter due to the reorganization of its structure and resulting in the size of particles easily available to microbes (Zhao et al. 2010). In addition, Cast is rich in minerals like C, N, P, K, and S (Yi-Wei et al. 2012) and might be dominated by the bacteria belonging to the *Flavobacterium sp*, *Myroides sp*, *Aeromonas sp*, *Sphingobacterium*, and *Myxococcales* which are responsible for activities like biodegradation, denitrification, and mineralization (Liu et al. 2012).

13 Applications of Vermifiltration

13.1 Treatment of Domestic Wastewater

Sinha et al. (2010) had utilized earthworm vermifilter for degradation of organic waste matter present in sewage water and analyzed the reduction of BOD (90–98%), COD (60–40%), and TSS (90%). A group reported mean removal rate of COD 83.5%, BOD 89.3%, and SS 89.1% and used *Eisenia andrei* in their filter bed. Wang et al. (2010) studied that vermifilter was effective for removal of inorganic waste matter from domestic wastewater with the average removal rate of TCOD, BOD, ammonia nitrogen ($NH_4^+{-}N$), and phosphorus removal by the system being 78.0, 98.4, 90.3, 62.4%, respectively. Xing et al. (2010), had shown the significance of vermifilter for domestic wastewater treatment with reduction of COD (57.55 to 47.26%), BOD (60–89 to 54.78), SS (77.90 to 62.60%), TN (11.9 to 9.8%), and NH_4 -N (62.31 to 21.01%), and they further improved the working efficiency of vermifilter, i.e., COD (75.54%), BOD (58.37%), and SS (64.61%) (Xing et al. 2012).

In 2013, Liu et al. conducted a study on a full-scale vermifilter for domestic wastewater and reported average removal efficiency of COD, BOD, NH4-N, and TSS was 67.6, 78.0, 92.1, and 89.8%, respectively, during their 17 months of study. Wang et al. (2016) studied the effect of the earthworm, *Eisenia fetida*, on the removal of COD (81.3%), NH_3 (98%), and total N_2 (60.2%) and total phosphorous (98.4%) from domestic sewage wastewater during 131 days. Arora et al. (2014) had also reported that the concentration of BOD and COD decreased with a mean removal efficiency of 84.3 and 73.9%, respectively, during 10 weeks of study. Wang et al. (2016) investigated that vermifilter could remove BOD, COD, and SS up to 81.3, 83.5, and 93.5%, respectively, in domestic wastewater. Kumar et al. (2015) performed a comparative study to check the efficiency of two different epigeic earthworms, i.e., *Eisenia fetida* and *Eudrillus eugeniae*, for the treatment of domestic wastewater and it was revealed that *E.fetida* was more potential for the treatment of wastewater during the vermifiltration process. A summary of different studies applying the vermifilters for treatment of domestic wastewater is given in Table 2.

13.2 Treatment of Industrial Wastewater

Treatment of industrial wastewater is very important for the control of water pollution. One of the simple and low-cost technology issues of vermifilters which is used to treat wastewater released from various industries. Various studies have been conducted to check the applicability of vermifilter for the treatment of wastewater released from different industries.

In 2010, Sinha et al. utilized vermifilter for the treatment of highly turbid liquid waste of the dairy industry. They had noticed that BOD, COD, TDS, and TSS had been removed by over 98, 80–90, 90–92, and 90–95%, respectively. The study by

Table 2 Application of vermifiltration in treating domestic wastewater. BOD = Biochemical Oxygen Demand; COD = Chemical Oxygen Demand; TN = Total Nitrogen; TP = Total Phosphorus; $NH_4{}^+{-}N$ = Ammonium Nitrogen

Wastewater	Earthworm species	Organic matter removal%	Nutrient removal %	Reference
Municipal	*Eisenia fetida*	COD-57.55-47.26%, BOD-60.89-54.78%, SS-77.9-62.06%	TN-11.9-9.2% NH_4-N-62.31-21.01%	Xing et al. (2010)
Domestic	*Eisenia fetida*	BOD-78.0% COD-67.6% TSS-89.8%	NH_4-N-92.1%	Liu et al. (2013)
Domestic wastewater	*Eisenia fetida*	BOD-84.8% COD-73.9%		Arora et al. (2014)
Domestic wastewater	*Eisenia fetida*	BOD-81.3%,COD-83.5%,SS-93.5%	TN 32.4%, $NH_4{}^+{-}N$ 55.6%, TP 38.6%	Wang et al. (2016)
Domestic	*Eisenia fetida* *Eudrilus eugeniae*	BOD-88%, TSS-67%,TDS-75% BOD-70%, TSS-67%,TDS-60%	$NH_4{}^+{-}N$-86%,	Kumar et al. (2016)

Ghatnekar et al. (2010) involves the use of three-tier vermiculture biotechnology coupled with vermifiltration technology for the treatment of liquid effluent generated by gelatin manufacturing. They analyzed the effluent after each vermifilter treatment and reported a reduction of TS (77.56%), TDS (81.97%), COD (90.08%), and BOD (89.24%). Dhadse et al. (2010) also utilized vermifilter technology for Herbal Pharmaceutical wastewater and noticed an efficient decrease in BOD and COD up to 89.77–96.26% and 85.44–94.48%, respectively, during 2 days of Hydraulic Retention Time (HRT). A group used a vermifilter bed with *Eisenia fetida* for the treatment of toxic wastewater rich in hydrocarbons released from the petroleum industry. They reported the body of earthworm acts as a biofilter and removed BOD by 90%, COD by 60–80%, and TDSS by 90–95%.

Lim et al. (2014) utilized earthworm *Eudrilus eugeniae* with two vermifilter beds made of soil and rice straw for treatment of palm oil mill effluent (POME) and recorded soluble COD and volatile solids (VS) removal after treatment in the range of 56.9–87.9% and 19.7–27.1% with rice straw and soil filter beds, respectively. Samal et al. (2018) designed a vermifilter that involves macrophyte Canna indica and earthworm *Eisenia fetida* for the treatment of dairy wastewater. They observed hybrid macrophyte-assisted vermifilter (MAVF) systems had removed 83.2% COD and 57.3% TN. Singh et al. (2018), developed a horizontal subsurface flow vermifilter to optimize the parameter involved in the removal of organic waste matter present in brewery wastewater. They achieved 96.24, 21.57, and 43.3% removal of COD, TN, and TP, respectively, with an HRT of 26.66 h. Miito et al. (2021) recently conducted

Table 3 Application of vermifilters in treating industrial effluents

Industry Effluent	Earthworm	Organic matter removal%	Nutrient removal%	Reference
Gelatin industry	*Lumbricus rubellus*	BOD-89.24%, COD-90.08%, TDS-77.56%, TS-77.56%		Ghatnekar et al. (2010)
Herbal pharmaceutical	*Eudrilus eugeniae*	BOD-89.77- 96.26%, COD-85.44–94.48%		Dhadse et al. (2010)
Petroleum	*Eisenia fetida*	BOD-90%, COD-60–80%, TDS-90–95%		Sinha et al. (2010)
Palm oil mill	*Eudrilus eugeniae*	COD-56.9–87.9%, VSS-19.7–27.1%		Lim et al. (2014)
Dairy	*Eisenia fetida*	COD-83.2%		Samal et al. (2018)
Brewery industry	*Eisenia fetida*	COD-73.88%	TN 8–21%, TP 40–60%	Singh et al. (2018)
Dairy		TS-21%,TSS-68%,COD-45%		Miito et al. (2021)

a study of six months to evaluate the efficacy of pilot-scale vermifilter for treatment of dairy wastewater and analyzed reduction in the TS (21%), TSS (68%), and COD (45%). All these studies show that the Vermifilter technology is applicable for the removal of organic matter and nutrients from wastewater released from different industries. A summary of different studies applying the vermifilters for treatment of industry based wastewater is given in Table 3.

14 Application and Identification of the Proteins Expressed in Coelomic Fluid

The coelomic cavity of earthworm, i.e., coelom, consists of coelomic fluid containing free wandering cells, named coelomocytes, originating within the mesenchymal lining of the cavity and several proteins. These proteins of coelomic fluid are secreted from adjacent tissue (intestine) and by coelomic cells. The coelomic fluid plays a crucial role in providing immunity to earthworms. Many of proteins of coelomic fluid are reported to be involved in defense mechanism against soil pathogen by having hemolytic, hemagglutinin, proteolytic, and antibacterial properties. Several proteins reported in coelomic fluid are lysozyme, fetidins, lysenins, eiseniapore, and coelomocyte cytolytic factor-1.

After knowing importance of coelomic fluid of earthworm in its immune response, many researchers have tried to identify, characterize, and isolate the molecules responsible for these activities. Lysozyme is a protein that cleaves β 1–4, glycosidic bond between N-acetylmuramic acid and N-acetyl D-glucosamine residues and thus is bacteriolytic. Many studies have been done for isolation and characterization of lysozyme-like molecule in annelids including in coelomic fluid of earthworm. It has been shown that lysozyme-like activity in *Eisenia* coelomic fluid on injecting pathogenic Gram – and + strain in earthworms and maximum activity was reported after 4–5 h of injection. Fast Protein Liquid Chromatography was performed for its purification, and its amino acid analysis was also performed.

In 2009, a group assembled cDNA coding for lysozyme in earthworm by RT-PCR and RACE system and showed that this lysozyme shares high homology with other invertebrate lysozyme. Previously, a protein called lysenin of 41 kDa was also extracted from the coelomic fluid of an earthworm, *Eisenia fetida*, using techniques like anion exchange HPLC and size exclusion HPLC, and molecular weight was determined by SDS-PAGE. This protein was responsible for causing contractions in an isolated rat aorta. It was reported that lysenin a hemolytic protein in coelomic fluid of earthworm could bound to sphingomyelin a phospholipid present on cellular membrane and inhibit lysenin-induced hemolysis if sphingomyelin is present in vesicles.

Another group had purified two protein named fetidins of MW 40 kDa and 4 kDa from dialyzed coelomic fluid by anion exchange chromatography and was responsible for hemolytic and antibacterial activity against soil pathogens. In 1997, a cDNA was made encoding fetidin. A group isolated and characterized three proteins from coelomic fluid of *Eisenia fetida* called H1, H2, and H3 with MW of 46, 43, and 40 kDa, respectively, in 1998. IEF of these proteins indicate that these proteins exist in different isoforms with pIs between 5.1 and 6.2. H1 and H2 have been reported to have hemolytic activity while H3 has both hemolytic and hemagglutinating activity. It was concluded that coelomic fluid of earthworm of *Eisenia fetida* contains a cytolytic molecule called as coelomocyte cytolytic factor-1 (CCF-1) of 42 kDa which may exert lytic activity on mammalian cell lines and is responsible for defense system in earthworms.

There are several methods developed for the extraction of coelomic fluid from earthworms.

1. **Warm Water Method**: The collected earthworms are washed in distilled water to remove soil and dust residues and then placed on filter paper to remove water from their surface. The dried earthworms are then transferred to a water beaker containing water. The temperature of the water should be maintained at 40° C for at least 30–45 min. After 30 min, water is collected and centrifuged at 5000 rpm for 10 min. The supernatant is then removed carefully and sterilized through a 0.2 μM syringe filter. The filtrate is carefully collected in a sterile microfuge tube and stored in aliquots at $-20°$ C for further analyses.
2. **Heat Shock Method**: The cleaned and dried earthworms are transferred to a Petri plate. A bag of hot water (40–45° C) is placed over the earthworm on the Petri dish to generate stress conditions for the earthworm so that coelomic fluid is released through the dorsal pores on the body due to heat shock and was collected in a sterile clean dry test tube. The collected coelomic fluid is then centrifuged at

5000 rpm for 10 min to deposit the debris, and the clear supernatant is sterilized through a 0.2 μM syringe filter into a clean dry sterile microfuge. The filtrate is stored in aliquots at −20° C for subsequent use and analyses.

3. **Cold Shock Method**: The earthworms are washed in distilled water and dried on filter paper to remove water from their surface. They are then transferred in a ziplock bag. A bag of ice was kept over the ziplock bag for at least 30–45 min so that worms feel the drop in temperature due to ice above them. The coelomic fluid is made to release through the dorsal pores on the body due to the cold shock and collected in a sterile clean dry test tube. The collected coelomic fluid is then centrifuged at 5000 rpm for 10 min to deposit the debris, and the clear supernatant was sterilized through a 0.2 μM syringe filter into a clean dry sterile microfuge. The filtrate was stored in aliquots at −20° C for further use and analysis.
4. **Electric Shock Method**: The earthworms are washed in distilled water and dried on the filter paper to remove excess water droplets. The earthworms are then placed on Petri plates and subjected to electric shock. The worms are kept under shock like this continuously for 30 min, and then, the fluid is collected. The collected fluid is centrifuged at 5000 rpm for 10 min to sediment larger particles and other debris. The supernatant is carefully removed and filter-sterilized through a 0.2 μm (pore size) syringe filter. The filtrate is stored in aliquots at −20° C for subsequent use and analyses.

15 Applications of Vermifiltration in Improving Constructed Wetlands

Tomar and Suthar (2011) combined for the first time the vertical subsurface flow constructed wetlands (VSFCW) with *Perionyx sansibaricus*, the locally present earthworms to create a small-scale vermi-biofiltration reactor. They reported that the vermin-biofiltration system is more efficient than VSFCW in terms of contamination removal efficacy. The study showed a significant decrease in TSS (88.6%), TDS (99.8%), COD (90%), NO3− (92.7%), and phosphate PO42− (98.3%) levels in the wastewater treated by this hybrid of two technologies. Vermifiltration was more efficient in terms of removal of major pollutants from wastewater.

Before this study in India, there already were a few groups, e.g., in Germany and Thailand who had tried to combine vertical surface flow constructed wetland (VSFCW) with vermicomposting earthworms to achieve better treatment of wastewater. The study reported that earthworms could thrive during warm and sunny as well as winter periods in a VSFCW setup. Study also predicted that the effect from plant cutting could lead to the earthworms staying deeper below the ground. The lab scale study done by this group showed that in terms of the solid reduction, the set with earthworms receiving a hydraulic loading rate of 12 cm/d exhibited better performance than the one without. Moreover, in their pilot-scale study, they reported that applying earthworms into the constructed wetlands was strongly suggested as they had the potential to reduce the accumulated sludge within the VSFCWs under a strong load of high-strength wastewater. Also, the configuration was either equally efficient or more as compared to using VSFCWs alone, especially in parameters such as the BOD removal efficiency of VSFCWs receiving raw domestic wastewater.

16 Conclusion

Vermifiltration technology which is a sustainable, low-cost method can be used as an alternative to conventional methods of wastewater treatment in developing countries where equipment are not available and technology is less developed. To achieve high-efficiency treatment, different filter media along with two or more species of earthworms can be used. There is a need to study gut microbes of earthworms and their symbiotic relationship to achieve better filtration of different types of pollutants present in wastewater. In addition, the Mucus secreted by earthworms plays important role in decomposition and humification and modifying microbial profile in vermifiltration system. The Cast produced by earthworms has higher nutritional value and water holding capacity and can be used in agricultural fields.

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Treatment of Metallurgical Wastewater by a Combination of Zero Valent Iron and Coagulation Technology

Khac-Uan Do, Thi-Lien Le, and Thuy-Lan Nguyen

1 Introduction

1.1 Metallurgical Industry

Metallurgical industry plays an important role in production of metals from ores or other raw materials (Kruzhanov 2018). Metallurgical processes include several techniques, i.e. processing alloys and metal ingots, and changing chemical compositions. Those techniques could create metals with suitable structure and property for using requirements. Metallurgical industry is divided into two main categories: ferrous metallurgy industry and non-ferrous metallurgy industry.

Metallurgical processes to produce metal from ore include three steps, i.e. (i) ore purification; (ii) fabrication of raw materials by removing impurities; (iii) refining the raw materials to obtain the desired purity metals. A general metallurgical technology diagram is shown in Fig. 1.

As shown in Fig. 1, there are three methods of making metals from ores, i.e. fire refining, hydrometallurgy and electrolysis. In fire refining, heat was used to recover metal from its ores (Jiang and Xu 2017). This process includes the following steps: (i) selection of ore, (ii) drying to separate free water, (iii) calcination to dehydrate the bond (at a temperature higher than 200 °C) and (iv) sintering and smelting to

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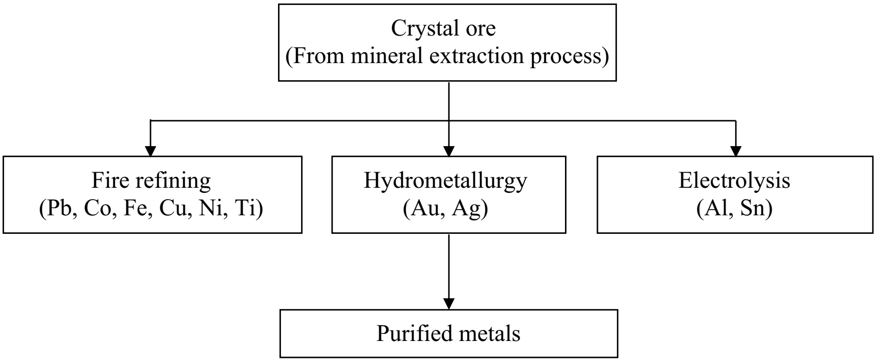

Fig. 1 Schematic diagram of general metallurgical technology

separate slag and impurities in ore. In hydrometallurgy process, liquid was used to dissolve metals. Metals were then separated from impurities. This process included the following steps: (i) extraction of ores, (ii) dissolution in solvents and (iii) separation of metals from solution. Electrolysis method was carried out based on the principle of dissociating metals to opposite electrodes (Jin and Zhang 2020). Metals were then separated from impurities in solution.

1.2 Metallurgical Wastewater

At metallurgical plants, wastewater was mainly discharged from (i) cooling water for ore incinerators, (ii) cooling water furnace gas and (iii) product cooling. Basically, this amount of wastewater was recycled and reused in the production process (Wu et al. 2017). Therefore, only a very small amount of wastewater was discharged into the environment. In the hydrometallurgical and electrolysis plants, the amount of wastewater generated was small (Rodriguez et al. 2007). However, wastewater could contain many toxic chemicals from mineral extraction, electrolysis, analysis and electrolytic sludge treatment (Elwakeel et al. 2020).

It should be noted that at the experimental and the semi-industrial scale, the amount of wastewater was generated small. However, the composition of pollutants in wastewater was very complex. For example, wastewater in ore extraction could contain inorganic impurities with high suspended solids content. Wastewater in pyrometallurgy could contain the impurities of ores and smelted metals. Wastewater in hydrometallurgy could include a dissolved solution containing impurities in dissolved, suspended and metallic forms (Dudeney et al. 2013). In metal processing, water was used to clean metal surface. It was used to prepare chemicals in the form of solutions for cleaning, polishing and coating. In this process, wastewater could contain many kind of pollutants such as rust, heavy metals, grease, caustic soda, acids and detergents (Qasem et al. 2021). The characteristic of wastewater of metallurgical industries in general and of metallurgical experimental facilities in particular could contain high concentration of heavy metals (Ocheri 2020). Therefore, recovering metals in the wastewater could be necessary before discharging.

1.3 Treatment of Metallurgical Wastewater

Currently, the neutralization method by using lime solution has been used widely in removing heavy metals in metallurgical industry wastewater (Zhang and Duan 2020). However, this method contained many disadvantages, such as low treatment efficiency, generating a large amount of sludge and treated water containing high hardness (Agoro et al. 2020). $FeSO_4$ could be used to remove Cr^{6+} and other heavy metals (Yin et al. 2020; Shah 2020). In the reduction process, Cr^{6+} was converted to Cr^{3+} at pH of less than 1.5 while other heavy metals were treated at pH of 7–9. Heavy metals could be removed by electrocoagulation combined with microelectrochemistry (Vidu et al. 2020). In this combination process, the optimal conditions should be at pH of 5 and the initial iron concentration of 50 mg/L.

Some heavy metals (i.e. Cu^{2+}, Pb^{2+}, Zn^{2+}) could be absorbed by *Saccharomyces cerevisiae* yeast (Ferdous et al. 2016; Machado et al. 2009). In this process, S. cerevisiae yeast could absorb heavy metals at pH of 5 in the sequence Pb^{2+}>Cu^{2+}>Zn^{2+} with the initial concentration of 50 mg/L (Chen et al. 2008; Civilini et al. 2006).

It should be noted that chemical and physical treatment technology has been the most effective technology for wastewater treatment containing heavy metals (Yan et al. 2016; Zewail and Yousef 2015). However, metallurgical wastewater including many metal ions with different valences and a small flow rate has not currently considered. Many types of technologies (i.e. mineral extraction, metallurgy technology) were used in the metallurgical experimental facilities. The flowrate and the composition of pollutants in wastewater were varied and very complex. It must be treated effectively before discharging into the environment. However, managing and treating this source of wastewater could be very difficult and expensive. Wastewater at metallurgical research facilities has been seen as a big problem that needs to be addressed (Do et al. 2022). So far, membrane technology has been studied and applied in municipal and domestic wastewater (Banu et al. 2009; Chen and Uan 2013; Do et al. 2009, 2013; Uan et al. 2013; Shah 2021). Membrane filtration could enhance the performance of the treatment system (Do and Chu 2022; Do et al. 2018). Membrane technology could be also applied to metallurgical wastewater treatment (Amosa 2017). Besides, biochar has been used as a low-cost adsorbent that could be used for wastewater treatment (Vu and Do 2021; Vu et al. 2021a, 2021b). Biochar could be applied for metallurgical wastewater treatment. Therefore, it is very necessary and urgent to find out the suitable treatment method for this wastewater source. Nano Fe^{o} could be used in removing heavy metals (Chen et al. 2020; Yang et al. 2019; Zhang et al. 2020). This could be considered as a suitable method to treat small wastewater flow but high toxicity from the metallurgical experimental facilities (Brasili et al. 2020; Li et al. 2019; Maiti et al. 2019). This method could produce less sludge and could recover useful metals.

1.4 Objective of the Work

The purpose of the study is to provide an assessment of the wastewater source and the characteristics of metallurgical wastewater. An analysis of metallurgical wastewater treatment systems was examined. An appropriate technological process was developed to improve the efficiency of heavy metals in wastewater from metallurgical research and experimental facilities.

2 Current Situation of Wastewater Treatment at Metallurgical Experimental Facility

2.1 Pretreatment of Metallurgical Wastewater

Actually, wastewater from the metallurgical experiment facility was collected and treated. Several treatment methods were applied for different wastewater sources (Ba 2015). Wastewater from the mineral extraction workshop was mainly generated from gravity selection stage. Wastewater after washing contains many suspended residues and metal ions (As^{3+}, Pb^{2+}, Cd^{2+}, Cr^{6+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Fe^{2+}). Wastewater treatment process in the mineral extraction workshop is shown in Fig. 2.

As shown in Fig. 2, wastewater generated from the mineral extraction workshop was flowed through the collection ditch system in the workshop. Several small sedimentation tanks (i.e. with dimentions of 1000 × 1000 × 1000 mm) were arranged on the collected ditch system to settle and collect sludge. Wastewater was then flowed into the settling tank. PAC solution was also added into the settling tank to increase the

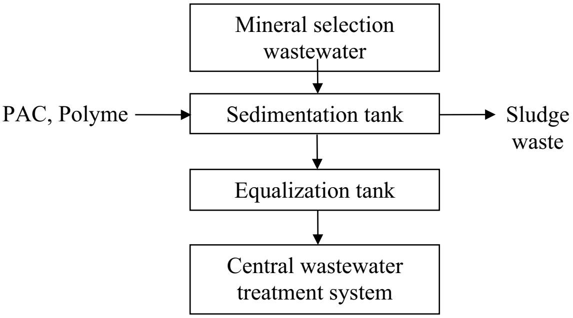

Fig. 2 Wastewater treatment system in mineral extraction area

ability of removing suspended sediments and metals in the wastewater (Kumar et al. 2019; Zhou et al. 2014). Wastewater after settling tank was collected to the neutralization tank. Limestone was used to neutralize the pH of the wastewater. After that, neutralized wastewater was flowed to the concentrated wastewater treatment system.

In the electrolysis workshop, a small amount of wastewater was generated at the sludge treatment stage. The generated wastewater was also collected into the concentrated wastewater treatment system. Wastewater was not generated from the metallurgical workshop. In this area, wastewater was generated only from the wet system for dust removal and slag cooling. This amount of wastewater was also collected into the concentrated wastewater treatment system.

2.2 Concentrated Metallurgical Wastewater Treatment

Wastewater generated from the physicochemical analysis center was characterized by acidity. It was passed through the limestone treatment tank to neutralize the wastewater. It was then flowed into the concentrated wastewater treatment system. The concentrated treatment system is shown in Fig. 3.

A combination of pretreated wastewater, domestic wastewater and rainwater was collected to the limestone treatment tank (Ba 2015). This mixed wastewater was characterized acidic. Therefore, it was neutralized by limestone (Do et al. 2022). After that, it was passed through the dolomite powder treatment tank. In this tank, small pellets of dolomite rock played a role in neutralizing wastewater. In addition, coagulation chemical (PAC) was added to remove metal ions (Oraeki et al. 2018). After that, wastewater was flowed to a settling tank to separate the sludge. Finally,

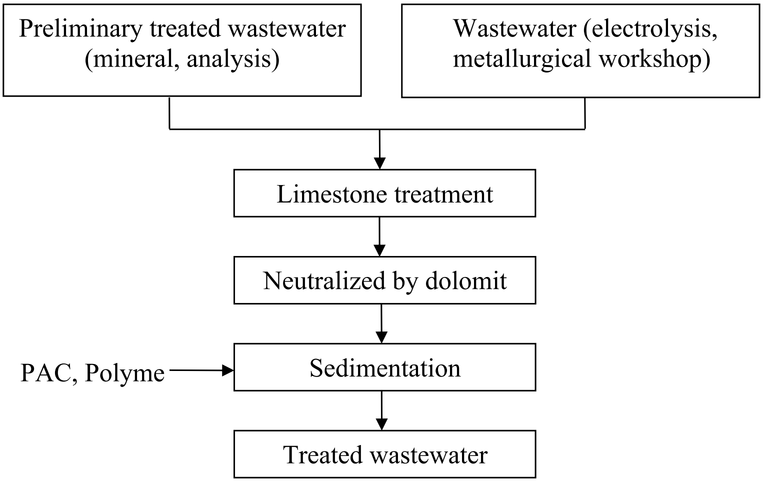

Fig. 3 Centralized wastewater treatment process of experimental metallurgical facility

treated water was overflowed into the receiving water reservoir. It should be noted that the wastewater from the experimental metallurgical facility has charactered by many metal ions and strong acidity. The quality of wastewater treated by the existing treatment technology did not sometimes meet the permissible discharge standards. Besides, a large amount of sludge was generated from this process because a large amount of limestone and dolomite powder were used. Therefore, it would be needed to develop an alternative technology to treat wastewater to ensure the allowable standards. In addition, the amount of sludge should be minimized while the quality of the treated wastewater is meeting the discharging regulations.

3 Development of Metallurgical Wastewater Treatment Technology

3.1 Propose a Metallurgical Wastewater Treatment System

The treatment technology for metallurgical wastewater is proposed in the following Fig. 4.

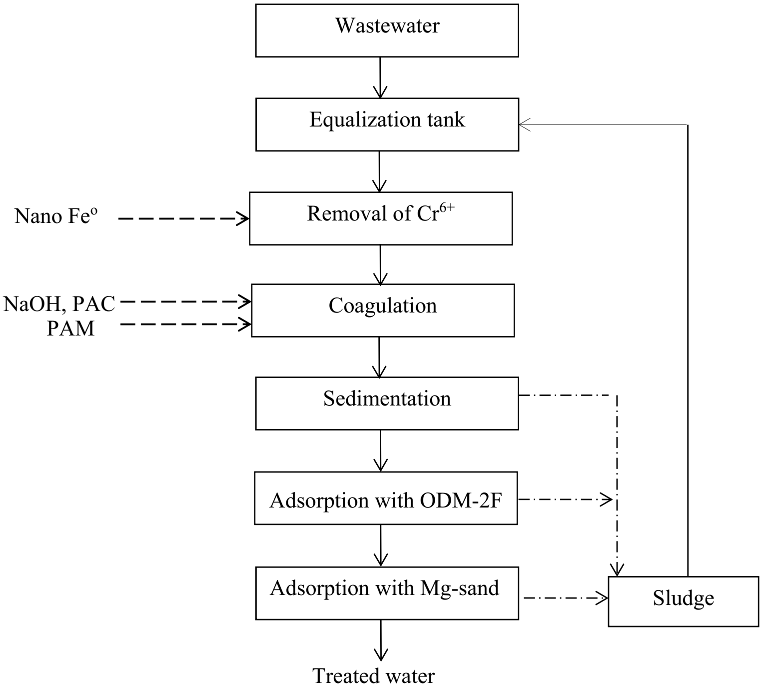

Fig. 4 Diagram of metallurgical wastewater treatment technology

As shown in Fig. 4, wastewater generated by the metallurgical experimental facility was flowed through a screen to separate the garbage before coming to the equalization tank. The screen could remove effectively the large solids (i.e. paper, wood, nylon, leaves) to protect the pumps and equipments in the next treatment stages. Wastewater was then automatically flowed into the equalization tank. An air supply system was installed to reduce the odor of wastewater and to prevent sedimentation in the tank. As a result, the flowrate and composition of wastewater were stabilized before entering the next treatment work.

Wastewater in the equalization tank was pumped to the Cr^{6+} reduction reaction tank. At low pH, Cr^{6+} was usually existed in the form of bichromate $Cr_2O_7{}^{2-}$. It is orange in color and highly toxic. More importantly, it was not precipitated when pH was raising. Therefore, Cr^{6+} must be reduced to Cr^{3+} to ensure treatment effectively. Cr^{3+} could be precipitated at pH of 7. Bichromate reduction was carried out at pH of 1.5 by using a reducing solution of nano Fe^o (Kakavandi et al. 2014). The reduction reactions of Cr^{6+} to Cr^{3+} were as follows (Kang et al. 2017; Mitra et al. 2017):

$$Cr_2O_7^{2-} + 3Fe^0 + 14H^+ \rightarrow 3Fe^{2+} + 2Cr^{3+} + 7H_2O3$$

$$Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow 2Fe^{3+} + 2Cr^{3+} + 7H_2O$$

After reducing Cr^{6+} to Cr^{3+}, the wastewater was flowed to the coagulation tank. In this tank, main process was placed to separate heavy metals from the wastewater by creating the insoluble hydroxides. Therefore, reducing agents and NaOH were introduced into the tank to raise the pH and precipitate the metal ions. A mixing system was used in the tank to mix chemicals with wastewater in the homogeneous situation. In addition, flocculation reaction was carried out to increase the separation of suspended substances in the wastewater. PAC (polyaluminium chloride) flocculation chemical was used at the dosage of 250 mg/L. PAC could help to create larger colloidal flocs to increase the settling rate (Barakat 2011; Kim et al. 2018). Besides, PAM (polyacrylamide) was used as an auxiliary for coagulation process to enhance the flocculation sedimentation efficiencies (Song et al. 2021; Xiong et al. 2018).

After coagulation, metal ions in wastewater were formed insoluble precipitates. They were capable of settling by gravity settling mechanism. Therefore, wastewater was then flowed to the settling tank to remove the flocs formed in the coagulation tank. After settling, wastewater was continuously filtered through a manganese sand (MnO_2) adsorption tank and a ODM-2F (containing diatomite, zeolite, benonnit) adsorption tank in order to ensure complete removal of metals.

3.2 Technical Characteristic of the Chemicals and Materials

Chemicals used in the continuous process model include a 35% HCl solution, 10% NaOH solution and nano Fe^o. Nano Fe^o was prepared by four steps, i.e. (i) take 4 g of $FeSO_4.7H_2O$ to dissolve with 50 mL of distilled water by shaking at 150 rpm for

20 min, (ii) add 0.1 g of PAM in 1000 mL of distilled water and leave it for overnight to make 0.01% PAM solution, (iii) add 2 g of $NaBH_4$ in 18 mL of distilled water and (iv) mix 4 solutions at a low speed of 5 mL/min to make a nano Fe^o. Fe^o nanoparticles were separated by magnets. Finally, it was washed with alcohol and then put into a desiccator to use readily.

The technical characteristics of manganese sand (MnO_2) and ODM-2F are summarized in Table 1.

Manganese sand adsorbent material plays a role as a secondary treatment in the heavy metal removal process. Some heavy metals remaining in wastewater were oxidized and converted into precipitates as follows:

$$4Fe^{2+} + O_2 + 10H_2O \rightarrow 4Fe(OH)_3 \downarrow + 8H^+$$
$$2Mn^{2+} + O_2 + 2H_2O \rightarrow 2MnO_2 \downarrow + 4H^+$$

ODM-2F material was considered as a multi-purpose material. It could maintain pH of the wastewater stable. It could reduce nitrogen (i.e. nitrite, nitrate, ammonium) and organic compounds in wastewater. Oil could be adsorbed at high capacity of about 90 mg/g. Moreover, it could reduce effectively some heavy metals, such as copper,

Table 1 Specifications of filter materials

Filter materials	Properties	Values
MnO_2 sand	Particle size	0.9–1.2 mm
	Color	Black-brown
	Density	1400 kg/m^3
	pH	≥ 7
	Specification	25 kg/bag
	Origin	Vietnam
	Chemical compositions	MnO_2 25–45%; SiO_2 17–20%; Fe 20%; $MnCl_2$ 10–20%
ODM-2F filter media	Particle size	0.8–2.0 mm
	Color	Black-brown
	Density	650 kg/m^3
	Surface area	120–180 m^2/g
	Porosity	70%
	Absorption capacity	1.3 g/g
	Water immersion	90–95%
	Specification	25 kg/bag
	Origin	Nga
	Chemical compositions	$SiO_2 \leq 84\%$; $Fe_2O_3 \leq 3.2\%$; $Al_2O_3 + MgO + CaO = 8\%$

zinc, chromium and nickel. ODM-2F adsorbent could also play a role in removing Fe and As.

4 Calculation of Metallurgical Wastewater Treatment System

4.1 Mixing in Tube

Tubular stirrer was designed on the basis of using flow change and hydraulic mixing in line. A resistant PE tube of 10 mm diameter was used as a stirring system.

The design of a mixing device was used for a capacity of 4.5 L/h as an example. Pipeline mixing was selected with a mixing time (t) of 60 s.

Mixing tube length could be calculated as follows:

$$L = \frac{4 \times Q \times t}{\pi \times D^2} = \frac{4 \times 0.0045 \times 60}{3.14 \times 0.01^2 \times 3600} = 0.955\,(\mathrm{m})$$

The tube was divided into 10 zigzags with 10 cm long of each. The tubes were installed on a stainless steel rack system. It could be removed and installed with the frame system. Wastewater was pumped into the head of the tube. Coagulant solution was added into the tube as well.

4.2 Reaction Tanks

At a treatment capacity of 4.5 L/h, a Cr^{6+} reduction tank was built with a dimension of 220 × 220 × 140 mm. Hydraulic retention time of wastewater in the tank was 60 min. An aeration system was used to ensure the mixing of chemicals into the wastewater. Dimensions of coagulation tank were 240 × 220 × 140 mm. Wastewater was mixed in this tank by the aeration system. In this tank, coagulation and floculation were taken place with the addition of PAC and PAM (Liu et al. 2017; Song et al. 2019).

4.3 Absorbent Materials

Wastewater after treatment in the coagualtion tank was pumped to the manganese sand adsorption tank and the ODM-2F adsorption tank. The adsorption column was calculated as follows. The filtration capacity of the material was selected as 0,7 L/m^2s. The minimum cross-section of the adsorption column was determined below.

$$S = 1.3 \times \frac{1000 \times q}{3600 \times \mu} = 1.3 \times \frac{1000 \times 4.5 \times 10^{-3}}{3600 \times 0.7} = 0.0018\,(\mathrm{m}^2)$$

Therefore, the minimum radius of the absorption column was calculated as

$$R = \sqrt{\frac{S}{\pi}} = \sqrt{\frac{0.0018}{3.14}} = 0.023\,(\mathrm{m})$$

It was reported that the adsorption capacity of manganese sand and ODM-2F materials for heavy metals was 0.0012 mg/g (called λ).

Assuming that before entering the adsorption column, the remaining manganese content was 10 mg/L. After treatment, the manganese content was assumed as 0 mg/L. It meant, the manganese load for the adsorption column was as follows:

$$g = q \times \Delta C = 4.5 \times (10 - 0) = 45\,\mathrm{mg}/\mathrm{h}$$

Assuming that the number of recovery times was 1 time/h (called I). Then, the required volume of manganese sand and ODM-2F materials was as follows:

$$m_{MnO_2} = m_{ODM-2F} = 1.3 \times \frac{g \times I}{\lambda} = 1.3 \times \frac{45 \times 10^{-3} \times 1}{0.0012} = 48.75\,\mathrm{g}$$

The volume of adsorbent was estimated as follows:

$$V = \frac{m}{\rho} = \frac{48.75 \times 10^{-3}}{720} = 6.7 \times 10^{-5}\,\mathrm{m}^3$$

The minimum height of the adsorbent layer was as follows:

$$h_f = \frac{V}{S} = \frac{6.7 \times 10^{-5}}{0.0018} = 37\,\mathrm{mm}$$

4.4 Total Height of Adsorption Column

The total height of the adsorption column was determined as follows:

$$\mathrm{H} = \mathrm{H_d} + \mathrm{H_{vl}} + \mathrm{H_n} + \mathrm{H_{dp}} + \mathrm{H_{tg}}$$

in which, $\mathrm{H_d}$ was the height of distribution area ($\mathrm{H_d} = 10$ mm); $\mathrm{H_{vl}}$ was the thickness of adsorbent layer ($\mathrm{H_{vl}} = 37$ mm); $\mathrm{H_n}$ was the thickness of wastewater layer on the surface of filter material ($\mathrm{H_n} = 15$ mm); $\mathrm{H_{dp}}$ was the fallback height

Table 2 Operational procedures of the system

Steps	Implementation procedures
Step 1	Wastewater was pumped into a zigzag pipe system. This was aimed to mix wastewater with chemicals
Step 2	10 mg/L Nano Fe^o was slowly added into the Cr^{6+} reduction tank at mixing condition for 60 min
Step 3	Wastewater from the Cr^{6+} reduction tank was overflowed to the flocculation tank. 10% NaOH solution was added into the flocculation tank to raise pH of 8. At the same time, PAC coagulant was added at a dosage of 250 mg/L. Aeration was supplied for 45 min. Then, PAM solution was added at a dose of 3.5 mg/L. Aeration was provided for 30 min
Step 4	After the treatment, sludge generated in the flocculation tank was removed. Wastewater after treatment at the flocculation tank was pumped into the manganese sand and ODM-2F adsorption column (filtered from the bottom up). Wastewater was then analyzed to evaluate the performance of the system

($H_{dp} = 5$ mm). Finally, the total height was $H = 10 + 37 + 15 + 5 = 67$ mm. The adsorption column was fixed at the height of 67 mm and the diameter of 25 mm.

5 Operational Procedure of the Metallurgical Wastewater Treatment System

5.1 Operational Procedures

The operational procedure of the metallurgical wastewater treatment system is described in Table 2.

5.2 Sample Analysis

The influent and effluent samples were taken. Heavy metal ions such as As^{3+}, Pb^{2+}, Cd^{2+}, Cr^{6+}, Zn^{2+}, Mn^{2+}, Ni^{2+} and Fe^{2+} in the samples were analyzed following the standard methods (Rice et al. 2017) (Fig. 5) shows the samples taken at the factory and analyzed at the laboratory.

Fig. 5 Samples were taken and analyzed at the laboratory

6 Performance of Metallurgical Wastewater Treatment System

6.1 Analysis Results

The analysis results of wastewater before and after treating by the above technology are shown in Table 3.

As shown in Table 3, most of parameters in the influent sample (NT0) were much higher than the discharging regulation. The treatment efficiency was reached high when nano Fe^{o} reducing agent was used. The quality of the treated wastewater was met the limits of QCVN 40:2011/BTNMT (MORE 2011). It should be noted that during operation, the four wastewater samples after treatment (NT1, NT2, NT33, NT4) were taken at 60 min intervals. As seen for sample NT1, most of the pollutant

Table 3 Analysis results of wastewater before and after treatment

No	Parameters	Unit	Analysis results					QCVN 40:2011/BTNMT	
			NT0	NT1	NT2	NT3	NT4	Column A	Column B
1	pH	–	1.5	8.02	7.9	7.8	7.9	6–9	5.5–9
2	Pb	mg/L	316.2	0.8	0.6	0.4	0.4	0.1	0.5
3	Cu	mg/L	352.3	1.23	1.13	1,1	1.05	2	2
4	Mn	mg/L	507.2	1.05	0.82	0,8	0.8	0.5	1
5	Fe	mg/L	3795.1	3.78	3.11	3.09	2.96	1	5
6	As	mg/L	215.04	0.18	0.07	0.07	0.05	0.05	0.1
7	Cd	mg/L	0.77	0.07	0.03	0.04	0.03	0.05	0.1
8	Cr	mg/L	3.4	0.01	0.01	0.01	0.01	0.05	0.1
9	Zn	mg/L	0.7	0.1	0.1	0.1	0.1	3	3
10	Ni	mg/L	0.87	0.08	0.008	0.006	0.006	0.2	0.5

concentrations were decreased compared to the influent sample. However, some heavy metals (i.e. Pb, As, Mn) were still about 1.8 times higher than the values in the discharging standard. The pollutant concentrations in sample NT2 were reduced compared to sample NT1. Most of them were within the regulation. Analytical results for samples of NT3 and NT4 show that the heavy metal concentrations were decreased significantly. They were relatively stable over the time.

6.2 Effective of the Proposed Treatment System

It should be noted that during operation, the concentrations of heavy metals in wastewater were changed from samples NT1 to NT4. The changes have caused an unstable operation of the initial stage. Moreover, during operation, the chemical metering pump and the wastewater pump were not operated and quantified accurately. After 60 min, the pumps were well controlled to maintain constant chemical quantification. As a result, the system has gradually come into stable operation. Therefore, samples of NT2 and NT4 were taken to analysis of heavy metals. The obtained results shown that they were not changed much at these times.

The proposed treatment technology could reach high treatment efficiency (90%). The Cr^{6+} removal efficiency by nano Fe^o (10 mg/L) was very fast (98%) at pH of 1.5 in 60 min. After the Cr^{6+} reduction process, other metals in metallurgical wastewater were removed by using coagulation and floculation process. NaOH, PAC and PAM were used in the heavy metal reduction process (Ji et al. 2015; Zhang et al. 2021). In particular, NaOH was used to raise wastewater pH from 1.5 to 8. At the pH of 8, heavy metals were precipitated with an efficiency of about 98%. The PAC coagulant was used at the amount of 250 mg/L, while PAM was of 3.5 mg/L. Finally, the total efficiency of heavy metal removal in metallurgical wastewater could reach 98%. The heavy metals in the effluent were within the limits of the discharging regulation (QCVN 40:2011/BTNMT) (MORE 2011).

7 Conclusion and Remarks

Wastewater from metallurgical experimental facilities contains high heavy metal concentration. It was treated by mainly neutralization method, using limestone and $FeSO_4$. However, this method has many disadvantages, such as low removal efficiency, a large amount of sludge and high hardness of the treated wastewater. A continuous treatment system by a combination of reduction (using nano Fe^o), coagulation (PAC and PAM) and adsorption (MnO_2, and ODM-2F adsorbent) processes could enhance the removal of heavy metals in metallurgical wastewater. The obtained results show that heavy metals in treated wastewater were met within the limits of the regulation (QCVN 40:2011/BTNMT). However, in the proposed system, several operational conditions should be controlled; that is, aeration should be supplied for mixing PAC and PAM with wastewater. The air velocity in the mixing pipe should be maintained from 10 to 15 m/s. PAM should be added to the reactor after PAC addition for at least 2–3 min while reducing the gas velocity by 5–10 m/s. Nano Fe^o could be used as a reducing chemical for removing heavy metals in metallurgical wastewater. The proposed treatment process could be an alternative solution for treating small wastewater flow and high toxicity from the metallurgical experimental facilities. This process produced less sludge. Useful metals in sludge could be recovered. The proposed treatment system could be applied to the similar metallurgical experimental facilities. The proposed technology could be operated simply and easily.

Industrial wastewater could contain many different components such as organic, inorganic substances and various heavy metals. Therefore, it should be needed for further study on application nano Fe^o to treat inductrial wastewater (Ramos et al. 2020; Soni et al. 2020). Sludge generated from wastewater treatment process could contain a large amount of heavy metals. Therefore, it is necessary to recover the precious heavy metals in the sludge. It could help to reduce the amount of hazardous waste generated.

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Valorization of Sugarcane Vinasse for Fungal Biomass Protein Production: Potential Application as Fish Feed Ingredient

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1 Introduction

In recent times, there has been an increase in aquaculture activity as a result of the accelerated increase in the world population and the increase in global demand for fish and seafood. Since the production of feed for aquaculture represents about 50% of the production cost, achieving optimal nutrition is essential to reach profitable and good quality production (Craig et al. 2017). Fish feeding with prepared diets can be complete supplying all the necessary nutrients for their optimal development, or complementary, where the fish receive certain nutrients that they are not available in the medium or they are present in limiting way. Most fish farmers use complete diets, generally made up of the following components and percentage ranges: protein, 18–50%; fat, 10–25%; carbohydrate, 15–20%; ash, <8.5%; phosphorus, <1.5%, and trace amounts of vitamins and minerals (AVMA 2017). The nutrients in the feed will depend on which species of fish is cultivated and in what stage of life it is found. Therefore, the appearance of new commercial diets that promote optimal and healthy growth of fish will allow the growth of the aquaculture industry to continue to advance safely.

Fish meal and soybean meal are the main ingredients used to formulate the commercial feed for aquaculture. The fishmeal is obtained after the processing of raw fresh fish and byproducts of their processing, whose main nutrient is protein (Shepherd and Jackson 2013). Soybean meal is also a source of protein widely used in aquaculture. However, soybean meal has the disadvantage of the absence of some essential amino acids for fish such as tryptophan, added to the presence of certain substances that can be harmful to the development of fish such as inhibitors of proteases, saponins, lectins, oligosaccharides, and allergens (FAO/UNDP 1980;

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Drsjant-Li 2002). In addition to all this, the fact that the price of these commercial feeds reaches high values makes their use in fish feeding unprofitable. Due to this, in the last years, an intense search began to replace them, especially looking for lower-cost sources of protein.

Different materials have been suggested as alternative source of protein for fish feeds. Among that, the fungal biomass protein is gaining more and more interest from fish producers. The main advantage of the biomass of some filamentous fungi is that it presents an amino acid profile similar to that obtained in commercial feed, with a high content of the 10 essential amino acids required by fishes. Moreover, large amounts of biomass protein can be produced in a short period of time, since it does not depend on weather conditions, and it does not require large areas of land to obtain it. Another advantage associated with the use of fungal biomass protein is that these microorganisms present a great variety of non-specific enzymes, which allow the use of a great variety of substrates as raw materials for biomass production. In this sense, the use of organic waste as substrate for the fungi cultivation could minimize the biomass production costs at a large scale, promoting a sustainable development (Nasseri et al. 2011; Abu Yazid et al. 2017). Vinasse constitutes one of the most common organic residues in countries with a strong sugarcane industry, such as Argentina and Brazil. This residue, generated in sugar-ethanol plants, is a dark brown liquid due to the presence of melanoidins, with an acidic pH (3.5–5.0) and high content of organic matter that leads to a high COD (50–150 g/L) (Fitzgibbon et al. 1995; España-Gamboa et al. 2011). Therefore, the development of an integrated biotechnological process for the fungal biomass protein production from vinasse could reduce the economic pressures for fish farming, returning to the environment an effluent with less organic load.

Based on these facts, the present chapter reviews the nutritional properties of different fungal biomass produced from organic byproducts/residues to be used with aquatic purposes. Nutritional and toxicological aspects of fungus *Aspergillus* sp. V2 grown on sugarcane vinasse for use as fish feed ingredient are also considered.

2 Fungal Biomass Protein Production from Byproducts and Industrial Residues to Be Used in Fish Feeds

Among filamentous fungi, species of the genera *Aspergillus*, *Neurospora.*, *Rhizopus,* and *Fusarium* are categorized as GRAS (generally regarded as safe) and are promising candidates for use with food purposes (Karimi et al. 2019). Table 1 presents diverse reports in which byproducts and industrial waste were used for the production of fungal biomass protein to be used with aquaculture purposes. From the oldest researches of the year 1981 to the most modern, all seek to obtain added value from a byproduct or waste, while seeking to reduce the environmental impact that their disposal without treating would generate the environment.

Table 1 Production of fungal biomass protein from different byproduct and organic wastes

Fungal strain	Byproduct/waste used as substrate	Nutrients supplemented	Protein content (%)	Reference
Neurospora intermedia	Vinasse	–	57.6	Karimi et al. (2019)
Trichoderma harzianum	Rice polishing	0.1% $CaCl_2 \cdot 2H_2O$, 0.15% $MgSO_4 \cdot 7H_2O$, and 0.2% KH_2PO_4	49.5	Ahmed et al. (2017)
Aspergillus oryzae	Vinasse	SCOD:N:P ratio 100:5:1. $(NH_4)_2SO_4$ and KH_2PO_4	49.7	Nair and Taherzadeh (2016)
Rhizopus oryzae	Citrus waste free sugars	–	51.0	Satari et al. (2016)
Plerotus sajor-cajur	Vinasse	–	20.96	Sartori et al. (2015)
Neurospora intermedia	Lignocellulose	Thin stillage	50.0	Nitayavardhana et al. (2013)
Rhizopus sp.	Spent sulfite liquor	2 mL/L $NH_4H_2PO_4$ and 6.5 mL/L NH_3	50–60	Ferreira et al. (2012)
Mucor circinelloides	Corn ethanol stillage	Crude glycerol 10% (v/v)	30.4	Mitra et al. (2012)
Rhizopus oligosporus	Starch processing wastewater	Preculture medium: starch, 10 g; polypeptone, 5 g; yeast extract, 5 g; K_2HPO_4, 0.2 g; $MgSO_{4.}7H_2O$, 0.2 g in a liter of tap water	46.0	Jin et al. (1999)
Aspergillus oryzae	Palm oil waste	1.2 g/L KH_2P0_4 and 0.6 g/L $MgS0_4.7H_2O$	39.0	Barker et al. (1981)

Variability in the fungal biomass protein content shown in Table 1 is, in part, because the protein production is performed under different operational conditions, in terms of substrate used, incubation temperature, incubation time, etc. For example, Sartori et al. (2015) cultivated species of the *Pleurotus* genus on undiluted vinasse, resulting in biomasses with protein levels ranged from 15 to 21%. In contrast, fungi such as *Rhizopus oligosporus*, *Rhizopus oryzae*, *Aspergillus oryzae,* and *Neurospora intermedia* showed a protein content ≥50% when cultivated on vinasse (Nitayavardhana et al. 2013; Karimi et al. 2019). Hence, the protein levels will depend not only on the microorganism itself, but also on the substrate and culture conditions.

Despite advances on this topic, the main limitation of using fungal biomass protein as fish feed ingredient is associated with the production of toxic metabolites (mycotoxins) by some fungi species. Consequently, additional studies are required to ensure nutritional safety of fungal biomass regarding mycotoxins production.

3 Mycotoxins: Harmful Effects on Fish Farming Productivity

From a structural endpoint, the mycotoxins are a diverse group of compounds, mostly small molecular weight. These secondary metabolites are typically produced by some filamentous fungi under suitable temperature and humidity conditions, which have the ability to grow on different animal feeds, being potentially dangerous for both human health and animal health. Mycotoxins do not have a defined biological role in the development of fungi. However, from a structural point of view, these metabolites can vary from simple C4 compounds to highly complex compounds (Dinis et al. 2007). To date, more than 300 mycotoxins have been identified; however, scientific interest has been focused on those with carcinogenic and/or genotoxic activity. Although the contamination of animal feed with mycotoxins is a worldwide problem, this fact tends to be more frequent in developing countries, since the feed is manufactured under improperly conditions, favoring the mycotoxins occurrence. The regulations regarding the maximum permissible limits for mycotoxins in feeds vary according to each mycotoxin, but also according to the geographical location of these metabolites (Biomin 2011; Zain 2011). In turn, the symptoms caused by feed poisoning will depend on the type of mycotoxin, the amount and duration of exposure, but also on other variables, including sex, age, and the health of the individual exposed to the poisoning. The main mycotoxins and the producers' microorganism are shown in Table 2 (Bennett and Klich 2003).

Among mycotoxins, aflatoxins (AF) typically produced by *Aspergillus flavus* and *Aspergillus parasiticus* are the most studied and best characterized. The major AF are called AFB_1, AFB_2, AFG_1, and AFG_2 based on their fluorescence under UV light (blue or green). AFB1 is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains (Squire 1981). AF-forming *Aspergillus* species require temperatures above 25 ºC and moisture of 80 to 85% for growth; therefore, AF occurrence is most prevalent in regions such as South America, South and Southeast Asia, Africa, and the southeastern states of the USA. Although regulatory limits for mycotoxins are extremely varied, many countries follow FAO guidelines. These guidelines suggest that the acceptable level for total AF ($AFB_1 + AFB_2 + AFG_1 + AFG_2$) in animal feeds and ingredients is 20 ppb (FAO 2004).

AF contamination has been linked with both toxicity and carcinogenicity in human and animal populations. Acute aflatoxicosis results in death, chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions. The youngest fish are more susceptible to aflatoxicosis than adults, and some species

Table 2 Main mycotoxins and producers' microorganism

Mycotoxins	Producer microorganism
Aflatoxins	*Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus bombycis*, *Aspergillus ochraceoroseus*, *Aspergillus nomius*, *Aspergillus pseudotamari*, *Aspergillus versicolor*, *Aspergillus nidulans*, *Aspergillus parasiticus*, *Aspergillus oryzae*, and *Aspergillus sojae*
Citrinin	*Penicillium* spp. (e.g., *Penicillium citrinum*), *Aspergillus* spp. (e.g., *Aspergillus terreus and Aspergillus niveus*), certain strains of *Penicillium camemberti*, *Aspergillus oryzae*, *Monascus ruber*, and *Monascus purpureus*
Ergot Alkaloids	*Claviceps* spp.
Fumonisins	*Fusarium* spp., notably *Fusarium verticillioides*, *Fusarium proliferatum* and *Fusarium nygamai*, as well as *Alternaria alternata f. sp. Lycopersici*
Ochratoxin	*Aspergillus* spp., including *Aspergillus ochraceus*, *Aspergillus alliaceus*, *Aspergillus auricomus*, *Aspergillus carbonarius*, *Aspergillus glaucus*, *Aspergillus melleus*, and *Aspergillus niger. Penicillium* spp. (e.g., *Penicillium verrucosum*)
Patulin	*Penicillium griseofulvum*, *Penicillium expansum*
Trichothecenes	*Fusarium, Myrothecium*, *Phomopsis, Stachybotrys*, *Trichoderma*, *Trichothecium. Fusarium* is the major genus implicated in producing the nonmacrocylic trichothecenes. *Fusarium graminearum*, *Fusarium sporotrichioides*, and *Fusarium poae*. The macrocyclic trichothecenes are produced largely by *Myrothecium*, *Stachybotrys*, and *Trichothecium species. Stachybotrys chartarum*, and *Fusarium graminearum*
Zearalenone	*Fusarium Graminearum*, *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium equiseti*, and *Fusarium crookwellense*

Source Bennett and Klich (2003)

of fish are more sensitive to AF than others (Oliveira and Vasconcelos 2020). The liver is the primary target organ, with liver damage occurring when animal/humans ingest the feed/food contaminated with AF, especially AFB_1 (Zain 2011). The susceptibility of animals to aflatoxicosis also depends on dose, duration of exposure, species, age, sex, and nutrition. AFB_1 is considered the most abundant mycotoxin found in food, representing 60–80% of the total AF content. At the same time, it is considered the most hepatocarcinogenic substance for rainbow trout, inducing liver cancer (Agag 2004).

Chávez-Sánchez et al. (1994) analyzed the effect of AFB_1 on the variety of fish Nile tilapia, feeding them with different concentrations of the mycotoxin for 25 days. After that, it was observed that with the consumption of a low dose, the fish only reduced their growth rate and the amount of food consumed. However, it was observed that the liver was the most affected organ, observing histological changes such as fatty liver and characteristic neoplastic changes such as nuclear and cellular hypertrophy, nuclear atrophy, increase in number of nucleoli, cellular infiltration, hyperemia, cellular basophilia, and necrosis. Some changes in the kidney were also observed such as congestion, shrinking of the glomeruli, and melanosis.

Mwihia et al. (2018) analyzed the AF content in fish feed collected from different farms and feed production factories in Kenya, concluding that 84% of the feed was contaminated with aflatoxins at a value ranging from 1.8 to 39.7 μg/kg, and 18.5% presented values above what is allowed in Kenya for aquaculture feed. In the same work, the consequences of AF consumption were studied in two varieties of fish: rainbow trout and tilapia. Histological and macroscopic studies of the fish were carried out and alterations were observed in both species, mainly in the gastrointestinal tract. However, in general terms, it was observed that the trout suffered more injuries than the tilapia fish.

In the same line of research, Russo and Yanong (2013) studied the effect of toxins on Nile tilapia, observing that feeding the fish with diets contaminated with AFB_1 produced a reduction in the animal growth rate. On the other hand, it was observed that as the concentration of the toxin in the food increased, the effects generated in the fish increased. For example, when feeding 2.5 mg AFB_1 per kg of feed, during 8 weeks, a decrease in the weight gained by the fish and in the red blood cell count was observed. When the concentration was increased to 10 mg, abnormalities were observed in the liver. When the concentration was increased to 100 mg, there was a loss of weight in the fish added to chronic liver damage. At the end of this trial, 60% of the fish were killed. They also compared the response of rainbow trout with channel catfish to feeding with AFB_1, concluding that the former is much more sensitive to the toxin, observing that when feeding rainbow trout with a concentration of 20 ppb of AFB_1 for 8 months, 58% of the studied population produced tumors in the liver, and if the same feeding lasted for a period of 12 months, the appearance of tumors increased to 83%. However, when feeding channel catfish with a concentration of 10,000 ppb of AFB_1, a decrease in the growth rate of the population studied and few internal lesions were observed. To conclude, it was observed that prolonged feeding of fish with a low dose of AFB_1 induces the formation of tumors in the liver. At the same time, an increase in fish mortality can be observed, because the presence of these toxins in food alters the ability of fish to metabolize certain nutritional substances present in food, such as vitamin A, C, and thiamin. Finally, it was found that these mycotoxins generate an alteration in the immune system of fish, thus facilitating the infection of fish with bacteria, viruses, or parasites, thus favoring the generation of diseases produced by these vectors.

4 Nutritional and Toxicological Properties of *Aspergillus* Sp. V2 Biomass Cultivated on Vinasse for Use as Aquafeed Purposes: A Case Study

4.1 Effects of the Culture Conditions on the Growth of Aspergillus *Sp. V2*

The microorganism used in this work, named *Aspergillus* sp. V2, was isolated by Rulli et al. (2020) from a soil near a distillery in Tucuman Province. Once the microorganism was identified, the culture conditions were studied to optimize its development on sugarcane vinasse. It was tested the optimal vinasse concentration, vinasse condition (with and without sterilization), final spore concentration (1×10^4, 1×10^5, 1×10^6, 1×10^7 UFC/mL), agitation level (static, 120, 150, 180 rpm), and incubation temperature (30, 35, 40, 45 °C) (Rulli et al. 2020). The optimal conditions for the development of *Aspergillus* sp. V2 was between 50 and 100% sterile vinasse, inoculated with a final spore concentration of 1×10^6 UFC/mL, at 150 rpm and 30 °C (Rulli et al. 2020). However, it was decided to work with a 50% concentration since biomass obtained from undiluted vinasse resulted in a very colored product, making the washing process difficult. The results obtained by Rulli et al. (2020) coincide with many works found in the literature that study the development of filamentous fungi on industrial waste such as vinasse. For example, Reis et al. (2019) support that the use of undiluted vinasse for the development of *Mucor circinelloides* URM 4182 does not affect its growth. Regarding vinasse condition, the use of non-sterilized residue reduces the biomass concentration to below 50% compared to the use of sterilized vinasse (Fig. 1B). In this sense, both España-Gamboa et al. (2017) and Nair and Taherzadeh (2016) also demonstrated that the biomass production of *Trametes versicolor*, *Neurospora intermedia* CBS 131.92 and *Aspergillus oryzae* var. oryzae CBS 819.72 was significantly reduced by using non-sterile vinasse as substrate.

Rulli et al. (2020) also reported a higher value of biomass at 150 rpm. This was expected considering that the agitation allows, on the one hand, the better disposition of the nutrients in the medium to be used by the microorganism and, on the other hand, a higher level of dissolved oxygen that favors their development (Ibrahim et al. 2015). Finally, no growth of *Aspergillus* sp. V2 at temperatures of 40 and 45 °C was observed. Although the difference in the biomass obtained at temperatures of 30 and 35 °C does not present significant differences, 30° C was selected as the optimal since from operational point of view, a higher incubation temperature could become economically unfeasible if no clear benefit is obtained (Rulli et al. 2020). This is corroborated in Nair and Taherzadeh (2016), where *N. intermedia* CBS 131.92 and *A. oryzae* var. *oryzae* CBS 819.72 showed optimum growth in a temperature range of 25 to 35 °C.

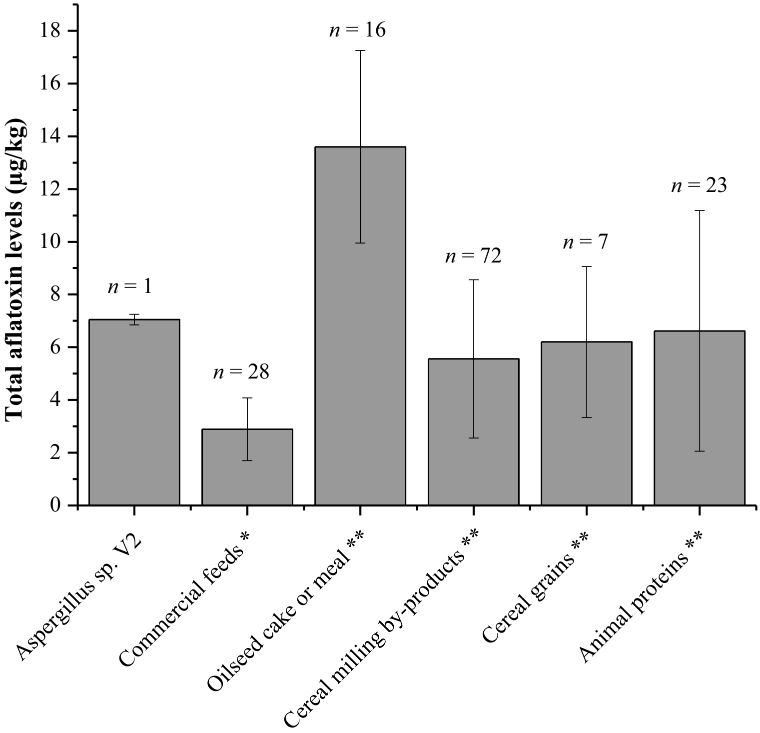

Fig. 1 Total AF levels determined by ELISA test for *Aspergillus* sp. V2 cultivated on vinasse with nutrients supplementation for 4 days (B_3) (Rulli et al. 2021) and for five categories of fish feeds reported in literature. *Source* Greco et al. (2015) and Mwihia et al. (2018)

4.2 Effect of the Incubation Time and Nutrients Supplementation on the Biomass Protein Production of Aspergillus Sp. V2

Once the optimal cultivation conditions were selected, Rulli et al. (2021) analyzed the effect of incubation time and the addition of exogenous nutrients to the vinasse on production and nutritional quality of the biomass. Biomass quality refers specifically to its total protein content (in terms of percentages relative to the dry biomass total) and protein productivity (expressed as milligrams of protein per liter of culture per h). As culture age largely determines the cell activity, protein content was measured in both exponential (4 days) and stationary growth phase (12 days). Parallel, the effect of the addition of an exogenous nitrogen and phosphorus to the vinasse was tested, since both nutrients are vital for fungal growth and protein synthesis. Thus, parameters were determined for 4 biomasses termed B_1 (4-day-biomass without nutrients supplementation), B_2 (12-day-biomass without nutrients supplementation), B_3 (4-day-biomass with nutrients supplementation), and B_4 (12-day-biomass with

Table 3 Content of the vital nutrients in B_3

Nutrient	Content (%)	Desirable range (%)	Reference
Protein	34.0	30–59	Soong et al. (2016)
Fat	4.7	1–10	Soong et al. (2016)
Carbohydrates	45.2	until 50	Kamalam and Panserat (2016)
Ashes	15.8	0.4–18	Soong et al. (2016)
Crude fiber	4.0	0.5–8	Soong et al. (2016)
Moisture	0.3	< 10	Portman (2017)

Source Rulli et al. (2021)

nutrients supplementation). The authors concluded that the best biomass for use as fish feed ingredient could be B_3, reaching a total protein content of 34.0% and a protein productivity of 14.2 mg/L h. Besides protein, nutritional value of any fish feed is also defined in terms of other vital nutrients. Therefore, Rulli et al. (2021) also analyzed B_3 for fats, carbohydrates, fibers, ash, and moisture, founding that all of them were within acceptable levels for commercial fish diets (Table 3).

4.3 Essential Amino Acids Profile of the Biomass Selected

The nutritional value of a fish feed depends not only on the quantity of the protein but also on the quality of this nutrient, which will depend on the composition of amino acids. More than 200 amino acids can be found in nature, but only about 20 amino acids are common. Of these 20, 10 are considered indispensable and are known as essential amino acids (arginine, histidine, isoleucine, methionine, leucine, lysine, phenylalanine, threonine, tryptophan, and valine) since they cannot be synthesized from novo by fish. Among the essential amino acids, methionine and lysine are usually the first amino acids found below the recommended limits. Fish feeds based on vegetable proteins such as soybean meal are often deficient in methionine, whereas those manufactured from yeast or bacterial proteins are limited in both lysine and methionine (Craig et al. 2017). In this context, when these products are used in replacement to conventional protein sources, it is convenient to supplement these amino acids to the final dietary formulation. Rulli et al. (2021) analyzed the percentage of each essential amino acid for B_3. They found that protein composition of the biomass analyzed corresponded to the composition of commercial protein sources, especially with soybean meal, containing almost all the essential amino acids within desirable fish feeding levels. Although the levels of leucine, threonine and arginine are below the values reported in commercial sources, almost all are within the necessary range for feeding fish. According to Soong et al. (2016), the minimum values required by fish of these amino acids are the following: 3.2, 1.8, and 3.1, respectively. Only threonine remains below the minimum value necessary for fish development, which can be supplied by supplementing the feed with other sources rich in this amino acid.

4.4 Occurrence of Aflatoxins in Biomass Selected

As mentioned above, the occurrence of AF in fish feed is a limitation for fish farming since it deteriorates the nutritional value of aquafeed. Taking into account that the occurrence of these metabolites can limit usage of fungal biomass as a potential fish diet ingredient, Rulli et al. (2021) determined the total AF in B_3 by using ELISA test. The authors founded that the total AF content in the fungus biomass was 6.8 ppb (or 6.8 ug per kg of dry weight). It is noteworthy that this value was below the maximum permissible limit reported for animal feedstuffs (FAO 2004). From this study, it was concluded that biomass selected could be safely incorporated to the fish diet, taking care that AF total concentration in complete feed not exceed 20 ppb (Rulli et al. 2021).

Figure 1 shows the total AF levels reported for B_3, comparatively with those reported in the literature for five categories of fish feeds.

While the first category corresponds to commercial feeds manufactured from different products (soybean, corn, wheat, soybean oil, fish meal, etc.), and in different proportions, the other four feed categories were farm-made. As expected, from analysis of the reviewed data, it was concluded that commercial feeds showed lower total AF concentrations than those farm-made (<3 μg/kg). The feeds manufactured from oilseed cake or meal showed the highest levels of total AF, with values higher than 13 μg/kg. Finally, the total AF concentration in B_3 was similar to that found in the three remaining categories.

5 Conclusions

Considering the increase in aquaculture activity, and the need to find new feeds for that this activity is more profitable, this chapter allows to conclude that the use of fungal biomass, of various species, grown on industrial residues represents a sustainable alternative. With this, it manages, on the one hand, to reduce the volume of waste with a high environmental impact, while manages to produce economically fish feed with a high nutritional value. Regarding *Aspergillus* sp. V2, it can be concluded that the biomass presented an optimal protein productivity using 50% vinasse added with exogenous sources of nitrogen and phosphorus, and incubated for 4 days. Selected biomass proximal analysis demonstrated a vital nutrients content suitable to achieve a healthy and balanced diet in the fish. It can also rule out the safety of the biomass obtained, as the AF values analyzed are below the limits established for feeding fish, thus allowing a safe use of it in fish farming. These backgrounds highlight the feactibility of using organic waste for the manufacture of added value fungal products, generating a decrease in its pollution load.

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Emerging Contaminants in Wastewater: Eco-Toxicity and Sustainability Assessment

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1 Introduction

Water is one of the most important components of life on the planet. However, numerous urban activities, including industry, farming, and health-care institutions, produce considerable amount of micropollutants and contaminate the water supply. These type of anthropogenic activities exacerbate the problem of freshwater scarcity that already exists. Over the last few decades, artificial sweeteners (ASWs), disrupting chemicals (EDCs), pharmaceutically active compounds (PhACs) and personal care products (PCPs) have been found in increasing amounts in the aquatic environment (Saidulu et al. 2021). Due to the absence of toxicological evidence and comprehensive guidelines, such compounds are designated as emerging contaminants (ECs). These hazardous compounds are widely distributed and can enter the aquatic environment through a variety of wastewater streams, including industrial effluents discharge, hospital waste, wastewater treatment plant effluents, agricultural runoff and so on. The concentration of ECs in the effluent is primarily determined by the sewer conditions, per capita water consumption, environmental persistence, usage patterns, watershed features and other factors. Although EC concentrations are very modest (ranging from ng/L to g/L), yet continuous exposure can have negative consequences for the sustainability of the ecosystem. According to recent studies, when ECs are released into the aquatic environment without being treated or just partially treated, they represent a considerable threat to the aquatic ecosystem. Lack of robust laws and inadequate data on the toxicity and fate of ECs are the main reason for this

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unregulated discharge (Lutterbeck et al. 2020). Interim aquatic standards have been established as a result of such recent concerns. Furthermore, international regulatory authorities such as World Health Organization (WHO), United States Environmental Protection Agency (USEPA) and European Union (EU) have compiled a priority list of pollutants that represent a significant threat to human health and aquatic life (Parida et al. 2021). The ECs are extremely resolute in the aquatic environment and are difficult to eliminate using the typical wastewater treatment methods. Because of their complicated structure and toxicity, many ECs have shown to be resistant to microbial activity, thereby making biological treatment methods ineffective. As a result, a variety of ECs along with their intermediate products end up in the effluents of the treatment plant. Various combinations of wastewater treatment methods, viz. biological treatments combined with membrane separation processes and advanced oxidation processes have been developed to improve the degradation of ECs. Even though such treatment methods have demonstrated good results with regards to the removal of ECs, however, high energy usage and cost of operation are the two key obstacles that must be overcome before they can be scaled up economically (Dhangar and Kumar 2020).

Many studies have been conducted on the treatment of ECs in various water matrices; however, they have been limited to a certain group or geographical location. Besides, few studies have evaluated the possible toxicity of ECs by comparing the maximum reported ambient EC concentrations to the toxicological data. Despite the fact that there have been several researches on the degradation of ECs using various treatment processes, only a few studies have compared the concentration of ECs in the wastewater to the regulatory guidelines. In addition, because of the complicated character and toxicity, the development of intermediate by-products throughout the treatment of EC warrants additional consideration (Mohapatra et al. 2016). In this respect, the current chapter seeks to offer an overview of global distribution and possible toxicity of ECs to various aquatic species. The risk quotient (RQ) values were calculated by combining the lethal concentration (LC50) and 50% effective concentration (EC50) of the chosen ECs from various studies. The current chapter also summarised the ability of traditional treatment techniques and other integrated treatment processes to reduce the wastewater concentrations below the permissible limits. Moreover, the sustainability assessment of such treatment methods was carried out to recognise the treatment alternatives that are both cost-effective and technically feasible on an industrial scale.

2 An Overview of Emerging Contaminants (ECs)

2.1 Sources and Pathways

The source-path-receptor model that allows for risk estimation towards the target receptors may be used to determine the fate of ECs. The sources and pathways of

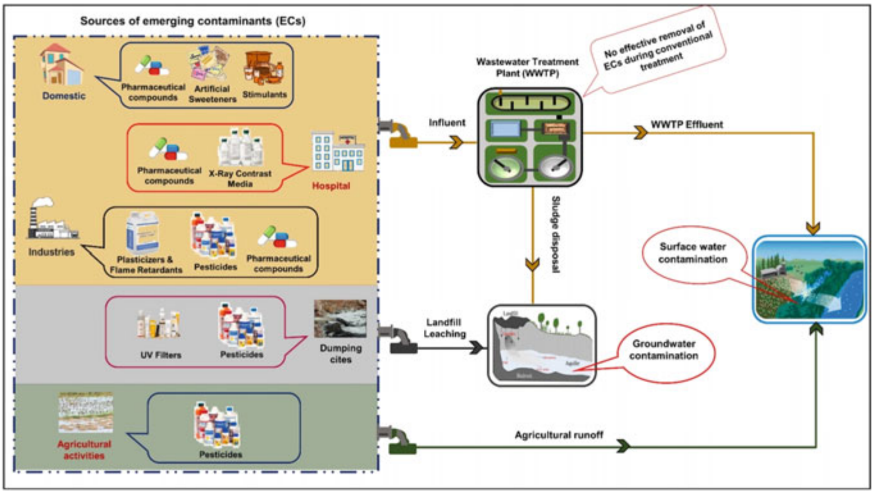

Fig. 1 Sources and pathways of various ECs into the aquatic ecosystem. Reproduced with permission from Parida et al. (2021) © Elsevier

ECs in the aquatic environment are shown in Fig. 1. Industrial effluents, agricultural runoff, hospital liquid waste, laboratory wastewater, landfill leachate and domestic sewage are the main sources of ECs. Human excretion of partially metabolised or non-metabolised PhACs can be found in domestic wastewater. Furthermore, the occurrence of ECs in the water bodies is determined by the consumption patterns in the human population. They can also be found in the effluents of traditional WWTPs, which are unable to handle the recalcitrant ECs (Rout et al. 2021). Landfill sites and septic tanks which produce highly concentrated leachate are still the major sources of ECs in groundwater, particularly when the groundwater table is shallow and aquifers percolate rapidly. Moreover, contamination of groundwater and surface water with EC occurs as a result of the use of fertilisers and other agrochemicals in agricultural fields. Most ECs have high adaptability in the environment and are difficult to biodegrade or hydrolyse under natural circumstances. PhACs are found in high concentrations in goldfish, mosquitofish and snails as reported by Ebele et al. (2017). This indicates that ECs might be biomagnified after being released into the aquatic environment.

2.2 *Regulatory Guidelines*

Owing to the lack of appropriate statutory guidelines, ECs are being released into the ecosystem without adequate treatments. Nonetheless, only a few proactive efforts have been made; for instance, the European Commission has revised its action plan to include EDCs as a key issue of pollutants. Also, the Risk Assessment and Directive (known for classifying harmful compounds) has been amended by the European Commission. Furthermore, under the Drinking Water Directive (98/83/EC), the European Commission has imposed strict limitations on the use of pesticides (Debnath et al. 2019). As there are no suitable regulatory guidelines for the quality of drinking water with regards to ECs, hence, the drinking water equivalent limits (DWEL) for each medication were computed based on specific governing parameters. DWEL takes into account an average person's daily water intake and body weight, which can vary depending upon the geographical location. When the reported EC concentrations were compared to the PNEC and computed DWEL values, it was found that the majority of ECs exceeded the PNEC and DWEL values. For instance, the average-computed PNEC and DWEL values for ciprofloxacin in Asia were 1.4 g L^{-1} and 29.5 g L^{-1}, respectively. However, the reported ciprofloxacin concentration was 31,000 g L^{-1}, which is greater than the PNEC and DWEL values. Thus, to reduce the adverse effects on the environment, the continual intake of ECs must be kept lower than the PNEC and DWEL levels (Saidulu et al. 2021). Additionally, the European Parliament Committee on Environment, Public Health and Food Safety proposed in 2007 that ECs, viz. bisphenol-A, carbamazepine and diclofenac should be added to the priority substance list. In USA, the Environmental Protection Agency (EPA) upgrades the priority list of compounds every 5 years based on the adverse effects on human health. Furthermore, the USEPA presented a study in 2015 categorising OP flame retardants as high-priority substances that necessitates significant research on their toxicological effects. In the aquatic environment, the EU has established a maximum permissible concentration for 34 ECs, including bisphenol-A, ibuprofen, mecoprop, ciprofloxacin and others under Directive 2008/105/EC. In majority of the cases, the reported concentrations of diazinon, ibuprofen, malathion, ciprofloxacin, diuron and carbamazepine found in the aqueous bodies were above the EU guideline limits. In addition, the World Health Organization (WHO) updated its Drinking Water Quality Guidelines in 2011 and included several such contaminants that had previously been overlooked (Parida et al. 2021).

3 Environmental Impacts of Emerging Contaminants (ECs)

3.1 Eco-Toxicity Effect on Human Health

Long-term exposure to ECs, even at low doses, causes a variety of abnormalities in human beings. Antibiotics are closely linked to human health protection and environmental concerns. Antimicrobial drugs, for example, result in the formation of antibiotic resistance genes (ARGs), which reduces the therapeutic ability of antibiotics against both animal and human infections. Estrogenic compounds, viz. 17-estradiol, can bioaccumulate in aquatic species and can reach the general population, thereby posing a serious health risk. Human embryonic stem cells can be hampered by the presence of atenolol and carbamazepine. ASWs-like sucralose and saccharin may cause inflammatory bowel disease in human beings by interfering with the digestive enzymes and gut bacteria (Tran et al. 2015). Wang et al. (2021) recently used in vitro tests to investigate the influence of OP flame retardants on human cells and observed that, exposure to such pollutants can considerably decrease the cell growth and protein synthesis, resulting in cell cycle arrest (Wang et al. 2021). TnBP has been shown to decrease the human plasma cholinesterase, while benzophenones have been shown to alter the hypothalamic-pituitary–gonadal (HPG) axis in animals. Caffeine has been linked to anxiety and panic attacks in human beings, as well as to hepatocellular, endometrial and colorectal cancer (Parida et al. 2021). Nevertheless, majority of the investigations that have been published are insufficient, thus, making it complex to deduce the toxicological consequences on human health. As a result, further study is needed to develop adequate EC regulation requirements in aquatic media.

3.2 *Eco-Toxicity Effect on Aquatic Life*

Algae and other photosynthetic microorganisms are the important components of the aquatic ecosystem's food chain. As a result, any changes to the regular functioning of photoautotrophic bacteria might have a major impact on the species present at different trophic levels. For the purpose of toxicity evaluation, acute toxicity data such as EC50 or LC50 were obtained from several studies for different aquatic species, viz. daphnia, fish, algae and other crustaceans. Therefore, RQ may be utilised to estimate the possible impact of ECs on the aquatic life. The ratio of the measured environmental concentration (MEC) of ECs to the expected no-effect concentration of aquatic species is called RQ (PNECaq) (Nika et al. 2020).

$$\mathrm{RQ} = \frac{\mathrm{MEC}}{\mathrm{PNEC_{aq}}} \tag{1}$$

Here, MEC stands for the average of highest calculated concentration of a component identified in effluents from various places, and PNECaq was calculated by multiplying the LC50 or EC50 by a factor of 1000 (Nika et al. 2020).

$$\mathrm{PNEC_{aq}} = \frac{\mathrm{EC_{50}\ or\ LC_{50}}}{1000} \tag{2}$$

For risk assessment, general risk rating characteristics were chosen from several studies. RQ ≥ 1 was reported to signify a potential high threat to the aquatic life. However, when the RQ value is between 1 and 0.1, it provides a moderate threat to the aquatic environment, and when it is <0.1, it poses a very low threat. Depending on the stated EC concentrations and PNECaq values, it was reported that, among the designated ECs, bisphenol-A poses the most ecological threat to fish, daphnia and algae with RQ values of 109.82, 25.72 and 105.7, respectively (Parida et al. 2021). Furthermore, with a highest RQ value of 15.2 for algae, ibuprofen may constitute a serious threat to the aquatic life. In Cyprinus caprio (freshwater fish) hepatocyte cultures, long-term exposure to ibuprofen can considerably enhance the CYP2K (cytochrome P450 enzyme) expression (Corcoran et al. 2012). According to Nie et al. (2013) few antibiotics can obstruct the photosystem II electron transport chain, hence, reducing the process of photosynthesis (Nie et al. 2013). Antibiotics can also impact the prokaryotic cells by inhibiting the production of cell envelope, protein synthesis and nucleic acid synthesis in aquatic species, via complex action mechanism. Table 1 represents the eco-toxicological effects of different categories of ECs.

4 Removal Mechanisms of Emerging Contaminants (ECs)

4.1 Biodegradation of ECs

Microbes deteriorate the ECs depending upon the catalytic activity of certain enzymes, which is eventually based on their genetic capacities. Besides, the genetic capacity of the microbes is heavily influenced by the toxicity of biomass normalised rate constant (Kbio) of different ECs. The biodegradability of a compound can be predicted using Kbio values. Kbio >10 indicates that the compounds are being biodegraded rapidly. However, when Kbio is less than 0.01, the biodegradation rate of the compounds decreases. Quintana et al. (2005) conducted a biological degradation study on a subset of PhACs (ketoprofen, ibuprofen, diclofenac and naproxen) and observed that only ketoprofen displayed metabolic biodegradation potential (Quintana et al. 2005). In addition, Sphingomonas Ibu-2 aerobic bacteria obtained from activated sludge were employed to degrade ibuprofen metabolically. Only heterotrophic bacteria were shown to be responsible for the metabolic biodegradation of some particular ECs that are innocuous to microorganisms. However, to change the non-growth substrate in cometabolic biodegradation, growth substrate, viz. propane, methane, acetate and ammonia is also required (Tran et al. 2013). Furthermore, the cometabolic biodegradation of β-blockers (sotalol, metoprolol and atenolol) was studied by Sathyamoorthy et al. (2013) who observed that the microorganisms responsible for atenolol biodegradation are associated with the ammonium oxidising bacteria (AOB) (Sathyamoorthy et al. 2013). AOB consist of a specific enzyme known as ammonia monooxygenase, which is accountable for the breakdown of several PhACs. Furthermore, autotrophic AOB are capable of degrading a wide range of ECs, including bisphenol-A and ibuprofen. Cometabolic breakdown can take place in either an aerobic or anaerobic environment. Nevertheless, majority of the investigation concluded that EC cometabolism under aerobic condition is more preferable compared to the anaerobic condition. Besides, a thorough knowledge of the cometabolism and metabolic routes involved in the biodegradation of ECs would

Table 1 Eco-toxicological effects of various emerging contaminants

Class	ECs	Eco-toxicological effects	PNEC (μg L^{-1})	ADI (μg kg^{-1} d^{-1})
PhACs	Ibuprofen	(i) Risk to aquatic environment with chronic toxic effects (ii) Postembryonic development among amphibians is hindered	0.01	110
	Diclofenac	(i) Reduces the hemacrotic values in fishes (ii) In Asian countries 'Gyps vultures' population is affected	0.05	1.6
	Naproxen	Lethal to aquatic species	0.33	4.6
	Ciprofloxacin	(i) The immune system of humans can be damaged (ii) Growth of human embryonic kidney cells (HEK293) will be inhibited	1.2	1.6
	Azithromycin	Terrestrial and aquatic organisms can be severely affected by resistant bacterial strains	0.05	10,000
	Atenolol	Human embryonic stem cell growth can be hindered	20	2.7
	Ketoprofen	Toxic to aquatic system	15.6	5
	Propranolol	Cell regeneration process will be affected	0.1	4000
PCPs	Salicylic acid	(i) Deleterious effects in fish gills (ii) Oxidative stress in mammals	1.28	150,000
	Propylparaben	Shows weak estrogenic activity	–	237
	Diethylhexyl phthalate	Pregnancy and miscarriage complications, when exposed to high levels	0.01	6.77
Pesticides	Atrazine	(i) It affects the hypothalamus by inhibiting luteinising hormones and prolactin levels (ii) Damages the adrenal glands	0.044	0.5

(continued)

Table 1 (continued)

Class	ECs	Eco-toxicological effects	PNEC (μg L^{-1})	ADI (μg kg^{-1} d^{-1})
	Mecoprop	Causes teratogen in rats at moderate to high doses	5.5	–
	Triclosan	Growth inhibition of algae (Pseudokirchneriella subcapitata)	0.07	12
	2,4-D	(i) Death may result in mammals and birds receiving single oral doses of exceeding 100–300 mg kg^{-1} body weight (ii) Affects androgenic synthesis and combines with testosterone	–	50
EDCs	Nonylphenol	(i) Feminisation of aquatic organisms (ii) Hormonal system of an organism will be interfered	6.6	150
	Bisphenol-A	Estrogenic effects in rodents and can increase the risk of breast cancer in humans	1.5	50
	Estrone-1	(i) Reproductive and sexual systems of fish and human can be severely affected (ii) It can affect the immune and nervous systems	0.018	0.001

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enable to develop and optimise different treatment methods for the improvement of biodegradation rates.

4.2 *Sorption onto Sludge Solids*

Another important regulatory mechanism for the fate of ECs throughout the wastewater treatment is the sorption process. The sorption of compounds occurs due to a variety of processes including electrostatic attraction, ion exchange, hydrophobic interaction, pore diffusion, van der wall forces, π–π interaction, inner-sphere complexation and hydrogen bonding. Physisorption has been identified as the primary mechanism for the elimination of organic contaminants, whereas chemisorption is the most prevalent method in case of metallic ions. In general, the solid-water distribution coefficient (K_d), physicochemical parameters and organic carbon content present on the sludge particles all influence the sorption of organic contaminants (Joseph et al. 2019). According to several studies, PhACs with a high log K_{OW} (>3.5) consist of a strong sorption affinity for the sediment particles in water. For instance, EDCs, viz. estrone-1, nonylphenol and bisphenol-A showed a strong sorption affinity onto the sludge solids, owing to their high log KOW values. The charge of compounds in an aqueous media may be determined by their pKa value and the charged compounds can significantly obstruct the biological degradation mechanism (Babić et al. 2007). Only, a few studies have used Kd values to determine the EC sorption affinity. For example, Stevens-Garmon et al. (2011) investigated the sorption affinity of 34 ECs onto the activated sludge solids at pH 7 and reported that the compounds having a Kd value less than 0.1 L g^{-1} SS such as diclofenac, atenolol and naproxen have demonstrated reduced sorption affinity (Stevens-Garmon et al. 2011). The value of Kd is controlled by a number of factors, including process conditions and solid phase properties. Furthermore, Xue et al. (2010) found that the lack of nitrate promoted the EC sorption onto the surface of activated sludge (Xue et al. 2010). The crucial degradation mechanisms, viz. biodegradation and sorption onto the sludge solids are shown in Fig. 2(a) and (b).

5 Techniques for Extraction of ECs

5.1 Activated Sludge Treatment Technique

Activated sludge treatment (AST), in which activated slurry includes a colony of microorganisms able of degrading different contaminants, is one of the most popular approaches for treating ECs. In the presence of dissolved oxygen (DO), all reactions were conducted, and the resulted sludge is recycled from the secondary settling tank to the aeration vessel to keep the balanced ratio of food to microorganisms (F/M). The deterioration of PhACs (ketoprofen, ibuprofen, and naproxen) in AST was shown to be higher than 80%, according to Martinez-Alcala et al. (2017). Several investigations have found that beta-blockers have negative elimination efficiencies (propranolol and atenolol). It might be attributed to the effluents' regeneration of starting compounds from metabolites. In particular, AST procedure was used to remove EDCs from an effluent treatment plant, and the rejection of estrone-1 was determined to be >98% (Kumwimba and Meng 2019).

5.1.1 Critical Influencing Parameters

F/M ratio, SRT, and HRT are to be considered as critical influencing parameters. The retention time of the liquid phase affects F/M ratio and the volume of reactor,

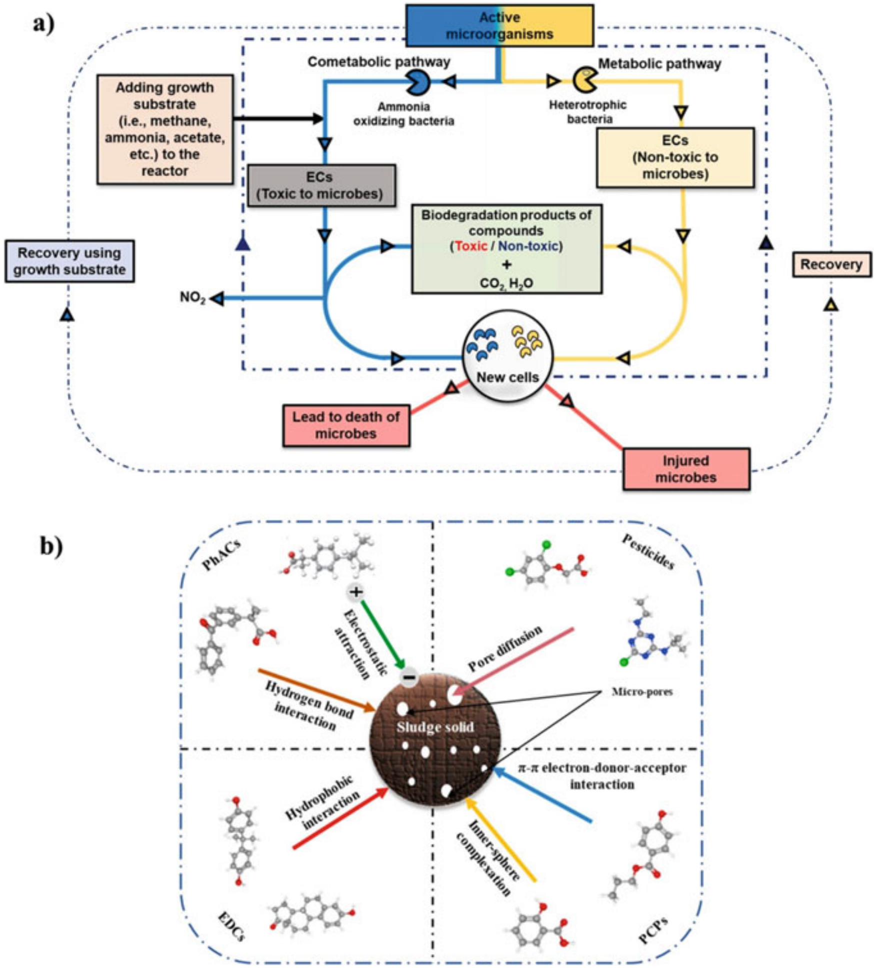

Fig. 2 Major degradation mechanisms **a** biodegradation and **b** sorption of ECs onto sludge solids. Reproduced with permission from Saidulu et al. (2021) © Elsevier

which is known as HRT. For maximal rejection efficiency of the selected pollutants, choosing the best HRT is critical. For instance, a rise in HRT enhanced the elimination efficiencies of certain ECs (naproxen, ketoprofen, ibuprofen, and Estrone-1). Findings demonstrate that increasing HRT enhances the chances of pollutants and bacteria coming into contact. SRT has been shown to have a considerable influence on bio-remediation and adsorption of pollutants during AST (Su et al. 2015). SRT may boost the flexibility in populations of bacteria, thereby increasing their capacity to metabolise the aimed ECs. According to findings, an average SRT of 10 days is adequate to achieve decreased EC effluent concentrations by ASP. Because microorganisms' metabolic activity in the digester grows with SRT, the biodegradation of

ECs will rise. The F/M ratio is important in ASP because it affects micropollutants removal, sludge settling and microbial growth. In ASP, the F/M ratio is stated to be between 0.25 and 0.5 g BOD g^{-1} ML SS d^{-1} (Gani and Kazmi 2016). Besides, lowering the F/M ratio leads to an increased EC degradation. For example, when the F/M ratio is 0.23 g BOD g^{-1}MLSS d^{-1}, DEHP removal effectiveness is greater than 80%, but when the F/M ratio is 0.67 g BOD g^{-1}MLSS d^{-1}, the rejection efficiency is severely decreased (< 30%) (Gani et al. 2019).

5.2 *Moving Bed Biofilm Reactor*

MBBR is a hybrid of coupled and suspended growth techniques wherein biofilms are developed on tiny suspended bio-carriers, removing organics and nutrients from a variety of wastewater sources. Furthermore, it has demonstrated effectiveness in the elimination of various ECs. MBBRs have several intrinsic advantages, such as reduced volume requirements, the ability to manage high OLR, homogeneous biomass distribution, lower HRT, the ability to manage high OLR, no dead space that causes clogging and channelling, less sludge generation and no rigorous recirculation requirements (Bakar et al. 2020). For ketoprofen, naproxen, acid, salicylic, ibuprofen, and bisphenol-A, MBBR has shown improved elimination efficiency (more than 85%). Using statistical hypothesis testing, Zupanc et al. (2013) compared the elimination of analgesics (ketoprofen, diclofenac, and ibuprofen) by MBBR and ASP and found that MBBR performed better (Zupanc et al. 2013).

5.2.1 Critical Influencing Parameters

HRT, carrier loading ratio, aeration rate and OLR are all factors to consider. HRT can affect the contact period between targeted candidates and the carrier's biofilm in MBBR. Furthermore, the exchange of contaminants' mass from the surrounding fluid to the biofilm can be influenced by HRT. Jiang et al. (2018) investigated the clearance effectiveness of specific ECs at various HRTs in a laboratory setting (6 to 24 h) (Jiang et al. 2018). At a HRT of 18 h, maximum EC elimination efficiency was reported (ibuprofen >92%, EDCs >70%, triclosan >80%, ketoprofen 80%, and salicylic acid >92%). The elimination effectiveness of atrazine was lowered from 73 to 64% when HRT was shortened from 24 to 12 h, according to Derakhshan et al. (2018) (Derakhshan et al. 2018). The study also reveals that MBBR exhibits the highest elimination for bisphenol-A, nonylphenol, salicylic acid, ibuprofen and estrone-1 during HRT period of 4 to 24 h. Furthermore, as compared to other comparable procedures, the MBBR demonstrated greater removal effectiveness of several ECs at low HRTs. The reactor performance and biofilm framework are influenced by the aeration rate. It was observed that increasing the aeration rate improves the performance of the reactor (Casas et al. 2015). For instance, the rejection effectiveness of phthalates was raised with an enhancement in aeration rate up to 60 L h^{-1}.

The result indicates that the rejection effectivity of phthalates was not enhanced. The removal of biofilms at higher aeration rates might be the reason for the above phenomenon (Ahmadi et al. 2015).

5.3 Trickling Filter (TF)

To sustain the microbial population, TF is another strategy that incorporates a set medium (plastic or various type of inert medium). The wastewater's influence finally spreads throughout the packed medium, where microorganisms destroy the organic materials as it trickles down. The TF has numerous benefits, including ease of use, low energy consumption, a self-cleaning system, and improved sludge thickening. However, just a few studies have looked into the use of TF to remove ECs. With TF, high clearance efficiency for ketoprofen (70%), ibuprofen (> 84%) and diclofenac (72%) were reported in PhACs. Salicylic acid, on the other hand, was shown to be practically completely removed in PCPs (Lin et al. 2009). Kasprzyk-Hordern et al. (2009) examined the removal of 55 specific ECs from wastewater using TF and AST. The average rejection effectivity of the AST process was found to be substantially greater than that of the TF method (Kasprzyk-Hordern et al. 2009). In another study, to increase the ejection efficiency for remaining antibiotics in sewage, Lamba and Ahammad (2017) developed the trickling filter by splitting the digester into two portions; the upper half is aerobic whereas the bottom half is anaerobic (Lamba and Ahammad 2017). When compared to AST, modified TF demonstrated higher efficiency in the context of eliminating microbial burdens and had reduced energy consumption.

5.3.1 Crucial Influencing Parameters

HRT and a lot of media are to be considered as intensifying factors. HRT affects different processes such as denitrification and nitrification. Ejhed et al. (2018) used TFs with varied HRT to track 24 distinct PhACs throughout the treatment of wastewater (Ejhed et al. 2018). The average elimination effectiveness of PhACs revealed a significant linear association with HRT, according to the authors. In contrast, no substantial effect of HRT variation on pesticide removal was detected. The best HRT for eliminating ECs in TF is between 36 and 60 h. According to Brandt et al. (2013), low HRT trickling filters tend to reduce PhAC and EDC extraction efficiency, in particular when packed substance does not contribute to significant retention of solid materials (Brandt et al. 2013). The loaded medium comprised in TF is beneficial to biomass development and microbial adhesion.

5.4 Constructed Wetlands

CWs are multi-component techniques that include plants, microbes, substrate and water. The substrate matrix also maintains plant development and microbiological activity, and it has a significant impact on the hydraulic method. CWs have been exhibited to be effective in removing ECs because they may convert many hazardous elements into nontoxic derivatives. Different removal processes, including volatilisation, sorption to solid media, plant uptake, biodegradation, photodegradation and photolysis, are used to convert specific pollutants. CWs are particularly successful (75–100%) in treating EDCs such as bisphenol-A, estrone-1 and nonylphenol. Except for diclofenac, practically all of the chemicals in PhACs had average elimination effectiveness of 75%. Nevertheless, maximum rejection efficiency was observed in a range of 20–90% over atrazine (Jain et al. 2020).

5.4.1 Crucial Influencing Parameters

HRT, seasonal fluctuations, flow regime and plant variety are all factors to consider. Several hydrologic patterns [surface flow (SF)] and directions of flow (vertical or horizontal) can be employed to create wetlands. Changes in the layout of wetlands can have a substantial impact on the efficiency with which toxins are extracted. 2,4-D and Salicylic acid were successfully eliminated in surface flow-built wetlands as compared to another variety, according to the research. In EDCs, the nonylphenol was removed the most, whereas, in vertical SFCWs, the nonylphenol was removed the least. Because unsaturated medium encourages larger oxygen transfer rates in SSFCWs configurations, resulting in higher removal efficiencies. In horizontal SFCWs, on the contrary, negative removals were found for EDCs (Dires et al. 2019). Horizontal flow-built wetlands (HFCWs) have displayed excellent effectiveness in terms of total EC removal. HRT is one of the most important design factors in CW systems since it influences the sizing of units and ejection proficiency. Researchers discovered that the best range of HRT for effective pollution elimination in a CW system is 2 to 5 days. Salicylic acid, naproxen and Ibuprofen were shown to be efficiently eliminated when HRT was equivalent to 5.1 days, as suggested by Hijosa-Valsero et al. (2011) (Hijosa-Valsero et al. 2011). With an increase in HRT from 27.5 to 137.5 h, the elimination efficiency of estrone-1 enhanced dramatically (40 to 84.3%). Several researchers, on the other hand, observed no substantial influence of seasonal fluctuations on EC removal at the field size. Most of the investigations have shown that various macrophytes can remove ECs, leading to the conclusion that macrophyte component selection is one of the key criteria for the successful elimination of specific pollutants in CWs (Brisson and Chazarenc 2009). Furthermore, as indicated by Matamoros et al. (2008), utilising Phragmites australis species successfully resulted in the elimination of several PhACs such as diclofenac, naproxen and ibuprofen, instead of employing Typha angustifolia (Matamoros et al. 2008).

5.5 Membrane Bioreactor

MBR is currently widely used as a possible biological treatment unit for the removal of different targeted contaminants due to higher effluent quality compared to other traditional treatment techniques. The key benefit of the MBR system is that it retains all of the biomass in the digester, resulting decoupling of the SRT from HRT and allows for a smaller reactor and/or greater OLRs. Influent wastewater parameters (such as organic matter concentration and pH), membrane features (chemical properties and membrane pore size) and operating variables (like SRT, redox variables, temperature, and HRT) all influence EC removal effectiveness in the MBR technique (Khan et al. 2020). Volatilisation, sorption and Biodegradation are the key removal processes in the MBR process. Various PhACs, like atenolol, naproxen, ketoprofen and ibuprofen, were efficiently eliminated by using MBR (more than 85%), and aerobic conditions were shown to be the most suitable for biodegradation of these chemicals. MBR has also shown greater removal efficiency for EDCs for instance estrone-1, bisphenol-A and nonylphenol. Salicylic acid was successfully extracted (over 90%) through MBR process in PCPs, while triclosan was virtually eliminated (Prasertkulsak et al. 2016).

5.5.1 Crucial Influencing Parameters

Among membrane properties, SRT and HRT, SRT is the most important factor which inhibits membrane separation during contaminated water filtering. Numerous findings have found that SRT has a greater impact on the elimination of specific ECs in the MBR operation compared to other variables. Kimura et al. (2007) studied the six acidic PhACs at various SRTs (7 to 65 days) and reported that the SRT for 65 days resulted in an improved degradation efficiency (Kimura et al. 2007). Employing the same reactor strategy Prasertkulsak et al. (2016) extracted PhACs from hospital wastewater at a short HRT of 3 h; in which mostly sorption of the non-colloidal particles were noticed (Prasertkulsak et al. 2016). The composition of the membrane and their minimal size of pore has both been demonstrated to have a substantial impact on the removal of ECs in MBRs. Silver (Ag) NPs-impregnated polysulfone membrane inhibited biofouling in the membrane reactor to some extent. The removal efficiencies are influenced by the membrane's surface charge. The nominal range of 0.004 to 1 m was chosen in most research for the elimination of ECs when it came to membrane pore size (Khan et al. 2020). Wijekoon et al. (2013) investigated the efficacy of the following reactor for the extraction of 29 different ECs and reported that at minimal membrane pore size (1 m), the average rejection efficiency for PhACs was <80% (Wijekoon et al. 2013). Also, the membrane pore size showed a significant impact on MBR fouling.

6 Integrated Systems for the Removal of ECs

6.1 Membrane Bioreactor-Based Integrated Systems

The membrane bioreactor (MBR)-based integrated systems involve membrane separation and conventional activated sludge processes (ASP). Previously, MBR has been successfully utilised for the removal of nutrients and conventional contaminants from the wastewater with superior efficiency. In the case of ECs, the MBR have shown good removal efficiency for certain type and class of ECs but with inefficient biological persistence, which demands process modification. In this context, researchers have worked over the development of integrated systems of MBR with different treatment methods like advanced oxidation processes (AOPs), membrane separation using ultrafiltration (UF) and reverse osmosis (RO), powdered activated carbon (PAC) and membrane distillation to increase the removal efficiency of ECs (Parida et al. 2021). Recently, Vo et al. (2019) demonstrated the sponge-MBR-ozonation integrated system for removing antibiotics namely ciprofloxacin, sulfamethoxazole, norfloxacin ofloxacin, trimethoprim, erythromycin and tetracycline which are commonly used for medication in hospitals. The traces of these antibiotics were frequently found in the hospital wastewater. The removal efficiency of the proposed integrated system was evaluated under variable fluxes. Among the seven antibiotics, norfloxacin, erythromycin, tetracycline and trimethoprim were successfully removed via sponge MBR coupled integrated ozonation system with more than 90% removal efficiency. However, in the case of ciprofloxacin, and ofloxacin it was found to be more than 80%. It was observed that the removal efficiency of the proposed integrated system was least in the case of sulfamethoxazole (66%). In addition, the concurrent denitrification and nitrification within the sponge under variable fluxes resulted in an increase in the total nitrogen removal within the sponge-MBR process. It was claimed that the proposed integrated system might be used as a potential technology for improving the removal of antibiotics from hospital wastewater. Similarly, Kovalova et al. (2013) developed a small-scale wastewater treatment facility for hospital effluent streams (Kovalova et al. 2013). The plant includes a primary clarifier followed by MBR and five post-treatment processes counting the ozonation, UV light with and without TiO_2, O_3/H_2O_2 and the powdered activated carbon (PAC). The wastewater streams comprise 56 different micropollutants of pharmaceutically active compounds, industrial chemicals and metabolites. The doses defined for the various integrated treatment of the hospital wastewater were 2400 J/m^2 for UV, 23 mg/L for PAC and 1.08 g of O_3/g of dissolved organic carbon for ozone treatment. It was observed that MBR combined ozonation treatment had displayed superior removal efficiency of more than 95% for 4800 ng/L of PhACs. Whereas, in the case of PAC and UV, the efficiency was lower up to 86% and 33%, respectively. The major drawback of using UV and ozonation treatment includes the formation of converted products/by-products that were mostly unidentified and capable of causing the toxic effects further. However, PAC does not comprise any degradation process, and the removal was conducted via an adsorption mechanism; therefore, it does not cause any toxic effects. Luo et al. (2017) have tried to compare the effectiveness of conventional MBR-RO systems and osmotic MBR-RO systems for the removal of 31 ECs (Luo et al. 2017). Out of the 31 ECs, 18 were hydrophilic, and the remaining 13 were hydrophobic in nature. It was found that both the osmotic MBR-RO and conventional MBR-RO-integrated systems had successfully removed all the 31 ECs, nutrients and other bulk organic matter. The removal efficiency was found to be >95% for almost all the ECs except for contaminants like diclofenac, atrazine and carbamazepine, with a lower removal efficiency of 70%. The major drawback of the osmotic MBR system was found to be the salinity build-up which unfavourably imparts membrane fouling and retards biological stability. From the previously reported literature, it was found that the MBR-based integrated systems have represented admissible performance with suitable removal efficiency for the removal of ECs. However, the membrane fouling, lower flux, higher maintenance and operation cost, and higher energy consumption are the major challenges for its utilisation for real-world applications.

6.2 Constructed Wetland and Aerated Lagoon-Based Integrated Systems

The constructed wetland (CW) is an integrated wastewater treatment technique that comprises substrate adsorption, plant uptake and microbial degradation of pollutants for their removal from wastewater. CWs may be modulated and planned in various modes hydrologic such as surface flow (SF), subsurface flow (SSF), vertical flow (VF) and horizontal flow (HF). In order to maximise the percentage removal of ECs via CWs, various researchers have integrated the CW through various other effluent treatment processes like membrane distillation, advanced oxidation processes (AOPs) like ozonation and UV treatment (Varma et al. 2021). Recently Lancheros et al. (2019) have demonstrated an integrated system between horizontal subsurface flow constructed wetland (HSSFCW) equipped with Cyperus ligularis and an ozonation reactor system for the treatment of PhACs such as naproxen and ibuprofen (Lancheros et al. 2019). The removal experimentations for these ECs were conducted using Cyperus ligularis macrophyte and ozone doses of 10, 15, 21 mg/L. The results showed that naproxen and ibuprofen had superior removal efficiency of >97%. However, statistical analysis revealed that, when compared to ozone dosages, the configuration of the integrated system had displayed a significant influence on removal efficiency. Although the suggested method had obtained a greater removal efficiency for ibuprofen, the effluent concentration was higher than statutory guidelines.

As compared to a single CW system, it was found that the integrated system had higher removal efficiency. Researchers have examined the use of aerated lagoons for improving the removal efficiency. Commonly, the aerated lagoons are classified as aerobic and facultative lagoons and comprise mechanical aeration for endorsing the biological oxidation process of wastewater in a typical pond-type system. However, the aerated lagoons require 3–6 times more energy in contrast to facultative lagoons (Chowdhury et al. 2010). Conkle et al. (2008) employed an aerated lagoon to remove PhACs from the urban wastewater in Mandeville, Louisiana (Conkle et al. 2008). The aerated lagoons were further accompanied by an integrated system using SFCW and UV irradiation. The results showed better performance for all the ECs with a removal efficiency of >95% except for carbamazepine (~55%). It was found that the hydraulic retention time was longer up to 30 days in the CW, which had credited higher removal efficiency to the system. Also, the integrated system was able to fetch the effluent concentration of caffeine, atenolol and paracetamol within the statutory guidelines values. Despite the superior performance for the removal of ECs, the proposed integrated system endures major challenges such as seasonal dependency and higher land requirements, which must be addressed before implementing these integrated systems. Except for the land availability issue, the use of CW for the removal of ECs provides significant advantages related to economic feasibility compared to other hybrid systems.

6.3 Advanced Oxidation Process and Adsorbent-Based Integrated Systems

The advanced oxidation processes (AOPs), with their great removal capability and short reaction time, have received significant attention in the field of wastewater treatment. During AOPs, a variety of oxidising radicals are created, which are proficient enough to decompose pollutants in an aqueous environment. Various AOPs, including ozonation, ultrasonic irradiation, photocatalysis, and Fenton oxidation, had been coupled with various biological treatment approaches to improve the ECs removal efficiency. Nguyen et al. (2013) demonstrated an integrated system combining the MBR with UV oxidation using a UV light of 254 nm wavelength or RO or NF membrane filtration unit to improve their removal efficiency for persistent biological and hydrophilic chemical compounds (Nguyen et al. 2013). This integrated method was able to remove over 85–99% of the 22 identified ECs. It was found that the removal efficiency was drastically enhanced for chlorinated chemicals like fenoprop, diclofenac and triclosan. The increased removal of ECs through the UV oxidation could be attributed to halogen substitution and subsequent dealkylation mechanisms. In addition, the MBR and UV oxidation-based integrated system illustrated outstanding performance for iopromide, paracetamol and iopamidol with removal efficiencies of 92%, >90% and 93%, respectively. Furthermore, the effluent concentrations of these pollutants met the regulatory guidelines in all situations. Ding et al. (2016) employed laccase-mediated oxidation in conjunction with soil absorption (SA) to remove 14 types of quinolone, sulfonamide and tetracycline antibiotics (Ding et al. 2016). The antibiotics (ECs) taken for the experimentation exhibits different chemical properties and structures. The simultaneous removal of ECs was made possible by the complementing actions of laccase-mediated oxidation and soil adsorption. It was found that after 15 min of reaction period, nearly 75% of removal efficiency was observed for a dose of 0.5 mg/L, and the complete elimination of ECs was obtained for a prolonged period of 180 min. Tetracycline was removed via both the SA and laccase oxidation process; however, laccase oxidation was the major mechanism for its removal in every case. Also, the tetracycline effluent concentration from the projected integrated system follows the statutory guidelines. Although most advanced oxidation-based systems are very competent, they have considerable maintenance and operational expenses. The primary expenses with Fenton-based approaches are connected with handling chemical reagents and power requirements. Still, in the case of photocatalysis-based systems, the main expenses are linked with energy needs, treatments, catalysts fabrication and disposal of produced sludge. Aside from that, ultrasound-based and ozonation processes are being used, which are energy-intensive, with significant operating and maintenance expenses.

ECs have also been successfully removed using various adsorbent materials in combination with other treatment approaches. Recently, Sajjadi et al. (2019) demonstrated metal–organic framework (MOF)-based heterogeneous Fe_3O_4@MOF catalyst to activate persulfate (PS) with the help of ultrasonic treatment for the degradation of diazinon (Sajjadi et al. 2019). The ultrasound-assisted integrated system successfully exhibited >94% degradation efficiency for the diazinon. In addition, a tiny amount of Zn and Fe elements were leached out during the degradation process. Also, the degradation of diazinon was encouraged by lowering the diazinon concentration, with an increase in the ultrasonic output power and Fe3O4@MOF-2 dosage. In another study, to remove of organic micropollutants, Shanmuganathan et al. (2017) have used an integrated system combining the submerged membrane filtration and granular activated carbon (GAC)-based adsorption (Shanmuganathan et al. 2017). The proposed system was capable of removing most of the hydrophobic organic compounds efficiently via the electrostatic adsorption process over the negatively charged surface of GAC, which may be due to the positive or neutral surface charge of the micropollutants. However, the removal efficiency was much lower for the hydrophilic compounds like sulfamethoxazole. The adsorption mechanism involved in the removal process includes mostly the non-covalent interactions such as $\pi - \pi$ bonding, hydrogen bonding, electrostatic attraction and van der Waals interactions. It was found that, with an increase in the adsorption process, the removal efficiency was enhanced up to 95% for most of the micropollutants. Overall, the adsorbent-derived integrated systems have provided a superior performance with regards to the degradation of ECs. Although these systems comprise lower operational expenses than the AOPs, the cost of adsorbents, disposal challenges and the regeneration process of used adsorbents are the key concerns that must be addressed for their efficient and cost-effective use in real-world applications (Majumder et al. 2019).

6.4 Sequencing Batch Reactor-Based Integrated Systems

The sequencing batch reactor (SBR) is the form of activated sludge process (ASP) that treats the wastewater generally in five different stages like a filling, reacting, settling, decantation and idle. SBR provides various benefits over ASP, including the capacity to integrate anoxic phases and aerobic in a single reactor, cost-effectiveness, flexibility degree and additional extensive applicability for the treatment of nutrients and heavy metals. However, SBR was unproductive for the treatment of hydrophilic micropollutants. Therefore, researchers have attempted to combine the SBR with various other treatments in order to improve EC removal. Wei et al. (2018) recently evaluated the removal of 26 micropollutants contained in synthetic wastewater, including organic and emergent contaminants, by employing a single aerobic SBR and an integrated system comprising of SBR and nanofiltration (NF) unit (Wei et al. 2018). It was discovered that the PhACs such as carbamazepine, ibuprofen and atenolol had removal efficiencies ranging from 30 to 85% when treated just by SBR, whereas SBR + NF integrated systems showed removal efficiencies ranging from 93–99%. Also, the SBR + NF-integrated system efficiently removed other remaining ECs like iopromide, sucralose, caffeine, acesulfame and TCEP. Gimeno et al. (2016) employed a combination of successive biological degradation and solar photocatalytic ozonation (Gimeno et al. 2016). The study concluded that photocatalytic ozonation resulted in higher mineralisation of ECs as compared to alone ozonation treatment. In addition, the ECs such as caffeine and acetaminophen (paracetamol) were successfully removed via biological treatment, whereas other ECs (carbamazepine and diclofenac) were efficiently removed through photocatalytic ozonation. Although the performance of SBR is not much satisfactory for the treatment of ECs, it persisted in lower expenses compared to the conventional AOPs, ASP and MBR methods. In general, the SBR-based integrated systems may contribute to improving its overall performance cost-effectively.

7 Sustainability Assessment of the Hybrid Systems Towards Removal of ECs

Although majority of the hybrid systems have demonstrated considerable efficacy in removing the ECs, however, long-term dependability is the most important factor to consider prior to scaling up of the treatment process. LCA may be used as a decision-making tool to assess the long-term viability of the integrated systems. The purpose of sustainability assessment is to reduce the negative environmental effects while also balancing the environmental and socioeconomic trends. LCA has previously been employed in several studies to identify the most relevant and critical features of the treatment methods throughout its operational phase. For the removal of PhACs and PCPs from wastewater, Tarpani and Azapagic (2018) examined the life cycle environmental effects of ozonation, nanofiltration and solar photo-Fenton processes (Tarpani and Azapagic 2018). Among the selected processes, NF process showed the least influence on 13 of 18 indicators, including fossil resource depletion, global warming potential, urban land usage, ozone depletion potential, photochemical oxidant production and so on. Nevertheless, membrane fouling is a major issue with the NF system, resulting in significant pressure drops and the need for additional energy to sustain the flux. Because the solar photo-Fenton process produces H_2O_2 and ozonation consumes a lot of energy, as such, both solar photo-Fenton and ozonation processes have low environmental sustainability. Moreover, for UV-assisted process, the source of photon formation is one of the critical variables in assessing its environmental implications. The UV lamp provides a larger environmental footprint than the solar light, even though it provides better removal efficiency (Foteinis et al. 2018). Foteinis et al. (2018) have investigated the environmental sustainability of several light-driven-based AOPs for the micropollutants degradation from wastewater (Foteinis et al. 2018). The long-term viability of the treatment process was shown to be inversely proportional to the operating time and directly proportional to its efficiency. Additionally, in all the light-driven-based AOPs, the energy usage from fossil fuels was the primary source of environmental pollution. MBR is another crucial process that is easier to integrate with various water treatment techniques because of its high removal efficiency of refractory contaminants. Nevertheless, owing to the higher energy usage and the expensive cost of membranes, various studies have revealed that MBR has a low environmental sustainability. Pesqueira et al. (2020) assessed the environmental effect of membrane-based technologies (NF, RO and MBR) for the degradation of EC (Pesqueira et al. 2020). Due of its high energy usage, the study claimed that MBR can cause more environmental damage than they mitigate. NF was observed to be more ecologically favourable, but RO showed the greatest potential for global warming. These issues can be solved by the fabrication and utilisation of low-cost membranes and renewable energy sources, such as solar light. Also, the combination of MBBR with MBR reduces the membrane fouling and improves the micropollutant removal. For adsorption process, the environmental influence can be greatly reduced by considering the regeneration and reuse option rather than constantly utilising fresh adsorbent. The reason can be attributed to low resource and energy consumption by the regeneration process. Land restoration, low energy requirement and eco-friendly nature of CWs make it a viable alternative for long-term sustainability (Wu et al. 2015). As a result, development of CW-based integrated systems (less environmental effect) for the degradation of contaminations is recommended. Nevertheless, the principal issue with CWs in urban areas is the need for a larger installation area. In all the aforementioned treatment processes, chemical consumption and energy usage are the two major features of the operating phase, both having a substantial impact on environmental sustainability. Thus, researchers must use LCA to develop a solution that balances resource usage, operating time, environmental sustainability, treatment cost and other pertinent variables. The sustainability assessment of the combined treatment systems for the degradation of ECs along with their benefits and limitations is shown in Fig. 3.

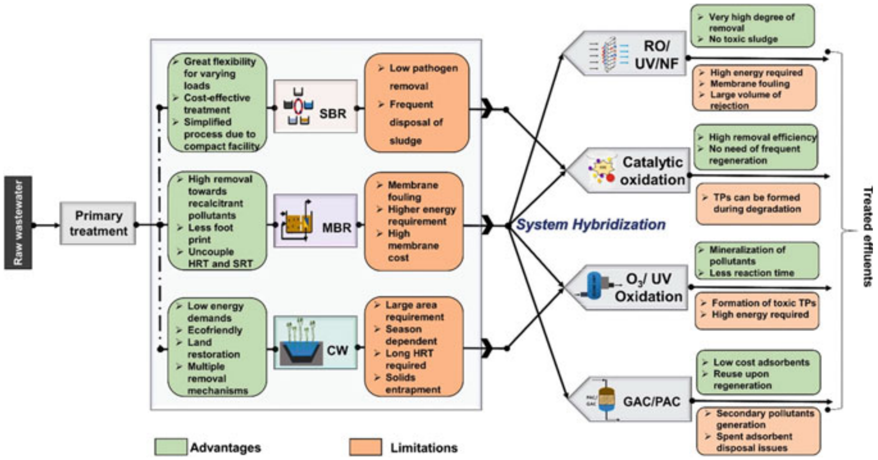

Fig. 3 Sustainability of different hybrid systems towards the degradation of ECs along with their advantages and limitations. Reproduced with permission from Parida et al. (2021) © Elsevier

8 Conclusion and Future Perspectives

The extensive utilisation of few compounds, viz. PhACs, PCPs, UV filters, ASWs and X-ray contrast media results in their widespread presence in the aquatic ecosystem. After subsequent utilisation, such compounds make their way into the aquatic bodies via various effluent streams. Despite the fact that these compounds were reported all over the world, their average concentrations in European and Asian nations were found to be rather high. These contaminants are released into the aquatic environment without proper treatment due to their overuse and regulatory limits. This leads to increased loads, which resulted in the disruption of biota. The toxicity of several substances on aquatic life, viz. crustaceans, fish, daphnia and algae was calculated using RQ values. Caffeine was reported to have the highest RQ value for algae (1100), thereby indicating a serious threat to the algal species. Crustaceans have shown to be more resistant to the effect of ECs than other animals. Under international regulatory guidelines, most of the traditional treatment methods were found to be ineffective in lowering the effluent concentrations. In general, the degradation of ECs was reported to be <70% throughout the traditional treatment methods such as SBR, CWs and ASP. Furthermore, excessive HRT and dumping of sludge containing ECs are the two important issues related to most of the traditional treatment methods. On the other hand, MBR + UV, MBR + RO, flocculation + ASP + UF and HFCW + Ozonation along with other integrated treatment techniques indicated around 100% degradation of ECs. When the LCA studies were compared, it was seen that among the several membrane-based processes, NF process was more environmentally benign, but RO showed a higher potential for global warming. In general, CW-assisted hybrid systems possess a very high level of sustainability and low implementation cost since they do not generate secondary pollutants. The current chapter provides a comprehensive overview of the efficiency of several hybrid processes in terms of eradicating various ECs along with their economic feasibility.

Despite the fact that these hybrid processes consist of very high degradation capacity, however, additional study on mineralisation is required to avoid the production of TPs, which are generally more hazardous than the parent compounds. Besides, adequate research should be conducted to reduce the costs of hybrid treatment methods, thereby allowing for easier upscaling choices. In addition, the technological viability of combining various AOPs with the biological processes necessitates substantial research in order to develop an appropriate hybrid treatment process. One of the most pressing concerns for the policymakers is the financial viability of the hybrid processes. The degradation routes and the metabolites of ECs are two very crucial parameters to address before implementing a system since these products can have bio-toxic and eco-toxic impacts on the environment. For advanced treatment processes, the impact of numerous design and operating factors should be rigorously analysed, which necessitates intensive utilisation those processes on a pilot scale. Advances in genetic engineering are necessary to select and improve the efficacy of microbial activity for the degradation of different ECs, thereby ensuring the economic and technical viability of the treatment process.

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Membrane and Disinfection Technologies for Industrial Wastewater Treatment

Deepti, Anweshan, Simons Dhara, and M. K. Purkait

1 Introduction

The rapid growth of the world's population foretells a significant increase in demand for water, energy and food (Deepti et al. 2020a). Increased wastewater discharge from numerous industries is now higher than ever due to population growth and lifestyle changes. Wastewater treatment removes organic matter and eliminates or disables microorganisms in wastewater to preserve the environment from pollution and to prevent the spread of illness. Given the possibility of dangerous and/or deadly substances present, it is critical to appropriately treat industrial wastewater before releasing it into the environment. Several attempts have been made over the years to implement various wastewater treatment methods, including physical, chemical and biological treatment systems. Existing technologies are also being improved to meet current discharge or reuse regulations (Deepti et al. 2020b). Membrane and disinfection technologies are two wastewater treatment technologies that have witnessed significant advancements throughout this time. Membrane and disinfection technologies have great demand in recent decades as a result of the advantages they provide in water and wastewater treatment. They have a lot of potential in industrial wastewater treatment because of their small-sized equipment requirement, low energy consumption and low capital cost (Singh et al. 2019; Yaranal et al. 2019).

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1.1 Environmental Impact of Various Industrial Wastewater

The industrial revolution and fast urbanisation have accelerated, resulting in massive amounts of wastewater being generated all over the world. Against the backdrop of worldwide water usage, the industrial sector uses an average of 22%. Nearly, 80% of the wastewater generated is dumped into water channels, causing contamination and posing a hazard to human health and aquatic life (Dutta et al. 2021). Iron and steel, chemical, food and dairy, textile, mining and quarries, paper and pulp, battery manufacturing, tannery, nuclear power, soap and detergent, power generation plant, fertiliser, pharmaceutical, petroleum and petrochemical and electroplating and metal processing industries are all major sources of metals like mercury, copper, zinc, chromium, cadmium, arsenic, iron, nickel and lead in wastewater (Patwardhan 2008).

Raw wastewater effluents are frequently discharged into water bodies as a result of inadequate wastewater treatment facilities, such as power outages, poor maintenance and a lack of educated and experienced labour. Water bodies, especially groundwater, become contaminated, posing health risks and polluting the environment. As a result, as the number of industries grows, more water and resources are consumed, resulting in a massive amount of wastewater being released. Therefore, industrial wastewater management has become a major concern around the world (Bora and Dutta 2019). There are various treatment techniques reported for industrial wastewater treatment such as adsorption, oxidation, coagulation, ion-exchange, electrocoagulation, membrane technology and disinfection technologies. A detailed discussion on membrane and disinfection technologies will be discussed in the subsequent sections.

1.2 An Overview of Membrane Technology

Among the wastewater treatment technologies, membrane-based separation technology, such as ultrafiltration, microfiltration, nanofiltration, reverse osmosis and electrodialysis, includes a number of different and characteristic separation processes and provides a more comprehensive solution by allowing treatments that address the particularity of various pollutant characteristics (Mohanty and Purkait 2011). Membrane technology has become more affordable in recent years, while its application in many treatment processes has grown. Membrane processes, in comparison with most traditional technologies, provide the flexibility to be retrofitted with various auxiliary processes in wastewater treatment and resource recovery without significantly affecting the product water quality or space requirements (Purkait and Singh 2018). Membrane processes are less susceptible to changes in water and wastewater quality, henceforth their operation is more stable than that of their conventional equivalents, particularly biological treatment systems. The integration of numerous membrane units boosts the efficiency of the membrane system, allowing it to handle multiple contaminants in a complex waste stream (Ahmad et al. 2021).

1.3 Disinfection Techniques in Brief

In order to inactivate pathogens and other contaminants, disinfection treatments are frequently used at the end of the wastewater treatment process. The use of disinfection methods through optimal doses is calculated based on the minimal water quality standards for the safe reuse of treated industrial effluent (Bergmann 2021). Common wastewater treatment technologies include ultraviolet irradiation, sodium hypochlorite, liquid chlorine, ozone and chlorine dioxide disinfection. Every disinfection method has its own set of benefits and drawbacks. The usage of a specific disinfection technique should be based on a thorough examination of both economic and practical considerations, like the volume of wastewater, safety conditions, disinfectant supply, investment, operation costs and level of operation management. Inactivating a wide variety of pollutants and limiting problems with effective disinfection technologies is highly suggested to achieve the minimal quality criteria for the safe reuse of wastewater in industry. This chapter discusses the underlying mechanism and application of membrane-based technologies, to treat industrial wastewater. Further, the main disinfection technologies, both traditional and advanced, for the treatment of industrial wastewater, as well as the probable generation of by-products, operating parameters and advantages and drawbacks are discussed in this chapter.

2 Membrane Processes for Industrial Wastewater Treatment

Industrial growth spurred by global consumption has increased wastewater creation. Industrial wastewater includes unwanted products, unsegregated products, unutilised reactants or raw materials and other pollutants. Most industrial wastewater elements are harmful, and some are even carcinogenic (Purkait et al. 2019). As a result, before it can be released into the environment, effluent must be properly treated in order to prevent its harmful consequences. The quality of effluent must be established and regulated in compliance with applicable municipal regulations. The following physical and chemical properties affect overall quality:

- Biological oxygen demand (biodegradable organics).
- Total suspended solids (TSS).
- Refractory organics [chemical oxygen demand (COD), total organic carbon (TOC)].
- Nitrogen.
- Phosphorus.
- Heavy metals.
- Dissolved inorganic solids.
- Oil and grease.
- Volatile organic compounds (VOC).
- Microbial organisms.

Treatment of toxic effluents for reclamation, re-utilisation and recycling is in high demand from an economic standpoint.

Membrane separation procedures vary in membrane type, design, feed component transport mode, process driving force and pore size. Components are accelerated toward the membrane surface by a driving force, some of which pass through the membrane. These processes have a wide range of applications with varying driving forces and separation properties. An overview of these methods is provided in Table 1. Despite being the most popular, pressure-driven membrane separation can only remove some contaminants. In pressure-driven membrane systems, a semi-permeable membrane can achieve large fluxes due to the feed solution's high pressure. Pressure-driven procedures, such as micro- and ultrafiltration, reverse osmosis and pervaporation, are the most technologically and commercially feasible. The driving force that generates permeate flow across membranes in industrial separation may be classified into four forms as shown in Fig. 1 (Nath 2017):

A. Hydraulic pressure-driven membrane process:
   - Reverse osmosis (RO).
   - Nanofiltration (NF).
   - Ultrafiltration (UF).
   - Microfiltration (MF).
   - Pervaporation (PV).
   - Membrane gas separation.

B. Osmotic pressure-driven membrane process:
   - Forward osmosis (FO).

C. Concentration gradient steered membrane process:
   - Dialysis.
   - Membrane extraction.

D. Electrical potential guided membrane process:
   - Electrodialysis (ED).

Assisted or carrier-mediated membrane transfer, liquid membrane separation, membrane contactors, membrane reactors, membrane distillation, charge mosaic membranes and piezodialysis are other membrane processes. The single-phase membrane reactors combine membrane filtration with chemical reactions. The reaction section and selective extraction of a reactant can boost conversions over the equilibrium value. Membrane reactors are suitable for achieving equilibrium-limited endothermic reactions.

Table 1 Distinctive membrane separation processes: operating principles, driving force and applications

Separation process	Driving force	Separation mechanism	Range of application	Pore size (nm)
Microfiltration	Pressure difference (ΔP)	Sieving	Sterile filtration clarification	10–10,000
Ultrafiltration	Pressure difference (ΔP)	Sieving	Separation of macromolecular solutions	10–100
Nanofiltration	Pressure difference (ΔP)	Solution diffusion	Separation of divalent ions from solutions	0.5–5
Reverse osmosis	Pressure difference (ΔP)	Solution diffusion	Separation of salts and micro-solutes from solutions	<1
Dialysis	Concentration difference (ΔC)	Diffusion	Separation of salts and micro-solutes from macro molecular solutions	<1
Electrodialysis	Electric potential difference (ΔE)	Selective transport of ions or molecules according to electric charge	Desalting of ionic solutions	<1
Supported liquid membrane	Concentration difference (ΔC)	Solution diffusion	Separation and concentration of metal ions and biological species	<1
Membrane distillation	Temperature difference (ΔT)	Vapour transport	Ultrapure water concentration of solutions	1–10
Pervaporation	Concentration difference (ΔC)	Solution diffusion	Separation of organics	<1

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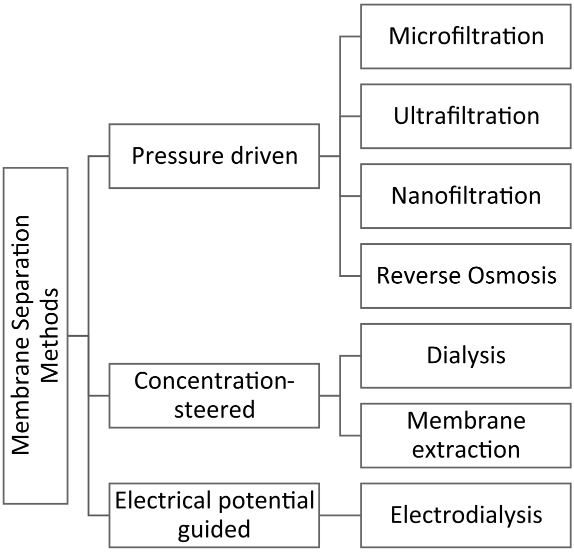
2.1 Ultrafiltration and Microfiltration

Sustainable water resource management is critical for the modern society and the economy. Increased water use has spurred significant economic and social growth as a result of industrialisation and human activities. Diverse solutions for cleaning and regenerating dirty streams have evolved. Ultrafiltration (UF) remains a safe, clean, cost-efficient and highly successful technology for separating a wide range of components and contaminants from water and wastewater since its commercialisation in 1970 for electrophoretic painting. Membrane filtering has been used for over

Fig. 1 Classification of membrane separation methods



a century to mechanically separate components. Benchold coined the term “ultrafiltration” in 1907. Hydrostatic pressure forces cause fluid flow over semi-permeable barriers. This separation procedure targets molecules and larger suspended particles (MWCO). Other factors to consider include molecule shape, charge and hydrodynamics (Changmai et al. 2019). While size exclusion is the basic method by which UF works, depending on the chemicals involved, interactions between the molecules and the membrane may prevent the process from operating at its full potential (Al Aani et al. 2020). Ultrafiltration is operated with a pressure differential of 1 to 10 bar. A typical MF membrane is primarily porous and symmetric, made from polymeric and inorganic materials, with pore size 1–100 nm. UF is a state-of-the-art separation technology that is employed in a wide variety of sectors. It was employed initially as a fractionation method in the late 1960s. Since then, UF membranes have been continuously improved, and their applications have grown to include chemical recovery, cell harvesting, dairy production, pharmaceutical applications, wastewater reclamation, water purification and juice concentration (Nandi et al. 2010). UF is commonly used in the treatment of water and wastewater. UF is frequently used to treat wastewater, eliminate bacteria and viruses and treat metal, paint and textile effluents. The technique is economical and straightforward since it consumes less energy, has fewer controls, uses fewer chemicals and is more efficient. Numerous firms can become more eco-friendly due to technological advancements (Widiasa et al. 2018; Sharma and Purkait 2017). Microfiltration is an effective method for eliminating colloidal colours from exhausted dye baths and subsequent rinses in membrane technology, whereas ultrafiltration is ideal for cleaning secondary textile effluent (Jana et al. 2010).

Initially, microfiltration was employed to purify water and air. Microfiltration (MF) outsells all other membrane processes combined. MF has several uses. This approach may be used to separate suspended particles, germs, cell fragments and huge colloids. Low-pressure cross-flow membranes can separate particles from 0.05 to 10 microns: sorting or coarse filtering. So bacteria cannot travel through the pores (0.1 to 10.0 microns). An MF membrane's permeability allows molecules in existing solutions to pass. A minimal pressure difference, i.e. <2 bar, is required for microfiltration (Deepti et al. 2020a). A typical MF membrane is porous and symmetric, made either from polymeric or inorganic materials, with pore sizes ranging from 10 to 1000 nm. The final uses of membrane materials directly reflect their characteristics (Lin et al. 2016). Membrane materials are chosen based on their mechanical and thermal properties, as well as the production process. Impeded diffusion of solutes and suspended particles are the fundamental transport mechanisms in microfiltration. MF membranes use sieving methods with varying pore diameters to retain larger particles than the pore width. Membranes with absolute ratings are ideal for sterile fluid, air and water filtration applications. MF purifies wastewater by removing suspended particles and insoluble metal hydroxide solids. It benefits businesses, including paper and pulp, textiles and leather tanning.

2.2 Nanofiltration and Reverse Osmosis

Nanofiltration (NF) is similar to reverse osmosis (RO), but the membranes employed are far more permeable, requiring less pressure (and hence less energy). Another differential is the removal of monovalent ions like chlorides. RO eliminates 98–99% of monovalent ions at 200 psi. Comparatively, nanofiltration membranes remove monovalent ions between 50 and 90%, depending on the membrane composition and manufacturing process. As a result, nanofiltration membranes come in many forms. Some types are incompatible with others. To selectively transfer liquid and solutes over a membrane, use NF. Nanofiltration combines the benefits of reverse osmosis with ultrafiltration. An NF membrane is a thin UF or RO membrane. This approach eliminates 1 nm solutes (Deepti et al. 2020b). Filtration occurs on an organic semipermeable membrane layer of selective separation. The pressure differential between the feed (retentate) and filtrate (permeate) sides of the membrane's separating layer causes separation. Molecular weights less than 200 Da can pass through the semipermeable separating layer. The NF membrane transport and rejection mechanisms are complex. The influence of numerous factors on transport mechanisms and membrane performance has been studied extensively. "Surface-capillary flow" and "solution diffusion" are crucial ideas (Nath 2017). Therefore, charged solutes, especially those smaller than membrane pores, will be excluded from membranes by the sorption surface-capillary flow hypothesis (Donnan exclusion). The effective charge density, pore radius and ionic strength of monovalent ions. However, monovalent ions make up between 0 and 50% of NF membrane monovalent ions.

The theory of solution-diffusion states that water and solutes (ions) dissolve into a porous layer called a membrane. The solute is primarily propelled across the membrane by concentration gradient forces. Previously, NF was used only in the potable water business to soften and remove organics and micropollutants. Nanofiltration is presently employed in a range of sectors for a variety of purposes, including fractionation, solvent filtration, water recycling and process stream treatment. When applied to wastewater, nanofiltration is still viewed as a purifying operation rather than a fractionation technique (Bruggen 2013).

Reverse osmosis, occasionally referred to as hyperfiltration, is a high-pressure process stream dewatering using RO to concentrate low-molecular-weight compounds in solution or cleanse wastewater. It can concentrate dissolved and suspended particles. RO is commonly used to desalinate brackish water. Water must travel across a membrane to be desalinated. Extra pressure must be applied to the water column on the salt side of the membrane, first to lower the natural osmotic pressure, then to push the water across. Reverse osmosis is a liquid-driven membrane technique that passes water but rejects micro-solutes such as salts and low-molecular-weight organics (1000 daltons). Osmosis is the movement of water from diluted permeate to concentrated feed. The required pressure is proportional to the concentration of the reject (concentrate) salt solution. Operating the system at 1100 ppm requires pressures of over 200 psi on the concentrate side. Systems with 33,000+ ppm seawater normally run at 800+ psi. Domestic reverse osmosis systems typically run at 50–70 psi (Nath 2017).

2.3 Forward Osmosis

An alternative to pressure-driven membrane systems, forward osmosis (FO) processes, has come to the forefront (Cath et al. 2006). In FO, a chemical potential difference between a concentrated draw solution (DS) and a diluted feed solution (FS) induces spontaneous water permeation across a semi-permeable membrane. In comparison with RO, FO has the advantages of reduced energy consumption (i.e. lower capital and operational costs) and less irreversible membrane fouling. FO utilises the osmotic pressure differential across the membrane active layer to draw water from the FS (low concentration) to the DS (high concentration) side, while the hydraulic pressure differential across the membrane (ΔP) is almost zero. The process becomes pressure retarded osmosis (PRO) ($\Delta P <$ osmotic pressure difference ($\Delta \pi$)) or RO ($\Delta P > \Delta \pi$) when hydraulic pressure is applied to the more concentrated solution side. On the other hand, pressure assisted osmosis (PAO) is powered by hydraulic pressure applied to the lower concentrated solution side. FO/PRO/PAO does not separate freshwater throughout the process, unlike traditional RO separation. Instead, the penetrated water goes to the DS side, where it must be separated from the dissolved solutes by a separate process if clean water is wanted as the final result. The membrane is at the heart of a highly efficient FO system. Because of the internal concentration polarisation (ICP) effect in the membrane support layer, the real driving force (true osmotic pressure gradient across the membrane active layer) during a FO operation is substantially less than the osmotic pressure difference between the bulk FS and DS. FO has been employed in municipal sewage treatment systems (Hey et al. 2018). In FO membrane systems, large ions may be rejected, and sewage can be concentrated by a factor of ten to fifteen (Gao et al. 2018). FO can be used in combination with RO to dilute saltwater utilising a wastewater stream in a hybrid process. Another research used a forward osmosis-membrane distillation hybrid technology to remove tetracycline from wastewater, with a rejection rate of 99.9% and a water recovery rate of 15–22%. In addition to water recovery, FO may be used in wastewater treatment for nutrient and energy recovery. Biogas production and nutrient recovery (e.g. phosphate, ammonia and potassium) are examples of these recovery processes.

2.4 Electrodialysis

Electrodialysis (ED) is an electro membrane process that separates ions by transporting them across selective membranes in the presence of an electric field, resulting in two streams of differing concentrations (Gurreri et al. 2020). When polymeric ion-exchange membranes (IEMs) were first commercially made in the 1950s, electrodialysis (ED) techniques were developed for the production of table salt and drinking water. Since then, electro-driven membrane separation of ions and salts using IEMs has become a prominent electro driven membrane method (Moon and Yun 2014). In the chemical, biochemical, food and pharmaceutical sectors, electrodialysis (ED) has been used to treat saline water and brines, treat metal-contaminated wastewater, recover valuable products, remove harmful components and perform concentration and purification operations.

Min et al. (2021) evaluated design aspects such as velocity variation, ion separation efficiency and concentration efficiency, as well as the fouling phenomena in the electrode plate for the treatment of plating wastewater using electrodialysis. The voltage and velocity variations were found to be associated with the linear flow velocity in the experiment. The thickness of the diffusion boundary layer on the surface of the ion-exchange membrane is influenced by the linear flow velocity, according to the study. They also found that, the separation efficiency of heavy metals separated in the electrodialysis process is lowered by electrodeposition, it is preferable to use the reverse electrodialysis process on a regular basis to minimise fouling (Min et al. 2021).

Lafi et al. (2018) studied the application of an integrated process for the treatment of textile wastewater utilising a combination of ultrafiltration and electrodialysis. All measures, including COD, conductivity and TDS, showed considerable decreases. They stated that the use of UF as a pre-treatment before ED, made it possible to treat the effluent without clogging the membrane during electrodialysis (Lafi et al. 2018).

3 Disinfection Technologies for Industrial Wastewater Remediation

3.1 Conventional Methods

3.1.1 Chlorine-Based Disinfection Techniques

Chlorination has been the most cost-effective technique of disinfection. Chlorination using bleaching powder is more appropriate for large-scale operations. Because it is less dangerous to handle, with suitable warehouse infrastructure, rather significant volumes may be hoarded. It also has a relatively high availability of chlorine per unit weight. When chlorine is added to water in just about any form, hypochlorous acid is formed (HOCl). Hypochlorous salts react with water, and hypochlorite ions (OCl^-) are produced (Lister 1952). Both hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) are potent disinfection agents in chlorinated water. On the other hand, hypochlorous acid is the more effective of the two. The pH level of the water determines the number of each organism in the water before chlorine is added. Hypochlorous acid will take precedence at lower pH values. What is known as free chlorine is a mixture of hypochlorous acid and hypochlorite ions. Free chlorine has a higher oxidation potential than other types of chlorine, such as chloramines, and is thus a more effective disinfectant. Chlorine is a cheap and readily available product, and it also does not degrade during storage.

The "chlorine demand" refers to the quantity of chlorine necessary to eliminate all contaminants. The amount of chlorine required is determined by the initial concentration of pollutants existing (Priya et al. 2020). The amount of chlorine supplied to the system exceeds the requirement for chlorine. Residual chlorine refers to the remaining chlorine in the water body. The residual chlorine protects the system against microbial contaminants in the downstream operations.

Hypochlorous acid (HOCL) and hydrochloric acid (HCL) are produced when chlorine gas is mixed with water. Hypochlorous acid is a weak acid that may be dissociated further into hypochlorite ions and hydrogen ions (Clasen and Edmondson 2006).

$$\mathrm{Cl_2 + H_2O \rightleftharpoons H^+ + Cl^- + HOCL} \tag{1}$$

$$\mathrm{HOCL \rightleftharpoons OCl^- + H^+} \tag{2}$$

Sodium hypochlorite is a yellowish liquid, which provides 10–15% chlorine availability and is an excellent disinfectant. But it has a shallow self-life and gets degraded over time. Sodium hypochlorite is corrosive, temperature, light-sensitive and often requires secondary containment to prevent any spill. Calcium hypochlorite is said to be much stable than sodium hypochlorite and also holds a higher concentration of chlorine per kilogram. The dissolution mechanisms of sodium and Calcium hypochlorite are as follows (Sieci and Gowej 2020):

$$\mathrm{NaOCl} + \mathrm{H_2O} \rightleftharpoons \mathrm{Na^+} + \mathrm{OH^-} + \mathrm{HOCL} \tag{3}$$

$$\mathrm{HOCL} \rightleftharpoons \mathrm{OCl^-} + \mathrm{H^+} \tag{4}$$

$$\mathrm{Ca(OCl)_2} + 2\mathrm{H_2O} \rightleftharpoons \mathrm{Ca^{2+}} + 2\mathrm{OH^-} + 2\mathrm{HOCL} \tag{5}$$

$$\mathrm{HOCL} \rightleftharpoons \mathrm{OCl^-} + \mathrm{H^+} \tag{6}$$

3.1.2 Ozonation

Ozone has a weak single bond and a strong double bond, making it a formidable oxidising agent. Because ozone can react as both an electrophile and a nucleophile, it has an interconvertible two resonance structure. Ozone is an unstable gas that interacts with the compounds in wastewater in one of two different ways: directly as molecular ozone or indirectly as secondary oxidants, such as hydroxyl radicals. These two distinct reaction pathways produce distinct oxidation products and are governed by various sorts of kinetics. To determine the oxidising effects of ozone and the rate at which it is consumed, the degradation kinetics of ozone should be studied.

Ozone can react with organic compounds directly or indirectly. The hydroxyl radical, which possesses an unpaired electron, is involved in the indirect reaction pathway (Das et al. 2021a). Hydroxyl radicals are extremely unstable, and they react with other molecules almost rapidly to get an electron. Because of its dipolar nature, molecular ozone interacts with unsaturated bonds, causing the bond to disintegrate. The degree of nucleophilicity or electron density of organic water pollutants determines how quickly ozone interacts with them. The sluggish dissolving rate and quick breakdown in the aqueous phase restrict the efficacy of ozone for the treatment of organics polluted wastewater (Das et al. 2021b). Micro-nano-bubbles (MNBs) are a revolutionary way for extending the ozone's reactivity in the aqueous phase, hence, speeding up the contaminant's treatment. Khuntia et al. used ozone MNBs to remove ammonia from an aqueous medium, which seems to be a primary cause of the unpleasant odour. They discovered that microbubble-assisted ozonation was a quick procedure that could be used on a broader pH range than biological ammonia degradation. In addition, industrial wastewater with high saline levels was chosen as the target pollutant, and the treatment efficacy of ozone MNBs was investigated. Ozone MNBs had a strong treatment impact on high-salinity wastewater, with COD dropping by 63% after 14 h of treatment. The number and size of MNBs have an influence on their mass transfer efficiency. Smaller bubble size results in higher internal pressure and a more extensive specific area. In contrast, more significant

MNBs result in a larger surface area and enhance the mass transfer flux from bubbles to the solution (Khuntia et al. 2013).

The coupling of ozone and hydrogen peroxide, often termed as the peroxone method, is arguably the best researched and utilised advanced oxidation technique besides ozonation (Koulini et al. 2022). Ozone breakdown to hydroxyl radicals is increased with low quantity of hydrogen peroxide. Hydroxyl radicals are generated by the reaction between the ionic form of hydrogen peroxide (hydroperoxide ion, HO^{-2}) and ozone. The optimal stoichiometry for this reaction is one mol hydrogen peroxide to two mol ozone. Apart from the reduced generation of harmful by-products such as bromate, the peroxone procedure has a greater efficiency and reaction rate than ozonation alone. Yet, unnecessarily high hydrogen peroxide acts as a radical scavenger, and hydrogen peroxide residue is toxic to the environment.

When different catalysts are combined with ozone, reactive hydroxyl radicals are produced with less ozone than when ozonation is used alone. Catalytic ozonation procedures are characterised as homogeneous or heterogeneous based on the catalyst's solubility in water. Homogeneous catalytic ozonation utilises metal ions as the catalyst, whereas heterogeneous catalytic ozonation utilises metal oxides (or metals) that are frequently immobilised on supports. Manganese oxides (MnO_2), aluminium oxides (Al_2O_3), titanium oxides (TiO_2) and zinc oxides (ZnO) are all examples of metal oxides that have been used in heterogeneous catalytic ozonation. It is a promising method for the degradation of, pesticides, surfactants, dyes, medicines, phenols nitrobenzenes and other persistent and harmful aqueous organic pollutants, as well as the remediation of synthetic and industrial wastewaters (Xiao et al. 2015).

3.1.3 Ultraviolet Irradiation

UV-based advanced oxidation processes (AOPs) have gotten a lot of attention lately because of their excellent precision, energy efficiency and environmental friendliness. UV approaches have recently been combined with EO to increase the formation of oxidants and, as a result, the degradation of various pollutants in aqueous media, including hydrocarbons, dyes, medicinal compounds and pesticides. Until now, chemical disinfectants or ultraviolet (UV) irradiation have been widely utilised in worldwide water treatment facilities. Ultraviolet irradiation has two significant upsides: (1) it is devoid of dangerous by-products of chemical disinfection and (2) it applies to many bacteria. UV can selectively destroy molecules containing nucleic acid by wavelengths spanning from 200–300 nm, particularly at 260 nm (Luo et al. 2022). Ultraviolet may also cause photo-oxidation, which causes the creation of reactive oxygen species (ROS), protein disruption and damage in cell membrane. UV light may be generated artificially using excimer lamps, mercury vapour lamps, light emitting diodes and xenon pulse lamps, among other technologies (LEDs). The most extensively used UV light generators in water disinfection are traditional mercury lamps, particularly the low-pressure mercury lamp (LP) generating just 254 nm when the medium pressure mercury lamp (MP) releasing a bunch of wavelengths. The United States Environmental Protection Agency (US

EPA) recommended mercury lamp-based UV disinfection for water treatment. Newfangled Ultraviolet light sources, like UV LEDs and excimer lamps, have recently advanced to the point where they can provide an alternative and extend the UV spectral spectrum and improve efficiencies over mercury lamps. Excimer lamps, specifically xenon pulse lamps, have been proven to create a much improved bactericidal impact over mercury lamps during water disinfection and clinical infections. Excimer, xenon and mercury lamps are gas-state UV generator, while UV LED comes under the class of solid-state UV emitter. This implies that UV LEDs with a range of wavelengths may be created dynamically by altering the solid composition of chips, which is considerably easy in comparison with altering the gas composition in gas-state UV tubes. Readily available UV LEDs can now generate UV wavelengths more than 250 nm. Ultraviolet LEDs with a wavelength of 280 nm have also been offered as an effective substitute to low-pressure (LP) UV light. Among the many UV light sources, UV LEDs stand out for their exceptional ability to emit a variety of wavelengths for optimal disinfection via semiconductor chip adjustment. Numerous studies have shown that UV LEDs with wavelengths ranging from 315–400 nm, UV-B (280–315 nm) and UVC (200–280 nm) are efficient at inactivating planktonic pathogens in seas. Generally, the wavelengths 260 nm and 280 nm provide a practical sterilising effect since they correspond to the maximal absorbance of DNA and protein, respectively.

Different pollutants have been treated using UV-EO techniques, including insecticides, medicinal substances, dyes, hydrocarbons, and synthetic urine. UV exposure, whether natural (sunlight) or artificial (UV lamps), can replenish EO by producing pretty active radicals and promoting the breakdown of oxidants such O_3 and H_2O_2 via the reactions specified in Eq. (7) and (8)

$$\mathrm{H_2O_2} + \mathrm{h}\upsilon \rightarrow 2 \bullet \mathrm{OH} \tag{7}$$

$$\mathrm{H_2O} + \mathrm{O_3} + \mathrm{h}\upsilon \rightarrow 2 \bullet \mathrm{OH} + \mathrm{O_2} \tag{8}$$

During UV-EO, other oxidants such as peroxocarbonates and peroxosulfates are generated in high numbers. The disintegration of peroxocarbonates, peroxosulfates, peroxophosphates and chlorine causes a large number of radical species. BDD (boron-doped diamond) anodes have a synergetic effect that favours the development of an oxidant mixture (e.g. hypochlorite, persulfate, peroxiphosphate, ozone, and percarbonate), which seem to be the significant contributors to the material's effective behaviour in wastewater treatment.

The use of UV-EO for dye removal in batch cells has been investigated. When compared to EO or photolysis separately, Montanaro et al. found that the UV-EO method using BDD/Ti electrodes in a batch cell performed better at eliminating coumarin. Because of the synergetic action of UV light, which promoted the electro generation of reduced residual active chlorine, peroxides and lowered chlorate synthesis, the UV-EO method caused in quicker dye removal and mineralisation (Montanaro et al. 2017).

UV-based AOPs efficiently eliminated different antibiotics, such as quinolone, macrolide, β-lactam, tetracycline antibiotics, chloramphenicol and a variety of sulfonamide antibiotics in wastewater as an emerging tertiary treatment procedure. Because of the obvious potency of UV-mediated AOP, antibiotics can be removed efficiently from mixed wastewater containing ions, and the influence of ions in ion containing water on ultraviolet-based AOP is minimal since inorganic ions rummaged free radicals and produced offspring radicals with certain oxidising abilities. UV-based AOP proved successful in destroying the antibiotic in natural water bodies. The majority of organic compounds and inorganic ions in wastewater had an effect on the functioning of UV-based AOPs via a sequence of processes like reactive species scavenging (Souza et al. 2013).

3.2 *Advanced Processes*

3.2.1 Solar Disinfection

Solar disinfection (SODIS) is a low-cost approach that has been widely proved to be successful in deactivating waterborne pathogens from all areas of sanitary concern, including chlorine-resistant microbes, as well as inactivating or degrading several chemical pollutants in water (Chaúque and Rott 2021). The usage of solar disinfection by communities has been associated to enhanced immunity and a 50% decrease in the prevalence of diarrhoea in children under the age of five. This method of action consists of a number of mechanisms that work in conjunction to inactivate bacteria both in drinking water and wastewater. Although UV-B is known to induce DNA damage, it has only a minimal impact in the inactivation of SODIS microorganisms because stratospheric ozone absorbs 95% of the UV-B spectrum. Furthermore, the materials used in the containers may further limit UV-B transmission. As a result, the UV-A component of sunshine is the principal SODIS inactivator. UV-A radiation causes the cell to produce reactive oxygen species (ROS), which leads to death of the cell (Moreno-SanSegundo et al. 2021). Temperature too has a significant impact on the solar disinfection process, resulting in a synergistic effect with sunlight that decreases treatment time as temperature rises, especially above 45 °C. The direct effect of temperature is due to bacterial protein denaturation, while the synergetic effect is due to faster phases in the entire mechanism of radiation damage. The pathways of synergistic bacterial inactivation by sun radiation are show in Fig. 2.

3.2.2 Electrochemical Processes

Electrochemical disinfection (ECD) is the process of eliminating bacteria by passing an electric current through the water being treated using suitable electrodes. The electric current induces the electrochemical generation of disinfecting species from the water itself or from species dissolved in the water at the phase boundary between the

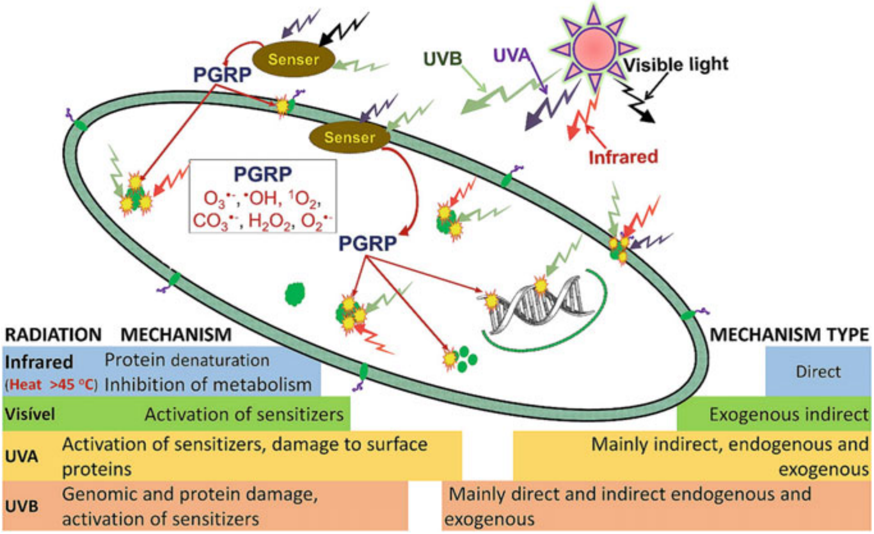


Fig. 2 Mechanism of inactivation of microorganisms by solar energy. Reproduced with permission from Chaúque and Rott (2021), copyright © Elsevier

electrodes and the water (Kraft 2008). The oxidation capacity of disinfectants in the electrode layer or the bulk of electrolytes is the basis of electrochemical disinfection. Intracellular enzyme system disrupture is frequently cited as the primary cause for the inactivation of microorganisms. In most of the situations, electrical field effects and pH-based influences may be ignored. Electrochemical disinfection would occur after coagulation/flocculation, sedimentation and filtering stages in water treatment unlike traditional chemical disinfection processes (Hand and Cusick 2021). ECD, also known as green technology, is environmentally and human-friendly since disinfectants are produced on-site. The most important reactant in the process is the electron, which is a relatively safe and clean component. The reaction mechanism of ECD is explained in two stages: First is the direct oxidation which takes place on the electrode surface and is defined by the quick death of microorganisms. Whereas the second stage is the indirect oxidation of water produced by intermediary processes that produce oxidising species such as the hydroxyl radical, atomic oxygen, ozone and hydrogen peroxide (Rahmani et al. 2019). These oxidizers are also called as reactive oxygen species (ROS). Portable equipment, minimal land requirement, ease of operation, simplicity, cost-effectiveness, creation of oxidising agents and no toxic chemicals are some of the significant advantages of this technology.

3.2.3 Photocatalytic Methods

Photocatalysis is a very effective and promising method that uses simply solar (or artificial) light and a photoactive substance. Because photocatalytic disinfection is a reaction between a photocatalyst and the target of disinfection (an aqueous medium), the materials of the photocatalyst and the properties of the aqueous media are both important components in the reaction (Habibi-Yangjeh et al. 2020). The heterogeneous photocatalytic operation begins with the absorption of used light with more energy than the photocatalyst band gap, which transfers electrons from the valence band (VB) into the conduction band (CB), resulting in the formation of charge carriers. The photoexcited charge carriers move to the photoactive material surface and participate in redox processes. The holes in VB and electrons in CB may both thermodynamically oxidise water molecules and decrease O_2 to form ROS. Disinfection and removal of toxins present in the environment occur as a consequence of the responses of bacteria and environmental pollutants with these radicals.

Materials with strong antimicrobial properties, such as chitosan, carbon nanotubes, fullerene and inorganic nanoparticles such as copper, silver, zinc oxide, titanium dioxide and others, have found widespread application as photocatalysts, but they still have limitations such as a fast rate of electron–hole pair recombination, a wide band gap that limits absorption (Pasini et al. 2021). Because of these constraints, practical application of the direct utilisation of nanoparticles in wastewater treatment plants is limited. Doping, as well as connecting and supporting two or more semiconductors, is some of the strategies utilised to circumvent these restrictions. The problems such as aggregation, electron–hole pair recombination and leaching can be resolved by supporting and linking a semiconductor with a suitable support or dopant that induces fine dispersion of semiconductor nanoparticles. The contact of two semiconductors generates an internal electric field, which increases the chance of e^-/h^+ separation. However, there are certain restrictions in the doping and coupling of two or more semiconductors, therefore, several novel ways to increase photocatalytic activity, such as metal oxide-based graphene composites, have been investigated (Dutta et al. 2019). Dutta et al. (2019) investigated the immobilisation of catalyst nanoparticles (NPs) on the surface of graphene as a two-dimensional support, which unlocked new prospects for the synthesis and design of a new group of catalysts. The high interest in graphene-based nanomaterials in wastewater disinfection stems from their availability in huge quantities, ease of functionalisation via chemical reaction, good water dispersion, high biocompatibility and lack of robust oxidants that are likely to produce harmful disinfectant by-products (DBPs) (Ibrahim and Asal 2017).

4 Challenges and Limitations of Membrane and Disinfection Technologies

Membrane and disinfection methods have been demonstrated to be a feasible solution for the treatment of industrial wastewater; nevertheless, there are numerous challenges to consider in terms of separation efficiency, fouling, cost, energy consumption and the generation of hazardous by-products.

Membrane separation methods are widely utilised in the separation, purification and concentration of various feed components. The membrane's efficiency and efficacy during the process degrades over time. This is related to membrane concentration polarisation and fouling. The two most significant disadvantages of membrane processes are concentration polarisation and membrane fouling. Fouling has remained one of the key concerns in wastewater treatment employing membrane technologies (Purkait and Singh 2018). In general, fouling occurs due to the deposition of undesired substances on the membrane surface, resulting in flux reduction. Foulants can clog membrane pores, resulting in internal fouling. Depending on the type of foulant, fouling can be classified as inorganic fouling (scaling), organic fouling or biofouling (Deepti et al. 2021). Scaling occurs when the concentration of inorganic ions in water exceeds the saturation level. As a result of this event, inorganic ions enter the nucleation stage, resulting in crystal formation and deposition on the membrane surface or pores. Several factors, including membrane characteristics, feed water composition and operating parameters like as pH and temperature, could all have an impact on the fouling phenomenon (Purkait et al. 2018). Several solutions have been developed to reduce fouling formation. Despite the introduction of numerous fouling mitigation methods, few of them have been used to treat actual wastewater. For industrial-scale membrane processes, a cross-flow configuration membrane module is preferred. Because the feed component does not directly affect the membrane surface, this greatly reduces membrane fouling and concentration polarisation and yields better outcomes in terms of effectiveness and efficiency. Polymer-enhanced filtering is another approach. This aids in the reduction of membrane fouling because it binds the feed components with the use of polymers that have binding targets for the feed components, preventing them from fouling the membrane (Jana et al. 2011). The increased size of the polymer-feed component complex prevents it from plugging the membrane pores, and the polymer restricts feed component adsorption on the membrane surface. As a result, cake layer production is halted, resulting in high membrane flow and higher separation efficiency. As a result, polymer-enhanced filtration is an excellent tool for retrieving small feed components and reducing membrane fouling. Other strategies, such as Micellar-enhanced filtration, the use of ultrasound, enhancing the hydrophilicity of the membrane material, and imparting a particular charge to the membrane in response to the feed, help to prevent membrane fouling (Deng et al. 2021). Aside from these procedures, the most often employed method is feed pre-treatment. Pre-microfiltration or ultrafiltration, pH modification, heat or chemical treatment, adsorption, chlorination and the addition of complexing agents are all examples of pre-treatment. It is a critical step in reducing membrane fouling (Changmai and Purkait 2018).

Similarly, several challenges and limitations have been reported in the disinfection of wastewater. First, in the case of photocatalysis, the difficulty is in recovering the suspended photocatalyst from the solution. This is due to the fact that the nanosized photocatalysts and photocatalysts functionalised with other carcinogen compounds may be poisonous, the suspended photocatalysts (nanosized) essentially be removed before the treated water is reused or discharged into the environment (Murcia et al. 2018). If the released photocatalyst has not gone through a complete reaction, it means that disinfection has still not occurred, and the photocatalytic disinfection could be more hazardous as catalyst is still possessing the adsorbed harmful contaminants. Second, the nanosized photocatalysts are fragile and prone to aggregation in actual water applications. Photocatalyst aggregation may obstruct the active surface area and limit photocatalytic efficacy. As a result, for an efficient photocatalytic degradation in wastewater treatment, those limitations must be overcome (Nasir et al. 2021). Similarly, there are some challenges in solar disinfection, such as the disposal of waste residue after wastewater treatment, which has a negative influence on the environment. As a result, a recycling strategy for handling waste residue must be established, increasing the overall economic value. The disadvantage of solar energy is that wastewater treatment plants work poorly at night, however, it can only treat wastewater during the day. The requirement for energy storage systems raises the total cost of the wastewater treatment plant. Wastewater treatment facilities must meet Environmental Impact Assessment (EIA) standards, they are site-specific; as a result, the facility lacks experienced staff for advanced performance. As the shift from laboratory level research to industrial level research continues, there is a lag in scientific revolution in the technologies adopted for treating wastewater using solar energy. In addition, the water produced by solar energy in wastewater treatment plants must be treated further for biological disinfection and to achieve clean odour (Pandey et al. 2021). There are many negative side effects when utilising ozone as a disinfection agent in wastewater treatment is considered. The probable mutation of nucleoids after ozone interaction, as well as hazardous transformation products, is critical considerations. Although some techniques, such as biological filtering with a medium of sand or activated carbon after ozone disinfection, have been found to be effective, they are associated with the danger of microbial regrowth (Foroughi et al. 2021).

5 Case Studies: Wastewater Treatment in the Industries

Deepti et al. (2021) investigated closed circuit reverse osmosis (CCRO) for the treatment of highly saline membrane rejected stream generated by the nanofiltration (NF) process at the Tata Steel industry, India. They stated that the volume of the reject stream was between 30% of the volume of the feed steam. The concentrated monovalent and divalent ions were present in the membrane reject stream (3–4 times the feed). The primary source of such extremely concentrated chloride and sulphate ions has been recognised as the use of sea water for coke quenching. They observed that all of the water quality parameters, including total dissolved solids, magnesium, sodium, manganese, chlorides, sulphates and other salts, are substantially beyond the allowable limit, necessitating treatment before reuse or discharge. The closed circuit reverse osmosis technology was shown to be effective in removing contaminants, notably chloride and sulphate salts (up to 95%). The recovery reported was as high as 82% at a steady-state flow of 7 L/m^{2}h at a transmembrane pressure of 7 bar (Deepti et al. 2021).

Ali et al. (2020) investigated nanofiltration as a treatment method to treat wastewater streams from a Danish anodising industry. Wastewater was collected from four rinse baths such as alkaline pickling rise, colour rinse, anodising rinse and acidic pickling rinse, as well as a centralised container in which wastewater from the different baths was blended and neutralised. Nanofiltration (NF) was performed on all wastewater streams at a low pressure of 3.5 bar with an Alfa Laval LabStak M10 cross-flow module, a cross-flow pump and two pressure gauges. They observed that removing larger particles before NF kept the flow steady for the mixed wastewater. The acidic pickling rinse, on the other hand, revealed a high rejection of cations and a slight decline in NF permeability over time (Ali et al. 2020).

Bilińska et al. (2020) investigated the treatment of the textile wastewater that originated from a dyeing of cotton fabric in a Polish dye factory by catalytic ozonation. The catalytic effect of activated carbon (AC) with ozonation was investigated. An Ozonek Ozone Generator was used to create ozone. A compressed gas cylinder provided the oxygen required for ozone production. A BMT 963 Vent ozone analyser was used to detect the ozone levels at the reactor's inlet and output. An internal cooling coil served as the reactor thermostat. The Elmetron C411 device was used to monitor the temperature and pH inside the reactor. They observed that the catalytic ozonation was better when compared to single ozonation. The presence of AC improves the colour removal process. In comparison with single O_3, the colourless by-products were oxidised more effectively through catalytic ozonation with AC addition. The use of catalytic ozonation resulted in a faster rate of mineralisation. The overall process oxidative potential was boosted via catalytic ozonation (Bilińska et al. 2020).

Martín de Vidales et al. (2017) studied electrochemical oxidation by conductive diamond, photolysis, sonolysis and also all the combinations of these three technologies for the treatment of two types of wastewater originated from pharmaceutical industry (organic synthesis) and also the output of a municipal wastewater treatment plant. Electrochemical flow cell was used for electrolysis experiments that operated in batch mode. The anode selected was a conductive diamond electrode (p-Si-boron-doped diamond) while the cathode was stainless steel (AISI 304). They observed that CDEO removes refractory contaminants from both industrial and urban effluents efficiently. Single-irradiated methods such as sonolysis and photolysis are ineffective at oxidising chemicals in wastewater such as metoprolol and caffeine. However, combining these irradiation methods with CDEO has a synergistic effect, resulting in a significant improvement in the performance of the electrochemical processes. They also stated that combining UV irradiation with other processes considerably more energy-efficient method (Martín de Vidales et al. 2017).

6 Summary and Future Perspective

Membrane-based technologies, as well as the main disinfection technologies, both traditional and advanced, along with their mechanism and significant applications in industrial wastewater treatment were discussed in this chapter. Membranes have recently proven to be viable processes for a variety of industrial and commercial applications. Simplicity, selectivity, flexibility, permeability, effectiveness, efficiency, stability, compatibility with other processes and environments, low energy requirements, ease of control, low cost and the ability to scale up easily make them an ideal process to use and explore for current and future applications. In terms of technology and processes, many potential applications are on the verge of significant improvements. In case of membrane science, many new materials, techniques and technologies are being introduced in order to improve efficiency and effectiveness. Membranes are an effective and efficient technology that is employed in a variety of applications. However many of the applications require further developments in membrane research, as they cannot be accomplished successfully or efficiently with current membranes. As a result, improved membranes and, in particular, membrane materials must be developed for use in demanding mixture separations or modern-day industrial applications. Membranes that can effectively separate biomolecules, enantiomers or isomers are needed. Biomimetic and smart membranes are also required to perform functions similar to those performed by biological membranes. Advanced membrane materials, such as responsive polymers, hydrogels, nanocomposites and carbon nanotubes, can be used to accomplish these advancements. In this chapter, various disinfection technologies, both traditional and advanced, are discussed. According to the technologies described, several ways have improved disinfection properties. Most disinfection technologies have emerged as viable candidates for wastewater disinfection and other uses at this time. However, significant advancements in the field, such as photocatalytic disinfection using metal oxide composites, have yet to be made. Furthermore, precise kinetic data is necessary for optimal photocatalytic disinfection system design. In addition, developing a good mechanistic model necessitates a knowledge of the basic reaction that underpins the disinfection process. Furthermore, the application of photocatalytic disinfection agents on a broad scale has remained unaddressed. To increase the potential of nanocomposites as photocatalytic disinfection agents, more research and suitable optimisation are necessary. Solar photo-fenton efficiency for bacterial inactivation, as well as solar water disinfection and heterogeneous photocatalysis, increases with larger solar energy doses. Despite this, the relationship between microorganism photo-inactivation rate and solar exposure time, energy received or dose administered is not totally understood today, as various factors such as biological complexity and solar radiation fluctuation play a role. As a result, the fundamental notion to address in terms of solar reactors is the optical characteristics of the wastewater by both biological and chemical components with respect to light penetration and UV absorption. The advancement of pollutant removal by membrane and disinfection technologies will be advantageous and valuable in controlling and safeguarding human and environmental health. Both technologies are projected to gain increased attention and profitability in this market area in the next years, especially if the technological and operational limitations can be properly addressed.

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Holistic Approach to Remediate Heavy Metals and Radionuclides

Sonia Sethi

1 Introduction

Toxic heavy metals and radionuclides have been released into the environment as a result of industrial activity. Chemical techniques to metal remediation are available, but they are frequently costly to implement and lack the specificity needed to treat target metals in the presence of competing ions. Furthermore, such technologies are ineffective for cost-effective in situ treatment of large-scale underground pollution. Biological techniques, on the other hand, have the ability to remove hazardous metals in a highly selective manner while also providing a high level of operational flexibility, since they may be deployed in situ or ex situ in a variety of bioreactor designs. Microorganisms that play a crucial role in the biogeochemical cycle of hazardous metals and radionuclides are used in many of these activities.

New or better metal bioremediation technologies have resulted from advances in understanding the functions of microorganisms in such processes, as well as the capacity to fine-tune their activities using molecular biology tools. The contamination of the terrestrial world by excessive amounts of recalcitrant and xenobiotic substances has been a prominent source of environmental toxicity in recent years. Human activities produce a significant number of organic and inorganic substances into the environment each year (Sonowal et al. 2018).

Heavy metals (HMs) are found in the environment in various oxidation states with varied levels of toxicity. Interference with cellular biomolecules causes the harmful effects of HMs. Their increasing exposure to HMs in living things poses a risk to their health (Sarma et al. 2017). The toxicity of HMs and radionuclides is yet unknown, and their soil penetration poses potential environmental and human health problems. Absorption, skin contact, soil-diet, inhalation, and oral intakes are among ways that hazardous chemicals are absorbed. Metals can induce cell damage and inhibition by

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acting as oxidising and reducing agents in environmental processes (Jaishankar et al. 2014). Higher amounts of HMs such as Pb, Se, Hg, As, and Cr can accumulate and create hazardous consequences.

Aside from HMs (As, Cr, Cu, Hg, Cd, and Zn) and radionuclides (238U, 232Th, and 40 K), the manufacturing units have also released a variety of harmful substances into the environment, including carbon monoxide (CO) and chlorofluorocarbons (CFCs), which have harmful effects and contaminate natural resources (Ahuti 2015). Toxic industrial wastes such as Cu, Zn, Cr, and Cd pose a risk of poisoning groundwater and soil. Industrialisation, modern agriculture, ever-increasing human activity, and unlawful dumping of industrial effluent have all damaged soil quality and harmed all living things. Many of these pollutants are known or suspected carcinogens and mutagens, and they may have an impact on ecosystem health. Arsenic (As), for example, is the most abundant metalloid on the planet (Jun Noda et al. 2015).

Arsenic may be present in both inorganic and organic forms in soil and groundwater. Pentavalent arsenate [As(V)] ions, such as $AsO_4{}^{3-}$, H_2AsO_4, H_3AsO_4, and $HAsO_4{}^{2-}$, are the most deadly inorganic arsenic compounds found in natural water, and they are more hazardous than trivalent arsenite ions, such as $H_2AsO_4{}^{3-}$, $HAsO_3{}^{2-}$, and H_3AsO_3. Nausea, stomach discomfort, bloating, and diarrhoea are some of the negative effects of As on humans. When arsenic is present in the environment, it comes into touch with the organism and penetrates the cell's tissues, causing injury.

As, Cd, Cr, Hg, Pb, Co, Cu, Ni, Zn, and Se have polluted more than 20 million hectares of soil throughout the world, with current soil concentrations above regulatory standards. Radionuclides (isotopes of uranium, cesium, chromium, strontium, tritium, plutonium, and radium) created during nuclear fission, explosion, and research operations damaged the biosphere, disrupted the food chain, and posed a health risk to humans. In one of the populous locations of northern Haryana, India, radioactivity of 226Ra, 232Th, and 40 K was measured, and average levels of radon and thoron exhalation were found to be 16.6 0.7 mBq kg^1 h^1 and 132.1 2.6 mBq m^2 s^1, respectively (Devi and Chauhan 2020).

Radionuclide concentrations in soil have been difficult to measure, thus some tree species, such as *Acacia auriculiformis*, have recently been employed to assess such levels. Uranium and other rare earth elements have been investigated in North Vietnamese mining locations. The radionuclide transfer factor from soil to *A. auriculiformis* has been examined. In both soil and *A. auriculiformis*, the quantities of 226Ra, 238U, 137Cs, 228Ra, and 40 K exhibited substantial fluctuation. This plant species has collected radionuclides in the soil and is hence suited for radionuclide biomonitoring (Duong et al. 2021).

The immediate health consequences from contact to radionuclides or irradiation causes headaches, confusion, and vomiting. With more encounter, an individual may also have low blood pressure, fatigue, fever, baldness, dizziness, disorientation, dysentery, and stools containing blood. The biological impacts of radiation can cause smaller heads or brains, poorly formed eyes, irregular growth, and mental retardation in foetuses, who are particularly prone to these consequences (Bogutskaya et al. 2011). According to studies, long-term exposure to radionuclides increases the risk of leukaemia, leucopenia, renal damage, and genetic damage, all of which can have fatal health consequences and be passed down to future generations (Mohner et al. 2006).

Surface capping, encapsulation, landfilling, soil flushing, soil washing, electrokinetic remediation, stabilisation, solidification, vitrification, phytoremediation, and bioremediation are some of the in situ and ex situ remediation techniques that have been developed to clean up contaminated sites with HMs and radionuclides (Cameselle and Gouveia 2019). In comparison to the rate at which metal(loids) are released into the environment, advancement in remediation tactics and technology, notably in the removal of metal(loids), has been slower (Sun et al. 2020). However, several commercial radionuclide remediation procedures have been shown to be ineffectual. When an electrical current is used to remove contaminants from the treated media, electrokinetic remediation, on the other hand, retains high removal efficiency (Purkis et al. 2021).

For the elimination of HMs and radionuclides, bioremediation is a viable approach. The removal of HMs and radionuclides via earthworm or microbe ingestion has been investigated in several documents. Toxic metals are absorbed by plant and microbial cell walls, however bacteria are known to be effective adsorbents for HMs ions. For the separation of these harmful chemicals from wastewater and soil, the adsorption approach is now frequently used. Recent research has looked into the use of several organisms (consortia) to remove HMs from soil. However, it is a natural process that should allow for the quick, effective, and targeted introduction of specialised therapies for accumulated pollutants in contaminated areas. PGPRs are employed as plant growth promoters in contaminated sites, which has improved phytoremediation technology (Ma et al. 2016).

Due to its good beneficial impacts, such as high quality, low cost, and ecologically sustainable services, enhanced phytoremediation technology is currently of great interest, notably for lowered concentrations of HMs and radionuclides in soil. Microbes involved in bioremediation cannot decompose the metal, although it can be changed from one oxidised state to another. As a result, microbial remediation might be thought of as a possible and future technique for converting HMs and radionuclides to a less dangerous form. This can be accomplished by applying the proper microbial consortia to the site. Phytoremediation has several advantages over traditional physical and chemical remediation technologies, including inexpensive investment, ease of operation, and in situ remediation, as well as being an environmentally beneficial method (Wang et al. 2020).

The development of technology for radionuclide bioremediation for the treatment of radioactive waste has been ongoing. To make these radionuclides less dangerous, they can be precipitated, concentrated, or immobilised. Many hazardous and toxic pollutants, including as HMs and radionuclides, have been identified as possible emergent bioremediation approaches. For a sustainable future, it is critical to understand how radionuclide mobility is controlled and how it enhances the elimination of harmful metals and the radionuclide detoxifying process.

2 Causes and Effects of HMs and Radionuclides in the Environment

HMs have been recorded in both industrialised and developing countries, and they have a long-term influence on human health. These HMs can be found in soil, water, and the air, posing serious environmental risks. The sources of such toxins in the global ecosystem are either anthropogenic or geogenic, resulting in animal and human interference as well as pollution of marine and terrestrial food chains. As a result, HMs are released into the environment by a variety of businesses, toxins, and volcanic eruptions, and they commonly return to the earth, polluting soil and water.

When heavy metals (such as Zn, Cu, and Cd) are present in the soil and water in both dissolved and suspended forms, environmental concerns might occur. Since their bioaccumulation in the food chain, all of these HMs have been hazardous to people, livestock, and plant species (Wang et al. 2021). HMs are very toxic to marine species at low concentrations, inducing alterations in the tissues of marine creatures including fish. Cytoplasmic enzymes are inhibited as a result of HMs exposure, and oxidative stress disrupts cell structure.

There are two types of HMs: essential and non-essential. Co, Cr, Cu, Mn, Fe, and Zn are necessary HMs, while Pb, Cd, and Hg are non-essential HMs. HMs were classed as extremely toxic, moderately toxic, or relatively toxic based on their toxicity levels. Cd has a better bioavailability and solubility than other non-essential HMs. Cd is an extremely poisonous heavy metal that, even at very low concentrations, can accumulate in the human body and disrupt essential metabolic processes.

Crop plants acquire HMs from contaminated soil and water, which then enter the food chain, posing a serious health danger to humans. Cd, for example, enters a human being through contaminated food and tobacco smoke. Cd is first attached to metallothionein when it enters the human body. Metallothioneins (MTs) are a huge class of proteins that contain a lot of heavy metals. The liver and kidneys store more than half of the overall quantity of metal in the human body. Another HM that is commonly utilised in a variety of applications is Cu.

Copper can be found in water tubes, pulp and paper, batteries, electronic equipment, mobile devices, fungicides, catalysts, and pesticides, among other things (Gossuin and Vuong 2018). Food, residues, and water can all be used to absorb it. Cu, on the other hand, is required for metabolic functions within the organism's body. Anaemia, bone and cardiovascular difficulties, impaired mental and sensory systems, defective hair keratinisation, lower levels of synapses, dopamine and norepinephrine, and inadequate myelination in the brain stem and spinal cord are all symptoms of copper deficiency. Apart from that, Zn has been employed in a variety of industrial processes. Zinc oxide (ZnO), for example, has been widely employed in biosensor and semiconductor materials due to its numerous benefits (Jaafar et al. 2011).

The harmful effects of radionuclides on the human body are a major source of concern. With increasing doses of radiation, clinical symptoms of acute disease include a decrease in lymphocytes, erythema of the skin, haematological damage, gastrointestinal damage, and central nervous system damage. Radiation-induced DNA damage (or another biological component) is necessary for cell viability. DNA replication, cellular multiplication, and survivability are all affected by radiation.

3 Remediation Techniques for Heavy Metals and Radionuclides Polluted Soil

Environmental management is based on the notion of a well-managed and clean environment. The two basic types of decontamination are in situ and ex situ decontamination. The ex situ method uses physicochemical techniques to remediate the soil, whereas the in situ method relies on plants and bacteria as important participants in the remediation process and is both cost-effective and environmentally beneficial. In situ approaches, on the other hand, are dependent on the bioavailability of HMs in soil. The two essential ideas underpin many remediation strategies. The first is to completely remove the contaminants, and the second is to use engineering technologies to convert the contaminants into less hazardous forms. The process of soil remediation is limited by physicochemical procedures, which consume a lot of energy and have a negative impact on the soil (Christopher et al. 2016).

The replacement of polluted soil with non-polluted soil is part of the physicochemical remediation of HMs contaminated soil. Thermal desorption is also a part of it. The heating of soil to volatile contaminants is known as thermal desorption. Low temperature and high temperature desorption are the two types of desorption. Immobilisation procedures and soil washing are used in chemical remediation technology. Solidification and stabilisation, in addition to physicochemical procedures, are clean-up strategies that slow the flow of toxins into the soil. The HMs are attached to inorganic binding agents including clay, zeolite, and Fe/Mn oxide, as well as organic binding agents like compost and bitumen, during solidification (Farrell et al. 2010).

Another step in the clean-up process is vitrification, which involves heating the soil to high temperatures to slow the migration of HMs. The vitrification process is divided into three categories: thermal, electrical, and plasma. The current was conducted through the soil using electrodes that were inserted into the soil in the electric approach (Kołaciński et al. 2017). Vitrification is the process of heating contaminated soil to a high temperature, which causes organic compounds and certain HMs to volatilise and be transformed into inert gas-like products.

All of these procedures are time-consuming and expensive, as well as being harmful to the environment. Biological remediation strategies for HMs and radionuclides, such as phytostabilisation, phytostimulation, phytotransformation, phytofiltration, and phytoextraction, are more cost-effective and environmentally beneficial (Martínez-Pascual et al., 2015). Plant use in the soil has a number of advantages, including adding nutrients to the soil and improving the microbial balance in the rhizosphere. Plants also aid in the removal of HMs and radionuclides from the soil, as well as restoring soil equilibrium. For example, due to its propensity to absorb and accumulate a high concentration of Cd, vetiver grass (Vetiveria zizanioides) has been claimed to be useful for the clean-up of HMs contaminated soil (Sarma et al. 2012).

One of the key mechanisms for detoxifying HMs is the bacterial community responsible for the transformation of HMs into soil via synthesis enzymes. Microorganisms are frequently affected by HMs and radionuclides, which alter major biological functions and reduce biotic behaviour and population growth. However, numerous arbuscular mycorrhizal has been proved to be able to deal with widespread HMs contaminations, according to current scientific publications in the literature. The conversion of HMs into less harmful or non-toxic forms is mediated by microbial redox mechanisms. The involvement of outer membrane c-type cytochromes (OM c-Cyts) and trans outer membrane porin–cytochrome proteins in bacterias ability to decrease HMs is well-documented (Ravinder Kumar et al. 2016).

These traits are found in bacteria belonging to the genera *Shewanella and Geobacter*, for example. HMs is also sensitive to oxidation by some enzymes. Other enzymes are involved in the cellular elimination of copper, in addition to multicopper oxidases like CueO, CuiD, and CopR. Nonetheless, Cr^{+6} is reduced to Cr^{+3} by chromate reductase (ChroA). The MerA protein aids in the reduction of Hg's toxicity to Hg^0. Under harmful situations, these proteins are up-regulated. Bacteria can also assist plants in absorbing HMs and aid in the detoxification process. *Geobacillus* was shown to convert harmful As^{3+} to less toxic As^{5+} by Majumder et al. (2013), who also discovered that other bacteria from polluted soil have the potential to convert toxic As^{3+} to less toxic As^{5+}.

4 Microorganisms: A Valuable Asset in the Removal of Radionuclides

Via biologically encoded alterations in the oxidised form, radionuclide-contaminated ecological habitats like soil, sediments, and water can be bioremediated. Radionuclide absorption, transport properties, and lethality can all be impacted by changes in speciation, such as mercury detoxification by methylation [Hg(CH3)2] (Wang et al. 2020). The active metabolising capacity of microorganisms is a key component of bioremedial methods for radionuclides. Radionuclides can be solubilised by a variety of methods, including direct and indirect enzymatic reduction through oxidation–reduction, change in pH and Eh (activity of electrons), biosorption by mass, biodegradation of radionuclide-organic complexes, or biosorption by biomass. Microbial activity during radionuclide biotransformation is influenced by electron sources and acceptors, diet, and environmental factors.

The oxidised forms of radionuclides are very soluble in water, making them mobile in groundwater, but the reduced species are highly insoluble and frequently precipitate out of solution. Enzymatic reduction of U(VI) on the surface of the bacterium *Shewanella putrefaciens* was reported by Maozhong Min et al. (2005). In the periplasm of *S. putrefaciens*, a c-type cytochrome with a mass of 9.6 kDa was discovered, which is essential for U(VI) reduction. The mutant was found to be unable to produce c-type cytochrome, resulting in an in vitro decrease of U(VI). In vitro, the isolated tetrahaem cytochrome c3 protein from *Desulfovibrio vulgaris* reduced U(VI) using U(VI) reductase and hydrogenase as its physiological electron donor. In vivo experiments revealed that a *Desulfovibrio desulfuricans* homologue (G20) validated cytochrome c3's participation in hydrogen-dependent U(VI) reduction. Lloyd and colleagues (2003) discovered a similar cytochrome (PpcA), a trihaeme periplasmic cytochrome c7 of the Fe(III)-reducing bacterium *Geobacter sulfurreducens*, that may be involved in U(VI) reduction in vitro.

Indirect reduction of soluble pollutants in sedimentary and subterranean environments by metal-reducing or sulphate-reducing microorganisms can activate enzymatic bioreduction of radionuclides. One strategy is to combine the oxidation of organic molecules or hydrogen with the reduction of iron Fe(III) or sulphur S(IV) as sulphate. Bioreduction of Fe(III) into Fe(II) and S(VI) into S(II) is possible (hydrogen sulphide, H_2S). Chemically, the result can be reduced further to produce individual or multi-component insoluble species. These metals' reduced forms are insoluble, and they can form reduced oxide or hydroxide minerals. Sulphate-reducing bacteria can also be induced to create hydrogen sulphide. Several bacteria, such as *Microbacterium flavescens*, developed unidentified substances such as organic acids, siderophores, and extracellular metabolites capable of dissolving and mobilising radionuclides into the soil when cultured in the presence of other radionuclides (i.e. U, Th, Am, and Pu). These chemicals could also aid radionuclide transport into cells (Seth G. John et al. 2001).

Because siderophore-producing microorganisms are so common, Pu(IV), Th(IV), U(VI), and Fe(III) exhibit molecular and biological similarities. The iron-sequestering compounds generated by these bacteria are essential for increasing radionuclide solubility and bioavailability. Pseudomonas aeruginosa produces extracellular chelating compounds that can bioaccumulate uranium, according to Seth G. John et al. 2001. When thorium and uranium were developed using these metals, this study inspired the development of various chelating agents. Seth G. John et al. 2001 used the siderophores enterobactin, desferrioxamin, carboxylate amino polycarboxylate, and catecholate ligands to solubilise hydrous PuO2(s). Among the other chelators studied, enterobactin siderophores were shown to be particularly effective in solubilising actinide oxides of plutonium.

4.1 Biosorption and Bioaccumulation

Through slime and capsule formation, positively charged metal ions are sequestered to negatively charged cell membranes and polysaccharides produced on the outer surfaces of bacteria. Metal sorption to intact cells is controlled by a variety of mechanisms and interactions that are still unknown. The role of carboxyls in metal cation binding to lipopolysaccharide (LPS) O-side-chains was investigated, and it was discovered that metal cations were most likely to bind to phosphoryl groups in the core-lipid ‘A’ of LPS, and that the negatively charged side-chains affected cell hydrophobicity in Gram-negative bacteria. Anastopoulos and Kyzas (2015) investigated the effective adsorption of radioactive U(VI) by the brown marine alga *Cystoseira indica* and found that pre-treating the alga with calcium improved the adsorption effectiveness of numerous radionuclides. Several bacteria have been identified as radionuclide biosorbents, including *Citrobacter freudii* and *Firmicutes* (Chojnacka 2010).

Metal sorption is important in microbe-metal interactions. *Citrobacter* initiates the precipitation of uranyl phosphate by interacting (electrostatically) with phosphate groups in the LPS. This connection protects an outer membrane phosphatase while also providing nucleation sites for mineral production (Chojnacka 2010). Panak and colleagues (2002) investigated the sorption of Pu(VI) by bacterial resting cells and determined that endogenous respiration induced changes in the oxidation state of Pu(V). Pu(VI) was largely linked to the phosphate groups on the cell surface, according to an extended X-ray absorption fine structure (EXAFS) analysis of Pu associated with the cells; biosorption effectiveness appeared to be positively related to temperature and may occur within hours. This procedure is much quicker than the previous one. However, due to the greater toxicity of radionuclides, biomass concentration in radionuclide-contaminated soil is often low. As a result, biosorption alone might not be enough to bioremediate radionuclides unless the amount of ground biomass is increased. Elegant technologies such as recombinant DNA technology and encouraged proliferation of microbes in polluted soil could boost radionuclide clean-up efficiency.

4.2 *Biostimulation*

Biostimulation is the addition of nutrients (carbon and other nutrient sources) to raise the number or activity of indigenous microflora that can be used in bioremediation (North et al. 2004). The most common radioactive contamination at nuclear complexes is uranium U(VI). Microbiologically, U(VI) can be immobilised from groundwater by converting it from UO_2^{2-} to insoluble U(IV) oxide. Biostimulation of U(VI) immobilisation is one of the potential ways for in situ U(VI) remediation. The in situ immobilisation of uranium via microbial conversion of soluble U(VI) to insoluble U(IV) can inhibit uranium mobility in groundwater (Vrionis et al. 2005). The addition of acetate to a uranium-contaminated aquifer successfully stimulates the growth of dissimilatory metal-reducing bacteria of the Geobacteraceae family, which speeds up the in situ uranium remediation process (Ortiz-Bernad et al. 2004).

The addition of microorganisms that can alter or destroy pollutants is known as bioaugmentation. The microorganisms that are added can be new species or mixed microbial communities that already present on the contaminated locations (Leung 2004). The small obligate anaerobe *Dehalococcoides ethenogenes*, which can reductively dechlorinate tetrachloroethylene to ethylene, has been effectively injected into the subsurface for an extended length of time (Holmes et al. 2006). Similarly, ongoing addition of microorganisms to a reactor site in the ground or ex situ treatment of contaminated groundwater can improve radioactive bioremediation. Organisms cultivated in the lab or in on-site bioreactors can be employed in ex situ therapies or injected back into the subsurface for in situ treatment.

Wu et al. (2006) discovered a unique approach to bioremediate sites contaminated with high uranium concentrations (>1000 m) at low pH and high nitric acid concentrations. The method involved employing a hydraulic recirculation system with an outer and inner loop to raise pH with the addition of carbon (ethanol) to support the growth of denitrifying and radionuclide-reducing microorganisms. An ex situ device was also created to avoid nitrogen gas, aluminium, calcium precipitation, and biomass from blocking the hydraulic recirculation system due to denitrification.

The concentrations of U(VI) and nitrate had been lowered to 5 m and 0.5 m, respectively, after one year under optimum circumstances. The level of U(VI) was lowered to 0.126 m after two years of preconditioning, well below the EPA's recommended range. Altering geochemical and hydrological conditions can efficiently biostimulate radionuclide-reducing microorganisms in situ, according to these findings. High U(VI) concentrations can be remedied by ethanol-biostimulated biofilm, which can effectively reduce U(VI) by 87%.

4.3 Biomineralisation

Metal ions can interact with microorganisms and immobilise them, preventing them from undergoing transformation. Some microbes produce biofilms in order to bind large amounts of metallic ions, which can then be used to precipitate insoluble minerals. Citrobacter sp. has been found to be capable of enzymatically producing metal phosphate deposits. By sorption to LPS and the action of an outer membrane acid-phosphatase, polycrystalline $NaUO_2PO_4$ accumulates in and around the cell wall of Citrobacter. Two gradients in the outer membranes drive mineral formation: an entering UO_4 and an outgoing PO4, resulting in the elimination of all U from the solution and the binding of 1 mg $NaUO_2PO_4$ per mg of the cell.

Chelating agents are found in wastes because they are commonly utilised in nuclear reactor and equipment decontamination, clean-up activities, and radionuclide separation. Several organic compounds have been employed to complex with radionuclides, including citric acid, hydroxyl-acetic acid, oxalic acid, tartaric acid, EDTA, diethylenetriamine pentaacetic acid (DTPA), nitrilotriacetic acid (NTA), and N-hydroxyethylenediamine triacetic acid (HEDTA). These metal chelates degrade aerobically or anaerobically, causing released ions to precipitate as water-insoluble hydroxides or oxides, slowing their migration into groundwater. Citrate has been proven to be effective as a chelating agent in decontamination because it creates highly soluble metal–citrate complexes that are easily destroyed by bacteria, resulting in metal re-precipitation. Bidentate, tridentate, and polynuclear complexes are examples of stable complexes.

Foulkes et al. (2016) present a unique process for reductive palladium biomineralisation employing formate as an electron source in aerobically growing Escherichia coli. This technology allows for the biorecovery of this precious element as well as the production of bio-Pd catalysts. Ferris et al. examined the oxidation of Fe(II) at circumneutral pH by natural Fe(II)-oxidising bacterial consortia in flocculent mats of bacteriogenic iron oxides (BIOS). When compared to chemical oxidation in solution, Fe(II) oxidation was faster in the presence of BIOS, indicating a complex interplay of Fe(II) oxidation reactions, including chemical oxidation in solution, chemical oxidation on hydrous ferric oxide surfaces, and bacterially mediated oxidation.

4.3.1 Biotransformation

In order to catalytically eliminate radionuclides like chromium, technetium, and uranium, microorganisms have been utilised (Cr). While reduced forms of U, Tc, and Cr are insoluble and usually precipitate into solution, reduced forms are dissolved in water and transportable in groundwater. Microbial isolates such Desulfovibrio desulfuricans, Geothrix fermentans, Deltaproteobacteria, and Clostridium have accumulated metals and radionuclides in extracellular environment (Brodie et al. 2006).

Uranyl carbonate that is soluble is transformed by metal-reducing organisms into the mineral uranite, which then precipitates. There has been tremendous advancement in the mechanisms of Fe(III), U(VI), and Tc(VII) reduction by the bacterium Geobacter sulfurreducens. Numerous types of bacteria convert the highly soluble chromate ion to Cr(III), which precipitates as Cr(OH)3. The Cr(VI)-reducing bacteria that have been isolated from chromate-contaminated waters, oils, and sediments include Arthrobacter aurescens, Pseudomonas aeruginosa, Pantoea agglomerans, and Desulfovibrio vulgaris (Horton et al. 2006).

4.3.2 Genetically Modified Microorganisms

For successful in situ bioremediation, a mix of ecological and microbiological knowledge, biochemical mechanisms, and field engineering design is required. Aside from ethical concerns, there are a number of other obstacles to successful recombinant strain design. Two key obstacles include competition for nutrients and other resources between the designed microorganism and other natural residents, as well as selection pressure provided by biotic and abiotic variables (Singh et al. 2011). To achieve desired results, it is necessary to select the appropriate bacterial strain in terms of growth potential and nutritional sensitivity.

It has been demonstrated that the decontamination system of genetically engineered bacteria is advantageous for metal bioleaching. In response to high Hg toxicity, the bacterial world has developed an astonishing variety of resistant strains.

Through a collection of Hg resistance genes (mer) in an operon, Hg reductase incites bacteria to convert Hg2 + into volatile Hg. It has been discovered that the limited Hg resistance mer operon breaks down mercury in three stages: mobility of Hg2 + within the cell, enzymatic NADPH-dependent transformation of ionic mercury to less hazardous inorganic mercury (Hg0), and finally regulation of the genes involved for mobility and conversion (Singh et al. 2011).

Hg^{2+} may be efficiently removed from electrolytic wastewater using genetically engineered *E. coli* carrying the merT-merP and MT genes at the same time. The most radiation-resistant bacteria known, *Deinococcus radiodurans*, was modified by expressing the merA gene from *E. coli* BL308. In the presence of radiations and high concentrations of ionic mercury, the recombinant was observed to proliferate. It was successful in converting Hg ions to less hazardous volatile elemental mercury. Deng et al. (2007) created genetically altered *E. coli* JM109 for bioaccumulation of Cd^{2+} from a heavy metals-polluted environment. Similarly, different genetically modified bacteria such as *Mesorhizobium huakuii, Pseudomonas putida*, and *Caulobacter crescentus* have been shown to accumulate Cd^{2+} by expressing fusion proteins for phytochelatin (PC), metalbinding peptide (EC20), and RsaA-6His, respectively (Patel et al. 2010).

5 Phytoremediation

Phytoremediation is one of the bioremediation processes that can be utilised as a heavy metal remediation alternative. Metal phytoremediation is a cost-effective, efficient, environmentally and eco-friendly ‘green’ method that employs metal-accumulating plants to remove hazardous metals, including radionuclides, as well as organic contaminants, from contaminated soils and water (Ali et al. 2013).

It’s expected that phytoremediation of mixed pollutants will be more challenging than phytoremediation of single-contaminant soils. The properties of the contaminants (such as organic compounds and HMs), the types of soil, and the nature of microorganisms in the rhizosphere all influence the choice of reliable procedures for mixed contaminants. In previous research, phytoremediation of cocontaminated soils with organic contaminants and mixed metals was described (Chirakkara and Reddy 2015). Some of the methodologies were developed at a regional level to assess the potential of phytoremediation at a site contaminated by heavy metals (such as Zn, Cu, and Pb) and organic hydrocarbons. Pine (*Pinus sylvestris*), red fescue (*Festuca rubra*), ryegrass (*Lolium perenne*), poplar (*Populus deltoides Wettsteinii*), white clover (*Trifolium repens*), and smooth meadow grass (*Poa pratensis*) were all investigated for phytoremediation of mixed pollutants.

The efficacy of HMs phytoremediation is often reduced due to chemical and physical connections between contaminants and their impact on bioavailability and flexibility (Chen et al. 2009). The coexistence of all types of contaminants has been found to affect phytotoxicity throughout the phytoremediation process, and as a result, remediation performance and plant development have been observed to be compromised. One of the main elements of efficient phytoremediation is the utilisation of numerous microorganisms in the mixed polluted soil. Plants have been proven to accumulate HMs, while other species (microorganisms) have been demonstrated to enhance the breakdown of organic pollutants in several trials.

Plant and microbe consortia, in which hyperaccumulator plants and microorganisms work together to eliminate HMs contaminants, are being employed as a new way to increase phytoremediation. It is a cost-efficient, environmentally friendly, and effective method of recovering damaged habitats, especially those that contain HMs. In the current circumstances, metal clean-up with bioenergy crops appears to be a successful strategy, as such crops are significant for economic purposes (Wang et al. 2015).

5.1 Phytostabilisation

Phytostabilisation is a method of reclaiming HMs from soil, allowing us to live a healthy lifestyle. Soil can be stabilised by lowering dust emission, and pollutants' bioavailability or solubility in the food chain can be decreased to prevent metals from blowing away. Phytostabilisation can be achieved by controlling metalloid contamination in some bioenergy plants, such as *Arundo donax* (Giant reed) and *Miscanthus sinensis* (Silver grass) (Sarma et al. 2017).

5.2 Phytovolatilisation

Plants that are exposed to HMs may convert them to a volatile state and release the resultant products into the environment. This approach was utilised to remove certain harmful HMs from polluted soils, such as Se and Hg (Sarma and Prasad 2016). Although this technology has been successful in the clean-up of toxic wastes, it has some limits because the metal is believed to be moved from one substrate (soil or water) to another (atmosphere).

5.3 Phytoextraction

Phytoextraction is a plant root process for absorbing contaminants from water or soil and aggregating them into biomass (shoots). *Brassica juncea*, which has a much higher overground biomass output, is suited for metal phytoextraction. In terms of phytoextraction by conveying, absorbing, and storing HMs in plant tissues, members of the poaceae family have a well-developed root system, making them the ideal model plant, according to a recent study (Ma et al. 2016).

5.4 Hyperaccumulator Plants

Because HMs can be removed rapidly, cheaply, and efficiently, the removal of HMs by hyperaccumulators has gotten a lot of interest as a way to clean up pollution. Metal hyperaccumulation is a trait seen in 664 plant species, accounting for 0.2 per cent of all angiosperms (Sarma et al. 2021). Uptake of Cd by *Solanum nigrum L.* and *Arabidopsis halleri*, uptake of Zn, Cd, and Pb by *Zeamays,* and uptake of Se by *Astragalus bisulcatus* and *Brassica juncea* are only a few of the findings on the usage of metal hyperaccumulators. Identification of hyperaccumulators is a crucial phase in the phytoremediation process that has yet to be completed. Identification of HMs-accumulating plants with advanced phytoremediation potential is the focus of much research.

6 Remediation of HMs by Live/decayed Microbes

The removal of HMs by live/dead microorganisms at a low cost has a number of advantages, including being trustworthy, useful, and having a high adsorption potential. Bacteria, fungi, and algae are typically found in or used with bacteria, fungi, and algae. Some HMs, on the other hand, may be harmful to some microorganisms.

6.1 Algae

HMs can be effectively removed from contaminated waters by algae. More HMs include dead algal cells than live ones. Several pretreatments improve algae's metal sorption ability. The most cost-effective technique of activating algal biomass is to use $CaCl_2$ (Bilal et al. 2018). According to the Freundlich and Langmuir adsorption models, *Fucus vesiculosus* is a highly efficient Pb adsorbent (II). Cu (II) can be removed from water by *Sargassum* (seaweed) (Barquilha et al., 2017). *Saccharina fusiforme* and *Saccharina japonica* were shown to have increased elimination capability for Zn (II), Cu (II), and Cd in aqueous solution (II). Microalgae have evolved a variety of extracellular and intracellular methods to cope with HMs toxicity, indicating the possibility for phytoremediation.

6.2 Bacteria

Bacteria are the most ubiquitous bacteria on the earth that can be utilised to reduce and recover HMs ions through bioreduction and biorecovery. The HMs ions are frequently adsorbed by functional groups on the bacterial surface, such as sulphate, amino, and carboxyl groups. HMs ions can connect these functional groups, allowing them to aggregate with significant adsorption capabilities. The capacity of bacteria to absorb heavy metal ions ranges from 1 mg/g to 500 mg/g. The mercury resistant strain Pseudomonas aeruginosa has a maximal adsorption capacity of about 180 mg/g mercury ions (Yin et al. 2016).

Bacillus sp. PZ1 and *Pseudomonas* sp. I3 are also capable of absorbing Pb (II) from wastewater. Dead and active *Arthrobacter viscosus* cells can reduce Cr (III) and Cr (VI) in an aqueous solution, respectively. Extracellular polymeric substances (EPS), which include proteins, lipids, nucleic acid, and complex polysaccharides, play an important part in HMs ion adsorption. By preventing HMs from entering the inner cellular region, the EPS on the bacterial cell's surface can protect the bacterium against toxicity. The presence of anionic and cationic functional groups in HMs such as Co, Cd, Hg, and Cu could effectively assemble on EPS.

6.3 Fungi

Fungi, such as *Trichoderma aureoviride*, *T. virens, T. harzianum, Penicillium* sp., and *Aspergillus niger*, may be the best bioaccumulators for removing Cd and Cr from contaminated soil (Martins et al. 2016). Fungi may remove heavy metals from the environment through a variety of ways (intracellular and extracellular sequestration), including integrating them into their mycelium and spores. *A. niger* was recognised as a great biosorbent for the removal of Pb when it came to biosorption (II). The ability of an indigenous fungal isolate *A. fumigatus* to bioremove Cr (VI) from field wastewater has been investigated. With the help of functional groups like imidazole, sulphydryl, phosphate, hydroxyl, and carboxyl, Cr (VI) can be absorbed by the acidic layer of *Termitomyces clypeatus* biomass. Cu (II) was extracted from *Saccharomyces cerevisiae* cell biomass using wastewater. *S. cerevisiae* can remove Zn, Cd, and Cu because it has an adsorption ability that can be enhanced with sodium chloride in high sodium circumstances.

For the restoration, recovery, revegetation, and management of severely impacted areas owing to anthropogenic activities, such as HMs and radionuclide contaminated sites, a complete approach (holistic approach) is required. Soil remediation techniques, including certain green measures like phytoremediation, can be efficiently implemented, and a suitable land cleaning process can be established. The effects of organic supplements, such as agricultural waste and sewage sludge, biochar, humic compounds, plant extracts and exudates, on phytoremediation have been found, and their effects on soil properties have been examined (Sarma et al. 2019).

7 '-Omics'-Implemented Radionuclide Bioremediation

An organism's genome contains all of its genetic information, which is translated to mRNA (transcriptome) for protein translation. The proteome of an organism is the full set of proteins that are expressed in the organism under specified environmental conditions, including enzymes. The structural and functional connections between proteins and other metabolites must be studied in order to find genes, proteins, and enzymes involved in radionuclide bioremediation. Advanced genomics and proteomics approaches can be used to identify and study potential genes and proteins involved in radionuclide metabolism (Singh et al. 2011). Recent advancements in next-generation sequencing, genomics, and proteomics have made it possible to express necessary proteins and enzymes in radionuclide-resistant organisms for bioremediation. Furthermore, genome-wide transcriptome analysis can help us gain a better knowledge of microorganism metabolism and physiology.

Many microorganisms' genome sequences are now accessible and can be utilised for genome organisation and comparisons using microarrays. Several genome-wide analyses of genes and proteins implicated in radionuclide reduction pathways have been carried out. In *Shewanella oneidensis*, DNA microarray study revealed 121 genes were upregulated during U(VI) reduction, compared to just 83 genes over-expressed with Cr(VI) reduction (Bencheikh-Latmani et al. 2005). *Thermococcus gammatolerans* was shown to be radioresistant among Archaea in a comparative genomics research, expressing thioredoxin reductase (tgo180), a glutaredoxin-like protein (tg1302), and two peroxiredoxins (tg1253 and tg1220), which allowed the organism to cope with radionuclide stress. When *Rhodopseudomonas palustris* CGA009 and *Novosphingobium aromaticivorans* F-199 were cultivated in the presence of radioactive cobalt, the expression of the NiCoT gene was found to be significantly elevated.

Omics technology is a molecular biological technique that allows for simultaneous study of biomolecules such as DNA, RNA, proteins, and metabolites from individual organisms as well as the entire community (Gutierrez et al. 2018). mRNA expression (transcriptomics) and whole community expression (metatranscriptomics) can be explored to study gene regulation in the anthropogenic environment (Roume et al. 2015). By examining the proteins utilising metaproteomics, anthropogenically produced proteins from contaminated areas may be monitored. Metagenomics studies microbial communities directly from the ecological environment, reducing the time and effort required for microbe screening and culture (Ghazanfar et al. 2010).

Metaproteomics is the study of proteins that allows anthropogenically produced proteins to be tracked from contaminated sites. Metabolomics is the study of main and secondary proteinaceous metabolites produced by organisms under various environmental conditions. Multi-omics techniques are frequently used to examine microbial communities because a single omics analysis cannot reveal the microbial community's functional activities (Meena et al. 2018). Understanding the processes involved in radionuclide bioremediation requires an understanding of specific genes and their protein products. Combining '-omics'-based techniques could aid in the identification of specific microorganisms capable of in situ radioactive bioremediation.

8 Nano-Bioremediation

Nano-bioremediation is a type of bioremediation that necessitates the use of specialised technologies. Its goal is to lower pollution levels while also reducing the negative effects on the environment. Nanotechnology is primarily concerned with the synthesis, manipulation, and use, combination, and reuse of materials that are smaller than a micron in size to individual atoms. This method combines nanotechnology and bioremediation in order to obtain higher remediation efficiency in less time while having a lower environmental impact (Prasad and Aranda, 2018).

Nanoparticles are useful in eliminating harmful metals from water and soil because they are small, have a vast surface area, and are extremely reactive. This nanoparticle-aided approach is a polluted site clean-up solution that is quick, inexpensive, and effective. Over the last few years, this approach has gotten a lot of attention. In recent years, there has been a rise in the production and implementation of nanoscale products that are aimed at environmental remediation. One such application is the use of nanomaterials to purify polluted soil and groundwater at hazardous waste sites that have been affected by chlorinated solvents or oil spills. The unique physicochemical, surface, and optical-electronic properties of manufactured nanoparticles enable them to address problems that were previously challenging to solve using conventional techniques. These properties also allow for the development of innovative approaches to create new methodologies, replace existing tools, and manufacture new materials and chemicals that are highly efficient and require minimal energy consumption.

9 Conclusion

Microbial transformations of radionuclides, heavy metals, and minerals are an important aspect of natural biosphere processes that can benefit human communities. As we all know, interactions between microorganisms and radionuclides are complex, and understanding the wide range of habitats in which these organisms live is difficult. The use of '-omics'-based approaches and Nanoremediation approaches to study the molecular mechanisms behind microbial transformation of radionuclides and exploiting them in applications such as bioremediation would aid in tracking the responsible microbial metabolic products towards cell-free bioremediation and further aid in the efficient removal of radionuclides from the environment.

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The Role of Nanotechnology in Bioremediation of Pollutants

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1 Introduction to Bioremediation

In the ancient times or recent past, the most common waste management practice was dumping of wastes into pits or landfills, but no complete degradation was witnessed, and hence to destroy the pollutants entirely became the need of the hour. Several technologies, for instance, base-catalyzed dechlorination, high-temperature incineration, UV oxidation were deployed to reduce the contaminant range but each one of the techniques possessed multiple demerits such as technological complexity, expensive solution for a small-scale application, etc. (Vidali 2001).

Bioremediation aims to destroy the contaminants and pollutants that are threatening to humans and the environment or at least transform them into less toxic forms by utilizing naturally occurring microorganisms (microbial remediation), plants (phytoremediation), and fungi (mycoremediation). These organisms can either be indigenous or non-indigenous. Indigenous organisms are the ones whose natural habitat is the contaminated zone and non-indigenous organisms are deployed into the target site from elsewhere (Okonko and Shittu 2007). This technique has a greater acceptance for it being a natural, cost-effective, and low technology demanding in comparison to the rest. The chief merit of this practice is that it can be directly executed at the contaminated zone.

The major loophole of this technique is that the organisms cannot detoxify a wider range of substrate and hence suitable methodologies are required to optimize the results. In recent times genetically modified organisms are implemented in places where indigenous organisms fail to ensure potent cleansing. This proved to be a

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worthy solution in remediating industrial effluents, municipal wastes, petroleum and oil spills, etc. (Kumar et al. 2018). Bioremediation is competent only under suitable environmental conditions which afford the fastidious growth of the organisms. Under these circumstances, the organisms enzymatically attack the contaminants and transform them into hypoallergenic forms, but to carry out these activities, manipulation of the environment is required to a certain extent.

1.1 Strategies Used in Bioremediation

The degree of saturation and the aeration in the contaminated region play an important role in availing a specific technique. The major classification of bioremediation is represented in Fig. 1. These techniques are of 2 types:

- In-situ technique
- Ex-situ technique

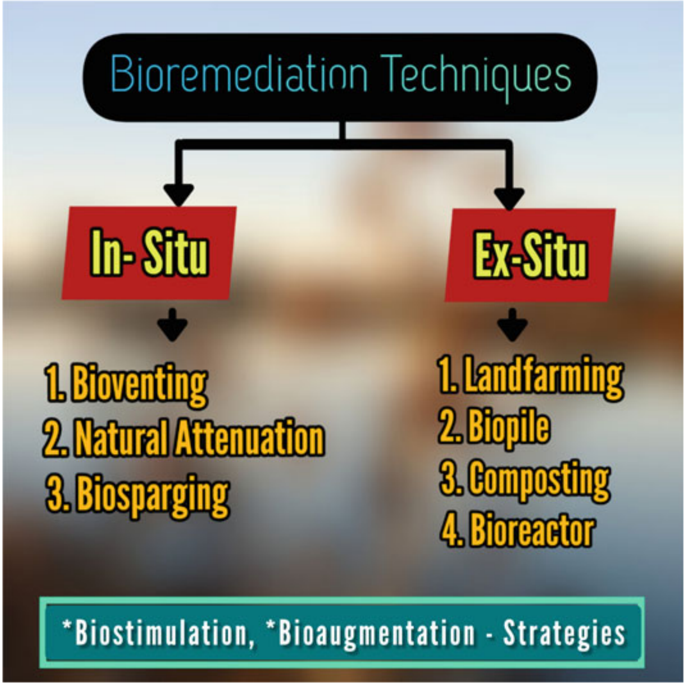

Fig. 1 Categorization of bioremediation

1.1.1 In-situ Techniques

This is adapted in regions where the groundwater and the soil experience the least interference. This technique is most preferred for its pennywise outcome by refraining from excavation and contaminant transportation. This process requires effective oxygen diffusion and is restricted to the depth of the soil, i.e., from 30 to 60 cm for the fruitful cleansing action (Boopathy, 2000).

The principle treatment techniques include.

Bioventing

This method facilitates the growth of the indigenous bacteria by providing nutrients and air via wells into polluted soil. A notable feature of this technique is that air is utilized only for the degradation of the contaminants. It is the best-suited method for cleaning deep under the soil surfaces and to break down hydrocarbons of low molecular weight (Chavez-Crooker and Obreque-Contreras 2010).

Biosparging

In this method, air under low pressure is administered beneath the groundwater table to increase the dissolved oxygen concentration and to escalate the growth of the indigenous bacteria which will aid in the degradation of the pollutants. This technique requires the stationing of a small diameter air injection with versatility in the design, thus making it a cost-effective practice.

Bioaugmentation

This technique employs both indigenous and non-indigenous organisms for decontamination. Although the utilization of exogenous organisms is advantageous, 2 main limiting factors must be taken into consideration.

a. Indigenous organisms possess an upper hand in degrading the contaminants in soils that were subjected to persistent exposure to biodegradable pollutants/waste.
b. In most cases, exogenous cultures don’t compete with the indigenous population to ensure satisfactory population size (Anjaneyulu et al. 2005).

In-situ Biodegradation

The contaminated soil is equipped with oxygen and nutrients through circulating aqueous solution. This amplifies bacterial growth and facilitates the speedy degradation of organic impurities.

1.1.2 Ex-situ Techniques

The major difference between this technique and the former is that it involves pumping of water and evacuation of soil from the polluted region. The cleansing action is performed on the shifted substrate (Paniagua-Michel and Garcia 2003).

The treatment techniques associated with this method are as follows.

Composting

Degradation of contaminated soil is executed by incorporating the soil with organic wastes like manure. This amplifies the microbial presence and elevated temperature speeds up the process, as increased temperature levels favor composting.

Land Farming

This method involves the coating of the excavated superficial soil, i.e., about 10 cm to 35 cm over a constructed bed followed by intermittent tilling until the indigenous aerobic organisms degrade the pollutants. Land farming isn’t a laborious process as it does not require regular monitoring and maintenance hence it is a potential cost-effective technique.

Bioreactors

An engineered containment system such as aqueous reactors or slurry reactors is exploited for treating soil and water. The vessel is designed in a manner to create a soil-liquid–gas combining condition. This results in increased remediation of water-soluble and soil contaminants as a liquid slurry containing contaminants and biomass of indigenous microorganisms. This is a better alternative for in-situ as the treatment can be monitored and controlled.

Biopiles

This technique is a combination of land farming and composting. This method chiefly involves the treatment of surface contamination using petroleum hydrocarbons. This method employs both aerobic and anaerobic organisms.

1.2 Classification of Bioremediation

Based on the type of organisms used, the bioremediation is classified into.

- Microbial bioremediation
- Phytoremediation
- Mycoremediation

1.2.1 Microbial Bioremediation

As per the definition microbial remediation refers to the application of microorganisms (or enzymes) for bioremediation (Gargouri et al. 2011). Microorganisms transform the pollutants into less toxic forms through their inherent metabolic processes. Applied bioremediation largely focuses on bioremediation using the natural catabolic pathways (Quintero et al. 2007).

Use of microbial consortia: The microbial consortium has a synergistic metabolism which proved to be much more effective in bioremediation than that of pure cultures. Three crucial reasons for employing microbial consortia are:

- The metabolic intermediate of one organism might be a food source for another bacteria in the consortia.
- Due to the involvement of various organisms, the process gears up.
- Appropriate trapping methods may be taken on board for an improved remediation process.

1.2.2 Phytoremediation

It is an eco-friendly phytotechnology that utilizes plants and trees for remediating herbicides, pesticides, oil spills, explosives, solvents from the soil, groundwater, streams, etc. (Meagher 2000). This technique is quite smart as the plant utilizes the contaminants and excludes the rest through its roots in unavailable biological forms; this is later degraded into beneficial products by the bacteria present in the soil converting them into useful bioavailable forms for the plants to utilize. However, phytoremediation has several disadvantages that must be taken into consideration like accumulation of metals, low biomass production, contaminants entering the food chain, etc. Phytoremediation involves several strategies such as phytostabilization, phytoextraction, phytotransformation, rhizofiltration, etc., for degrading the toxic contaminants into less toxic forms.

- Phytostabilization: It is a technique where the metal pollutants are transformed into less available forms to prevent the accumulation or migration of the metals into streams or rivers.
- Phytoextraction: The harvestable tissues of the plant accumulate metals and metalloids into it from the contaminated soil.

- Phytovolatilization: The plant exchanges the metal from the soil to the atmosphere in gaseous form while exchanging necessary gases.
- Phytotransformation: In this technique, the contaminant is chemically modified by enzymatic activity inside the plant shoot or root.

1.2.3 Mycoremediation

As the name suggests, mycoremediation employs fungi to degrade the environment. This technique is non-invasive, flexible, cheaper, and eco-friendly (Perelo 2010; Shah 2020). Fungi have a unique derogatory mechanism, i.e., it covers the entire substrate first and readily degrades it. The rate of degradation is directly proportional to the nutrient levels of the substrate. It is identified that fungi are capable of breaking down lignin, fats, sugars, pectin, cellulose, hemicellulose, keratin, etc., and white-rot fungi are capable of digesting lignocellulose. Some notable fungi in mycoremediation include *Irex lacteus, Lentinus tigrinus, Lentinus squarrosulus, Agaricus bisporus,* etc.

Mycoremediation takes place in 3 steps: biodegradation, biosorption, and bioconversion.

- Biodegradation: It is a process by which the substrate is broken down without causing any hazardous effects to the environment.
- Biosorption: It is the sorption of the contaminants from the polluted environment. It may be solid or gas or liquid. It is a very crucial process in retrieving metals from the polluted site. It happens in 2 steps:
  - Bioaccumulation: The pollutants are actively taken up by the cell and by intracellular components.
  - Biosorption: It is the process where the heavy metals and other contaminants are fixed to the biomass.
- Bioconversion: It is the conversion of the pollutants to their by-products.

1.3 Genetic Modification of the Remediating Organisms

Bioremediation, by nature, is a very slow natural process. This is because most chemical components in the pollutants are resistant to microbial degradation as microbes lack the necessary specific catabolic pathway (Menn et al. 2000; Shah 2021a, b). In most cases, strains of an exogenous organism are incorporated into the indigenous organism to speed up the process. With improved Genetic Engineering technologies such as site-directed mutagenesis, antisense RNA technique, PCR, pathway construction, modification of the genetic sequence of genetically modified organisms became an easy task. It is interesting to note that genetic modification creates new combinations of plasmids (organisms) that don't exist in nature. *Pseudomonas aeruginosa* (NRRL B-5472) and *P. putida* (NRRL B-5473) were the first-ever genetically modified strains that could degrade naphthalene, camphor, and salicylate.

1.4 Application of Organisms in Bioremediation

Industrial wastewater is composed of various components, among them the fundamental components include odor, solids, temperature, color, organic and inorganic contents, and heavy metals. It is important to note that, in most cases the darker the color of the effluent, the more hazardous compounds it contains, and the lighter ones (brown, yellow) indicate pre-treatment up to a certain extent. Dark gray or black color water indicates extensive bacterial decomposition under anaerobic conditions which is due to the formation of several sulfide compounds such as ferrous sulfide and pungent-smelling hydrogen sulfide and dimethyl sulfide (Asamudo et al. 2005).

1.4.1 Microbial Remediation

Removal of heavy metals: Anthropogenic activities led to increased levels of heavy metals with potent toxicity in the world. Several physical and chemical methods were proposed but they were unfruitful as they generated harmful by-products, were cost-effective, etc. (Microorganisms prove to be an excellent choice for remediation of heavy metals as they don't produce any harmful by-products.) A consortium of marine bacteria was capable of removing mercury in a bioreactor (Canstein et al. 2002). Secretion of exopolysaccharides by certain marine bacteria like *Enterobacter cloacae* helps in the chelation of heavy metals.

Removal of polyaromatic hydrocarbons: PAH is a great environmental threat as they are mutagenic, carcinogenic, and toxic. Many marine microorganisms can degrade the hydrocarbons by their usual metabolic activity and utilize the obtained carbon for growth. The efficiency of these bacteria can be increased by transferring the catabolic plasmid containing hydrocarbon-degrading genotypes of *Pseudomonas putida* to marine bacteria. Some new marine bacterial species like *Lutibacterium anuleoderans, Cycloclasticus spirillensus,* etc., are exploited to improve the degradation of PAH (Chung and King 2001).

Removal of oil spills: Microorganisms can readily degrade alkanes with C-10 to C-26 by enzymatic degradation. Complex structured hydrocarbons pose difficulty for microbial degradation although few organisms are capable of doing so, there isn't a satisfactory degradation as observed in simpler hydrocarbons.

Degradation of plastics: Plastics are used for a wide range of applications, right from packaging to construction. As much as its significance, it is a potent threat to the environment. Organisms belonging to genera *Moritella, Pseudomonas, Shewanella, and Psychrobacte*r isolated from the deep seas of Japan were capable of degrading E- caprolactam in plastics. Species like *Moraxella, Staphylococcus, Micrococcus* are

Table 1 Microorganisms present in pesticide compounds

Micro-sized organisms	Pesticide compounds
Enterobacter	Chlorpyrifos
Pseudomonas sp., Photobacterium sp.	Parathion methyl
Staphylococcus, Bacillus	Endosulfan
Pseudomonas putida	Ridomil MZ 68 MG
Arthrobacter sp.	Malathion

associated with Mangrove and are found to degrade 20% plastics (Kathiresan 2003). Table 1 depicts some of the microorganisms present in pesticide compounds.

1.4.2 Phytoremediation

Removal of heavy metals: Absorption of heavy metals contaminants by plants from soil is the major task of the recovery process. Where plants release organic materials, moisture, oxygen, and nutrients to the Earth for increased uptakes of pollutants, thereby altering the primary factors like redox and osmotic potentials and pH. This phenomenon facilitates an enriched environment for the activity of microbes and hence increases the tolerance level of plants in approach to the contaminants and pollutants (Sharma et al., 2013).

Algal biomagnification comprises a wide regime of metals like nickel, lead, zinc, tin, copper, silver, cadmium chromium, gold, manganese, and cobalt. Algae belonging to the marine, especially kelps such as *Ecklonia radiata*, *Laminaria japonica,* and *Durvillaea potatorum*, have increased capacity for adsorption for many heavy metal ions (Matheickal and Yu 1996).

The first step of remediation by plants is the pathway of heavy metals involving symplastic and apoplastic routes (Salt and Rauser 1995). The transport of heavy metals is facilitated by several transmembrane transporters involving several intracellular compartments and carries out the uptake operation from roots to aerial parts through transpiration pull in xylem vessels after which the contaminants are subjected to detoxification and sequestration.

1.4.3 Mycoremediation

Mushrooms and other fungal types use enzymes like laccases and post mushroom substrate for degradation.

- Removal of PAHs: Organisms like *Ganoderma lucidum* degrade acenaphthalene, acenaphthylene benzopyrene, anthracene, and fluorene using a laccase mediator system (Punnapayank et al.2009).
- Removal of pesticides: *P. pulmonarius* has high laccase activity which is capable of degrading PCP—a pesticide and wood preservative. *T. versicolar* can remediate

several pesticides like isoproturon, chlorpyrifos, chlorothalonil, etc (Jin et al. 2016).

- Decolorization of dyes: It is a known fact that dyes possess a complex structure which makes it difficult for bacteria to decolorize them but laccases in mushrooms have shown effective results in remediating dyes. Synthetic dyes like malachite green, brilliant green, congo red were successfully degraded by *Lenzites elegans.*
- Removal of heavy metals: Post mushroom substrate (SMS) was responsible for the degradation of heavy metals by biosorption process that contaminated the soils and not the fungal enzymes, e.g., SMS of *L. edodes* (Chen et al. 2005). SMS also plays an important role in remediating acid mine drainage which contains iron sulfate—a water pollutant as its major component.

1.5 Factors Affecting Bioremediation

Like every other phenomenon in the world, bioremediation is also controlled by 2 factors namely:

Biotic or biological factors
Abiotic or environmental factors

1.5.1 Biotic Factors

Biological factors aid in the degradation of organic and inorganic compounds by microbes with antagonistic association among microbes or protozoa and bacteriophage. The degradation rate is directly influenced by the concentration of contaminants and the proportion of the catalyst present for the biochemical reaction. The major biological interactions are (Boopathy 2000; Madhavi and Mohini 2012):

1. Enzyme activity
2. Interaction among organisms may be competition, predation, or succession.
3. Mutation of genes
4. Horizontal gene transfer
5. Population size.

1.5.2 Abiotic Factors

The success of the bioremediation process depends on environmental factors and cannot be scratched off. Factors like temperature, pH, oxygen availability play a key factor in deciding the extent of interaction between the organism and the pollutant. The factors that contribute to the remediation process are.

Availability of Nutrients

Every organism has its nutrient balance and it must be adjusted accordingly to maintain the C: N: P ratio, which boosts the metabolic activity of the organism thus increasing the remediation process. At times small concentrations of hydrocarbons also inhibit the degradation process and hence it is necessary to adjust the nutrients from time to time as nutrients from natural sources are available only in trace quantities. This is one of the major reasons which limits the degradation activities in aquatic organisms (Couto et al. 2014). Temperature: Temperature is a survival determining factor as it plays a crucial role in holding a stable hydrocarbon composition of the bacteria. Microorganisms do not show the same metabolic activity at all temperatures and exhibit maximum activity at their optimum temperature. Bioremediation slows down at low temperatures like in the arctic or sub-zero temperature zones; this is because the transport channels block and the cytoplasm freezes resulting in the inactivity of the organism.

The Concentration of Oxygen

Bioremediation may be carried out in either aerobic or anaerobic conditions depending upon the oxygen requirement of the organism. Therefore, it is necessary to ensure that the right levels of oxygen are supplied to the organism for satisfactory results (Macaulay 2014).

Moisture Content

The soil moisture content influences the growth of the organisms; it also has adverse effects on the bioremediating agents.

pH

pH is the measure of hydrogen ion concentration, as for temperature pH changes also have effects on the metabolic activity of the organism. Each organism has its pH and slight changes in acidic or alkaline nature may affect the metabolic activity of the organism (Asira 2013).

2 Prelude to Nanotechnology

The preflex “nano” refers to one billionth. It holds its origin from a Greek word for small—*nanos*. 10^{-9} of a meter makes one nanometer. It is hard to acknowledge the nanoscale, we can sense, hair of a human is 50,000 nm. A paper sheet measures about

100,000 nm while every second a human fingernail grows a nanometer. Microchips that are commercially used are featured up to 100 nm relating to new bacterial cells (Ratner and Ratner 2002).

2.1 Terms Related to Nano

The field of nano involves different terms with their particular applications. The following nano terms, proposed by the British Standards Institution, are being used, and they are:

- Nano Object: More than one peripheral nano dimensions possessed by a material.
- Nanoscale: Ranges 1–100 nm of size.
- Nanostructure: Region of nanoscale parts composed of interconnected constituents.
- Nanomaterials (NMs): External or internal structures with nano dimensions.
- Nanoscience: The study and science of a nano-sized matter dealing with the understanding of size-dependent properties compared to its atomic or molecular or bulk form.
- Nanostructure materials: Surface or internal nanostructure-containing materials.
- Nanotechnology: Control and manipulation of nanoscale dimensional matter by applying science knowledge in various industries.
- Nanoparticle: Three external nanoscale dimensions of a nano object. Terms like nanorods and nanoplates are employed instead of nanoparticles.
- Nanocomposite: Multidimensional structure comprising nano dimension at least with one of its phases.
- Nanofiber: A nanomaterial, consisting of nanoscale dimensions in two of its exterior phases with the third phase ranging in a larger dimension.

Natural nanomaterials, for example nanomaterials occurring in the human body like DNA, rubisco monomer, hemoglobin, glucose, micelle, ribosomes, enzymes, antibodies, etc., are related to the nanomaterials that belong to the natural world, without any corrections by humans and exhibit excellent properties with regard to their size.

A chemical property of a substance is dependent on its molecular structure. The nano-size of biological matter is due to the supramolecular organization. The interactions of water, light, and other phenomena lead to unique and noticeable properties which can be appreciated at the macro level (Jeevanandam et al. 2018).

2.2 What Is Nanoscience?

The compound word comprises two segments, where nano refers to the 10^{-9} scale and science refers to the related studies. Properties of an object vary with size range. It occurs differently in each variation concerning size as a function. Nanoscience refers to the study of an object's behavior at a nanoscale. The study of the nanoscale regime of a matter showcases its unique properties, which are further developed or studied in the field of science.

2.2.1 The Cosmos of Nanotechnology

Nanotechnology is a rapidly developing application. It has currently become the subject of much research and study. The innovations and improvements in nanotechnology have led to discoveries in many fields of which would be reducing the harmful effects from a source; reproducing to new methodologies; switching to an eco-friendly manner; manufacturing potential devices. Nanotechnology is booming up in the field of computer science and medicine. This subject is being implemented in various applications, as the characteristic feature "size" plays a dominant role (Rathi 2009).

2.2.2 Distinguishing Nanotech from Nanoscience

Nanoscience is a conflux of physics biology, physics, and material science, where it deals with molecular and atomic control of materials, whereas nanotechnology is the potentiality to construct a matter with various standards like observing, manipulating, assembling, manufacturing, and so on. Only a few reports are available, stating the history of nanotechnology and science, but no reports are available summarizing the development of nanoscience and technology from the beginning of the era.

2.2.3 Why "Nanotechnology"?

Nanotechnology is the application, characterization, production, and design of systems in control with size and shape in the nano regime. With a nanoscale material, it is possible to hold control over the features of a system like its chemical make-up, flash point, and many other properties. The main goal of nanotechnology is to offer the functionality of desired products by directing the molecules and atoms. The physiological, chemical, and biological properties of an atom, molecule, or bulk matter vary widely with nanoscale of the same material. With this prospect, nanotechnology offers many functions, features, and solutions to many standing problems.

The field is booming in the medical sector as it is providing solutions to many critical issues. Nanotechnology is already emerging in our lives and is unforeseeable in the upcoming era, as its range of applications is growing persistently.

2.2.4 Development with Nanotechnology

Nano-sized materials present in a wide range of products are being used in several applications, including mass-market consumer products. Among the best consumer products implementing nanotech are:

- Sunglasses—used as a protective layer.
- Textiles—implemented to manufacture windproof, waterproof, and stain-proof clothing.
- Sports equipment—applies nanoscience to increase the performance of torsion and flex resistance.
- Sunscreens and cosmetics—nanoparticle-based creams are used as anti-wrinkle agents, UV protecting layers, etc.
- Televisions—used in screen displays.

The usage of nanotech in several consumer products promoting it in the sections of research, education, and product development would enhance a country's economic growth. Care to be taken with these estimates because it refers to products "implementing nanotechnology" or "nanotechnology used in manufactured products," and not nanotechnology as such. It estimates the value of incorporating it into a particular industry like textile, aircraft, environment, etc.

2.2.5 Application of Nanotechnology in various Fields

The commercialized use of nanoscale products is widely diverse. Products developed from nanotechnology are applied in various industries such as biotechnology, environmental actions, defense, electronics, health science, textiles, and sports. A few examples of incorporating nanotechnology are listed below:

- Health care: Drug delivery enabled through nanoparticles are executed by delivering the nano agents to diseased cells to treat conditions like tumors and cancers.
- Aerospace: In modern helicopters and aircraft, fiber composites of carbon are incorporated for increased fuel efficiency and to reduce the weight carbon fiber composites are used. Also, the RADAR profile is decreased by these composites.
- Automobile: For the high performance of bikes and sports cars, tough and ultralight nanocomposites are used. Also, the nanoparticles are utilized for improving the efficiency in electric vehicles fuel cells, dirt-resistant paints, and anti-fog mirrors or windshields.

- Defense: Beneficial for the SMART weapons with better and light bulletproof guards and helmets with the incorporation of nanocomposites.
- Construction: "Self-Repairing" concrete and nanorods coated glass and windows which can be cleaned easily with water without the usage of soaps (Nalwa, 2000).

2.2.6 Properties of Nanomaterials

The nanoscale matter properties are unique when compared to the bulk matter counterparts. At the nano level, the size-dependent properties become more prominent. The following properties are witnessed upon switching to 10^{-9} size nanomaterials.

- Surface area

This property is associated with nanomaterials. Compared to a materials bulk form, the surface area of the same nanoscaled material is high.

- Quantum effect

This effect is more significant in nanomaterials. Although size comes into play, this effect depends on the semiconducting property of a material (Luke et al. 2020).

- Magnetic effect

This property can change with a material size range. Nano level of an element can drive the non-alluring to become magnetic.

- Mechanical strength

Excellent mechanical strength is exhibited by Nanoparticulate materials (Wu et al. 2020).

- Catalytic support

Enhanced catalytic performance is shown by various nanomaterials, where good dispersion of nano-active catalysts is shown by 2D sheets of the nanomaterials. Boosted catalytic activity is shown by D sheets nanomaterials (Liu et al. 2020).

- High heat and electrical strength

Fascinating heat and electrical strength are showcased by the nanomaterials when compared to the bulk matters.

Example: Graphene (nanomaterial) from Graphite (bulk matter) (Krishnan et al. 2019).

- Activity against Microbes

Few nanomaterials possess the capacity to act against pathogens-related conditions and are antifungal, antiviral, and antibacterial (Makvandi et al. 2020).

From the above features, we can witness nanomaterials are boosting performances in various fields and are possessing great applications as a consequence of their valuable and unique properties.

2.3 Classification and Types of NMs

2.3.1 On Basis of Dimension

In 2007, Skorokhod and Pokropivny classified nanomaterials based on a new scheme based on the electron movement along the dimensions. The classified composites on such basis are 0D, 1D, 2D, and 3D nanomaterials. Where nanomaterials entrapped in dimensionless space are regarded as 0D nanomaterials, whereas 1D, 2D, and 3D NMs have their electron movement along with different combinations of x, y, and z axes (Pokropivny and Skorokhod 2007).

2.3.2 On Basis of Origin

Nanomaterials (NMs) are also classified based on their origin as natural or synthetic.

Natural NMs

These nanomaterials are produced by natural activities through anthropogenic conditions or biological species. They are found everywhere in the globe such as the lithosphere including soils, rocks, magma, and lava at the evolution stage; the hydrosphere comprising lakes, oceans, and groundwater; the biosphere including microbes, higher organisms like humans; and the atmosphere which covers the whole troposphere (Hochella et al. 2015).

Synthetic NMs

These are nanomaterials amalgamated from biological, physical, chemical combinations or produced by engine exhaust, smoke, and mechanical grinding. The challenge faced with synthetic nanomaterials is to predict their interaction with the environment when compared to natural NMs (Wagner et al. 2014).

2.3.3 On Basis of Constituting Materials

Nano-sized materials are differentiated into four classes on the basis of materials they contain.

Organic NMs

It includes nanomaterials originating from organic matter excluding carbon. Organic nanomaterials are transformed to desired structures by assembling and designing molecules to dendrimers, liposomes, polymer, and micelles nanoparticles through weak non-covalent interactions (Jeevanandam et al. 2018).

Inorganic NMs

NMs consisting of metals like silver or gold nanoparticles and oxides of zinc and titanium nanoparticles are categorized under inorganic-based nanomaterials.

Carbon NMs

Configurations such as ellipsoid, tubes and spheres generally contain carbon NMs. These nanomaterials include C-nanofibers, graphene, fullerenes, C-black, or nanotubes. They are produced through methods like laser ablation, CVD, and arc discharge.

Composite NMs

Nanomaterials constituting multiphase are categorized under composite-based nanomaterials. They are present in combinations of nanoparticles either with bulk or other nanoparticles and larger type materials or complicated structures. They can constitute the fusion with metal, carbon, or organic-based nanomaterials. Examples: hybrid nanofibers, metal-organic frameworks, etc.

2.4 Nanomaterials' Sources

Sources of nano-sized materials on the basis of origin are classified into Incidental, Engineered, and Natural nanomaterials (Jeevanandam et al. 2018).

- Engineered NMs
  Nanomaterials required for certain applications with special properties which are manufactured by humans are called engineered nanomaterials such as nanoparticles in healthcare and biomedical products.
- Incidental NMs
  Nanomaterials obtained as a by-product incidentally from an industrial process are known as incidental nanomaterials. It includes nanoparticles produced from

fumes of welding, exhaust from vehicle engines, processes such as combustion, dust storms, volcanic eruptions, etc.

- Natural NMs
  These nanomaterials are found to be existing in the bodies of plants, insects, humans, and animal organisms. It includes nano-viruses, nano-organisms, nanobes, etc.; the difference between incidental and naturally producing nanomaterials are not classified well and therefore incidental nanomaterials are considered as a subdomain of naturally produced nanomaterials.

2.5 Methods of Nanoparticle Synthesis

Nanoparticles are manufactured by various physical, chemical, and biological methods involving many techniques under them. They are (Yadav et al. 2017).

2.5.1 Physical Methods

- Laser Vaporization
- Laser Pyrolysis
- ECR Plasma Deposition
- Magnetron Sputtering
- Ion Beam Techniques
- High Energy Ball Milling
- Melt Mixing
- Laser Ablation

2.5.2 Biological Methods

- Using Plant Extracts
- Using DNA
- Using microbes
- Using Protein Templates

2.5.3 Chemical Methods

- Co-Precipitation
- Microwave Synthesis
- Wet Reduction
- Langmuir–Blodgett synthesis
- Hydrothermal synthesis

- Microemulsion
- Sol–Gel Method
- Sonochemical Synthesis
- Solvothermal Method

2.6 Action of Nanoparticles to Control Pollution

In this contemporary world, environmentalists are very much devoted to finding a solution to the increasing pollution level. Research activities are being conducted to eliminate the toxic pollutants from the surroundings especially concerned with water bodies. The combination of the need for clean water and increasing usage of nanotechnology has led to experiments of water treatment with nanomaterials. The fundamental ideology to eradicate pollutants on a molecular basis is to split a particular matter from a combination mixture. Currently, the method of thermal partitioning is being employed for this process, which is based on the principle of phase-heat changes. Nevertheless, the cost and its inefficient procedure are the crucial demerits. The alternate method of energy extraction creates energy utilizing combustion, where most of it is dissipated and ends up producing undesired by-products which are to be cleansed and disposed properly.

The abovementioned problems in each recovery method could be overcome by utilizing nanoparticles with high efficiency of above.

2.7 Nanoremediation: A Conventional Remediation Operation

Nanoremediation is a conventional process which employs the nanomaterials or nanoparticles for reformation of the environment including ground and undesired water (Patil et al. 2016). Bioremediation through nano technique encloses the utilization of nanomaterials which react with the pollutants to detox and transform them. These nano-sized materials possess properties that enhance both catalysis and reduction of chemicals to diminish the contaminants of interest (Karn et al. 2009).

To decrease the contaminants, the implementation of nanotech is just arising to read the field of environment. Nanoparticles hold a wide diversity of materials like dendrimers, bimetallic particles, nanoscale zeolites, metal oxides, enzymes, and carbon nanotubes which are used in the remediation process (Mehndiratta et al. 2013). Hence, this technology employs new methods to manufacture NMs for the recovery of the environment. This process provides economical solutions to the testing problems for habitat remediation.

With the emerging advancements of nanotechnology, contamination can be reduced, but care should be taken with the ordinance on the size and complexion of the nanoparticles. For example, nanoremediation has applied crop production from polluted lands, where the strategy is to enhance its sustainability. It includes the practice of.

- Agro bio-tech;
- Engineering with rhizosphere;
- Molecular biology; and
- Nano bio-tech (Abhilash and Dubey 2015).
- Few nanomaterials are used to rectify groundwater or wastewater pollution because of certain specialized characterizations (Bora and Dutta 2014). This chapter mainly insists upon the utmost utilization of nanotech on the above-mentioned content, i.e., in remediation of wastewater having its sources from anthropogenic activities.

2.8 The Science of Bioremediation With Nanoparticles

For different reasons, various nanomaterials are to be used in the process of biodegradation; for example, it implements the use of carbon root nanoparticles, one metal nanoparticle, two metal-based nanoparticles, etc., due to (i) a nanoparticle diffusing or penetrating zone effect where microparticles are forbidden (ii) increased reactivity toward redox-vulnerable adulterant. Oxide-overlay Fe^0 is capable of forming a peripheral sphere with pollutants similar to carbon tetrachloride (CT). The Fe^0 Oxide layer enhances the electron transfer and hence CT is decayed into carbon monoxide and methane, whereas benzoquinone and other aliphatic chlorinated hydrocarbons have deteriorated into chemicals with less toxic values (Nurmi et al. 2005). In addition, nanotubes of TiO_2 through a photoelectrocatalytic interaction can be used to degrade pentachlorophenol. Single-metal NPs like Palladium (0) are employed in reductive dechlorination catalysts. Nano-sized particles can be laid on the matrix of the cell wall of *Shewanella oneidensis* and can be excited with radicals through the addition of various donors of electrons in a microbe pruning analysis consisting of Palladium (II). Followed by the excited Palladium (0) are laid down on *S. oneidensis,* the cells come in touch with the chlorine compounds. The reaction with PCP is catalyzed by the radical on the Palladium (0), leading to the eradication of the chlorine molecules (Windt et al. 2005). The potentiality of nanomaterials to eradicate pollutants is progressing and will rapidly result in revolution of the environment in upcoming decades.

2.9 Caliber of Nanomaterials Making Them Suitable for Bioremediation

It is well-known that, under the circumstances of bioremediation, the exploitation of nanotechnology can lead to increased efficiency in a much more economical path. Nowadays NMs are portraying a variant range of applications in bioremediation techniques. Nanoparticles are specifically used with determined properties for various purposes in the recovery process. With their remediation property, they are casted as a photocatalyst, surface activating agent, adsorbent, and carrier for enzyme immobilization.

2.9.1 Photocatalysis

Photocatalysts absorb radiations of certain wavelengths from the sun or other light sources, resulting in the acceleration of chemical reactions. The process of photocatalysis is an effective way to remove organic compounds from water. Advanced oxidation process facilitated with photocatalysts is an important decontaminating activity, as the eradication value of toxic and non-biodegradable substances is high. The technique is implemented with the modification of nanoparticles for wastewater treatment. Titanium-based photocatalytic reactions have been approached on a greater basis (Zhang 2003).

2.9.2 Surface Activation

Nanoparticles combine with enzymes covalently facilitated through surface activation. Hence, it is considered an essential task. Ex: carbodiimides, glutaraldehyde, and chlorides of cyanurides, where carbodiimides support the formation of a material at an intermediate by binding the carboxyl group.

2.9.3 Enzyme Immobilization

Immobilization applies the repeated usage of enzymes economically in remediation, however few conditions are not altered with the binding site of the enzyme. This property is served by nanoparticles owing to their increased surface areas per unit mass, fewer limitations with dimensions, increased capacity to load enzymes, and mass transfer resistance (Yogalakshmi et al. 2020). For example, immobilization of laccase through nanoparticles like SiO_2/Fe_3O_4 and so on.

2.9.4 Adsorption

Nanomaterials have an elevated ratio of surface area with volume, enhancing them to be an increasingly efficient adsorbent to eliminate contaminants. Organic and heavy metal compounds can be separated by dendrimers, ensuring good adsorption.

The mechanism of adsorption includes electrostatic interactions, complexation, hydrophobic effects, and bond formation between hydrogen atoms. Studies show that dendrimers have a hydrophobic interior portion which is utilized for adsorption of organic compounds and an amine or hydroxyl group attaches to the exterior portion for adsorption of heavy metals. Oxides of metals like Fe, W, Zn, and Al are effectively being used in water treatment due to their adsorbing capability (Yogalakshmi et al. 2020).

2.10 Implementation of Nanomaterials in Bioremediation

2.10.1 Nano Iron Structures and Their Derivatives

Arsenic (III) is a highly mobile and toxic species in anoxic groundwater; this component is removed by zero-valent iron NPs, while Arsenic (V) is eradicated on usage of NZVI as a barrier in colloidal form (Kanel et al. 2006). Chromium (VI) and Lead (II) are rapidly immobilized and detached from aqueous mediums by reduction process using NZVIs called ferragels. Halogenated organic compounds are degraded by reactive Fe walls, constructed along the pathway of the undesired groundwater. Likewise, dehalogenation of trichloroethane (TCE) is carried out in the surface of Ni–Fe nanoparticles in one-third ratio. Zero-Valent metals eliminate pentachlorophenol from aqueous solutions either by sorption or dechlorination reactions.

2.10.2 Dendrimers

The word dendrimer is a Greek derivative, “dendri” meaning “tree” and “meros” meaning a part or a fraction of a tree. These are monodisperse and highly branched macromolecules (polymer). These molecules have a distinct architecture consisting of 3 compartments: Core, Interior cells, and Peripheral groups. These NPs have numerous voids which facilitate their reaction with the substrate and that’s why dendrimers-NPs composite is widely applied to enhance catalysis. These composites have potential application in dye removing operation due to its less toxic nature, high surface area, and notable reaction speed.

2.10.3 Nanotubes of Carbon (CNTs), Nanocrystals, and Related Nanomaterials

The extraordinary and versatile properties of carbon-based nanomaterials facilitate new technologies to resolve and identify several usages such as antimicrobial agents, flux-membranes, sorbents, filters, renewable energy technologies, etc. The separation of ethylbenzene from aqueous solution has become easy after deploying hybrid CNTs (HCNTs), single-walled CNTs (SWCNTs), and multi-walled CNTs (MWCNTs). SWCNTs show rapid and efficient adsorption of ethylbenzene when compared to MWCNTs and HCNTs and hence are capable of maintaining high water quality. This is the prime reason for its utilization in eradicating diseases caused by ethylbenzene. Currently, cyclodextrins are used in water treatment, in the form of cyclodextrin-co-hexamethylene-/toluene-diisocyanate polyurethanes and modified carbon nanotubes are developed to reduce the pollutants' levels in the aqueous medium (Li et al., 2010).

2.10.4 Single-Enzyme NPs

Enzymes are widely used in the recovery process owing to their specific and highly effective effects. Despite these advantages, they lack stability and have short catalytic activity time. In most cases, the catalytic activity and stability of enzymes are lost due to oxidation which makes them less beneficial. Therefore enzymes are attached to magnetic iron NPs to increase their longevity, reusability, and stability. This is also an effortless idea as the enzymes can be separated from the substrate or product just by the application of a magnetic field. Currently, to unify core magnetic nanoparticles (MNPs), peroxides and trypsin are used (Fig. 2).

Chemically synthesized NPs have some demerits like self-aggregation water. To eliminate this effect, bio-inspired nanoparticles and microbes' usage are practiced. This technique proves to be eco-friendly and sustainable. The nanoparticles synthesized from fungal, plant sources, and enzymes of microorganisms prove to be a reducing agent for the metal complex salts that produce metallic nanoparticles.

For example, bio fabricated iron oxide produced from *Aspergillus tubingensis*, found in Sunderbans was able to detoxify the wastewater by removing heavy metals like Lead (II), Nickel (II), Copper (II), and Zinc (II) by adsorbing the metal ions over their surface through endothermic reactions. Biogenic nanoparticles effectively degrade azo dyes and textile effluents. 25 mg/L of biogenic nanoparticles were able to degrade congo red, malachite green, and direct blue - 1 up to 97.07%, 90.55%, and 88.42% (Noman et al. 2020).

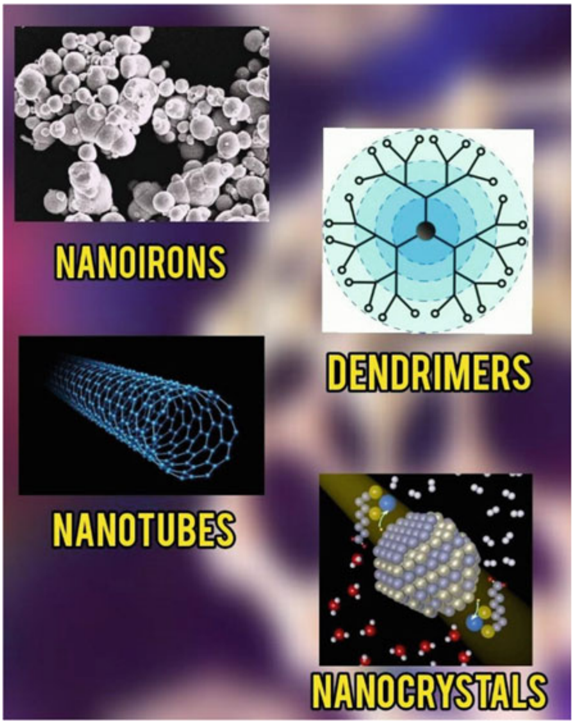

Fig. 2 Different forms of nanomaterial applicable in bioremediation

2.11 Nanoremediation of Wastewater—Modus Operandi

2.11.1 Oil Spill Treatment

Conventional methods have many demerits like increased risk of fires due to localization and leftover ashes in source site and causing fatal effects on human health and marine life. Nanoscience presents sustainable resolution to improvise post response by performing following attributes in NMs (Mehta et al. 2015) (Fig. 3):

- Pickering Emulsifiers: Pickering emulsifiers are formed when a microbe or a nanoparticle adsorbs oil–water into its interphase preventing the fusion of the droplets. The adsorbing particles form a monolayer which prevent coalescence and form a stable emulsifier. Another important factor that decides the stability of the emulsifier is the wettability of the particle to be adsorbed, i.e., it must be partially hydrophobic and must possess an angle of contact greater than 90° (Okazawa and Bron 1979).
- Nano-Based Oil Hinder: It was introduced in the year 1970 to enable in-situ burning of oil. These consist of amphiphilic particles having affinity for air–water intrusion. These elements adsorb the surfaces and reduce the surface tension significantly thereby confining oil slick.

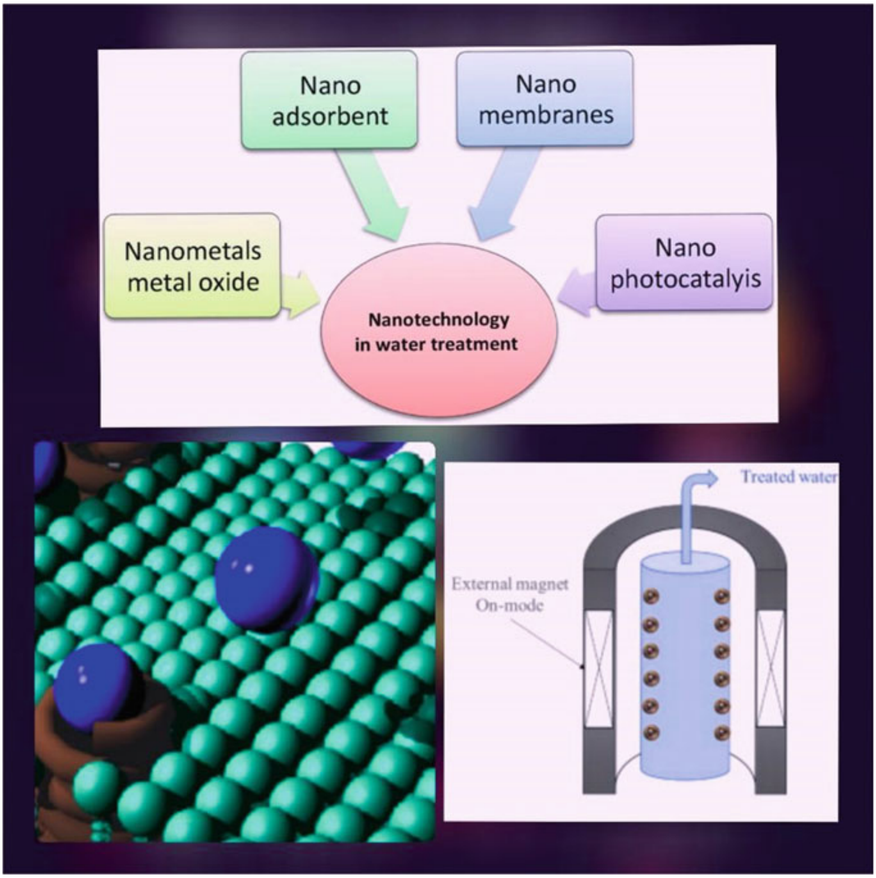

Fig. 3 Different methods to execute nano forms in industrial water treatment

- Magnetic Nanosorbents: Sorbents by nature are insoluble in both oil and water and their effectiveness depends upon various factors for instance oil retention, removal, rate of sorption, etc. Nanosorbents are designed with hydrophobic, high selectivity for effective remediation. The high surface area volume ratio facilitates increased sorption and oil-retention properties. The selectivity property of the sorbents ensures the restriction by sorption of the medium and prevents sinkage (Qiao et al. 2019).

2.11.2 Nano Photocatalyst

The word photocatalyst means decomposition of a substance in the presence of light. A photocatalyst alters the reaction rate without any direct intrusion during ongoing reaction. The increased surface ratio and shape features enable it to be used in water recovery. The nano photocatalyst works by the principle of expanding its oxidation ability of the particular element produced at the top layer of the material which is involved in the eradication of the contaminants. Cleansing of azo dyes, chlorpyrifos, nitroaromatics, etc., nano photocatalysts of zero valence-based metal, bimetallic, and semiconductor types are used. The significantly used nano photocatalysts are SiO_2, ZnO, TiO_2, and Al_2O_3, however, TiO_2 is the most preferred catalyst due to several meritorious reasons like non-toxic nature, high chemical stability, cost-effectiveness, easy availability, etc. (Dubinsky et al. 2013).

Advantages and Disadvantages of Nano Photocatalyst

– Advantages

  - Complete degradation of the pollutant is witnessed.
  - The catalyst can be reused.
  - The quantum-sized nature of the catalyst provides effective cleansing activity.

– Disadvantages

  - It has potentially toxic effects.
  - Catalyst recovery is a difficult task.

Future Perspective of Nano Photocatalyst

Synthesis of new photocatalysts with greater efficiency, eco-friendly nature, and huge stability is the need of the hour. It is also necessary to incorporate several new techniques like thermodynamics, electrocatalysis, photocatalysis, adsorption, etc. for increased efficiency.

2.11.3 Nanomembranes

Nanomembranes are created by the unique association of different types of nanofibers. This material is used to eliminate undesired particles from the wastewater. This elimination technique is also used as a reverse osmosis technique because it occurs at high speed (Jhaveri and Murthy 2016). Water porous membranes with a composite support layer are preferred for wastewater treatment as it is well equipped in ultrafiltration, nanofiltration, reverse osmosis, etc., the composite layer is usually a C-based material with either oxides of graphene or carbon nanotube.

Advantages and Disadvantages of Nanomembranes

- Advantages
    - Nano filters utilized for water safety measurement.
    - It does not require any supporter ion exchanger.
- Disadvantages
    - Fouling of the membrane occurs after using the membrane a few times.
    - The stability of the membrane can't be ensured for a long duration.

Future Perspectives of Nanomembranes

There is a need to improve the selectivity of the membrane in the first place. Secondly, the resistivity of the nanomembrane must be upscaled to utilize filters utilized to prevent fouling. New membrane types using grafting-based polymers can be generated to avoid fouling (Ying and Pumera 2019).

2.11.4 Nano and Micromotors

These motors ensure the conversion of energy from various origins into machine-driven force. This technique can be operated with and with the need of fuel (Jurado-Sanchez and Wang 2018). These have wide applications and possess increased power, regulated movement, self-mixing property, etc.

Advantages and Disadvantages

- Advantages
    - This technique can convert toxic contaminants into nontoxic forms.
    - These can be utilized for discharging remediation over long distances.
- Disadvantages
    - The lifecycle of micro and nanomotors are restricted.
    - There are high chances of Pt layer poisoning due to the reaction of contaminants in the wastewater.

Future Perspectives of Nano and Micromotors

It is necessary to incorporate novel materials like graphene to treat water; such materials increase the performance of the motor and present better results (Table 2).

Table 2 Mechanisms implemented to handle nano/micro motors

Nano/micro motors types	Mechanism	Implementation
Pt/Au nano motors	DNA Hybridisation—Ag nanoparticles, Silver-induced acceleration	DNA Identification, Ag ions detection
Pt micro engines—SAM modified	Hydrophobic interactions	Removal of Oil
Pd nanoparticles	pH Dependency	Monitors pH
Al, Zn micro motors	pH Dependency—Speed	Controls pH
Pt micro engines—Bubble Movement	Increased transport of the liquid, diffusion; Fenton reaction	Degrades organic pollutants, detoxification
Capsule motors—Polymer	Cargo towing induced by surface tension	Remediation of Oil

2.12 Supremacy of Nano-Bioremediation

Nanobioremediation can be deployed in places where the conventional degradation technique fails, for example, the degradation of dyes from effluents. The better alternative for the usual bioremediation process which is relatively slow is nanobioremediation. Nanoscience, nanotechnology, and biotechnology can be operated together in various fields like remediating agricultural wastes, textile dyes, heavy metals, and in the manufacture of electronics, medicines, etc. Alteration in quantum properties of nanoparticles paves way for enhanced remediation process and scope for future exploration (Yogalakshmi et al. 2020).

2.13 Challenges of Nanoremediation

The major challenge in this technique is to maintain the size, with increased reactivity the size of the material increases resulting in toxicity therefore it is important to study quantification of such risks and effects of the same. Although nanoparticles prove to be an effective remediation process, exposure to it causes lethal effects to human beings and so toxicology studies must be carried out for the same. For example, iron oxide nanoparticles restrict the ability of an organism to grow or reproduce. Although the urge to synthesize green nanoparticles seems promising, a check for predictable results must be taken care of. It is also observed that nanoparticles form deposition in the soil preventing the transport of fluids and resulting in clogging. The nanoparticles are also reported to hinder microbial growth and drastically eradicate them from the soil as they are a potent toxic material to them. It is crucial to consider these challenges and carry out further research on them to propose a sustainable outcome (Yaqoob et al. 2020).

2.14 Recent Trends in Nano-Based Remediation

2.14.1 Nanozymes

These are nano-sized particles in the range of 0–100 nm that exhibit enzymatic activity. These are exploited to connect nanoclusters, nanofibers, crystals, rods, etc. It has several merits as such it is very stable, easy to scale up, low-cost production, etc. Some important examples include metal-based enzymes and carbon-based enzymes (Maduraiveeran and Jin 2017). It is also regarded as a better alternative for natural enzymes as they mimic several enzyme properties. Nanozymes have an effective impact by degrading the dyes.

2.14.2 Single-Enzyme Nps

Single enzyme nanoparticles are in the development stage. MNPs are applied to external fields and are reused up to 5 times by preserving almost 100% activity. It is proved that about 96% activity of methylene blue under optimal conditions by the use of MNP - H_2O_2 (Shin et al. 2015).

3 Conclusion

It is crucial to understand that several anthropogenic activities involving the release of CFCs, Nitrogen oxides, CO, heavy metals, etc., cause damage to air, water, and soil, causing lethal effects to mankind, aquatic life, and the environment. It is estimated that there are about 246 microbial bioremediation and biodegradation methods yet due to certain safety standards, the utilization is limited. The bioremediation process is slow, laborious, and expensive at times, to overcome this, emerging mechanization—nanotechnology is utilized to solve the purpose. It is proven that nano-based approaches are effective and cheaper than conventional methods. Nanotechnology also offers versatile solutions to many major existing problems. Taking all of these data and facts into consideration, it can be concluded that nanoremediation will be the Gen-Z remediating technology. The future of nanoremediation is the innovation and novel proposals which have opened up new applications in this field. Several pieces of research led to practical applications in the economy with fruitful results. In the contemporary world, a technique must be versatile to fit for several purposes. On the other hand, the technique must be sustainable enough to support human needs and cross major scientific barriers.

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Nanobiotechnology: A Sustainable Approach for Marine Environment Bioremediation

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1 Introduction

The surge in world population resulted in increased demand for agricultural products for food production. The increased demand for crops has put pressure on the advancement of technologies and industries to create products that can maximize land productivity. Besides that, the industrial revolution expanded beyond agriculture and utilized many Earth resources, and as a result, the by-product or final fate of its products was found to be nowhere except the environment (Wiedmann et al. 2020). The toxicity of these wastes that are released and accumulated in the environment has raised concern about their terrible effect on the environment, particularly aquatic environments (rivers, lakes, and oceans), biodiversity, and human health as a in consequence (Ferronato and Torretta 2019). Proactivity is now needed to stop these dangerous wastes from leaching into the ecosystem and to cleanup the leached

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wastes to restore a the balanced ecosystem and remediate the environment. These toxic wastes are found in all manufacturing and farming practices such as fertilizers, pesticides, and herbicides, that are being used in agriculture. Besides, heavy metals, crude oil, and a variety of hydrocarbon derivatives are produced by all industrial activities. Among all the knowledge and approaches that have been created to remediate polluted environments, bioremediation is the most cost-effective and environmentally welcoming approach for pollutant cleaning (Adams et al. 2015). However, the use of microbes in bioremediation is influenced by other biotic and abiotic factors at the polluted sites being treated (Adams et al. 2015). Because contaminants reach environments as a mixture, not as a single contaminant, remediation is a challenging task. In the last two decades, a lot of advancement has happened in nanotechnology, and its diverse potential applications have made it a successful candidate for environmental cleaning, especially when it is coupled with microbial remediation. The nanomaterial could be formulated to precisely identify a specific chemical in the contaminated sites (Benjamin et al. 2019).

In this book chapter, we will discuss the green synthesis of nanomaterials using microbial cell factories, their ability to remediate various chemicals, and their interactions with other intrinsic or extrinsic microbes.

2 Nanomaterials in Bioremediation

In-situ remediation is very complicated to approach but with nanomaterials, this could be possible. Nanomaterials (NMs) have properties that make them different from their mass-phase counterparts in terms of their magnetic and catalytic characteristics as well as their surface-to-volume ratio (Mandeep and Shukla, 2020). These characteristics make NMs ideal in remediation by being fast in the remediation of contaminants and producing fewer by-products; for instance, nano-metal oxides have no secondary pollution residues and could be an effective approach in the remediation of heavy metals from water (Benjamin 2019, Shah 2020). In other words, the NMs can work either as adsorbents or catalysts. Another advantage is that NMs could be green synthesized by microbial activity, making them more sustainable and eco-friendly (Mandeep and Shukla, 2020). Thus, nanobioremediation (NBR) is described as boosting the bioremediation activity of microorganisms with the support of NMs (Cecchin et al. 2017). Not only could the NMs be used along with microbial bioremediation, but they showed the potential to work with other remediation method such as phytoremediation and could improve the effectiveness of the whole remediation process (Benjamin et al. 2019).

3 Green Synthesis (Biosynthesis) of Nanoparticles

Bio-based strategies that use live organisms or their products for creating nanoparticles (NPs) have grabbed attention due to their high efficacy and compatibility with the environment; in other words, they are greener. NPs formed by biogenic methodologies have superior stability regarding the cellular stabilizing capping metabolites (Singh et al. 2018). As mentioned earlier, NPs could be synthesized using microbial cells, which are more eco-friendly than the other NP synthase mechanisms. These bacterial cells are considered a microbial cell factory to produce these NPs (Salem and Fouda 2021). The green synthesis of NPs is not restricted to bacteria and can be achieved via the use of all other microbial forms. For instance, ZnO NPs were biosynthesized by two different fungi strains, *Aspergillus niger* and *Fusarium keratoplasticum* (Mohamed et al. 2019). The proper mechanism of how the NPs are synthesized by the microbes is yet to be identified, as there is not a universal way that can be applied to various kinds of microbes that have the capabilities for producing NPs (Salem and Fouda 2021).

4 Microbially Manufactured NPs

The traditional NPs synthesis is achieved by physical, chemical, or mechanical techniques such as ultrasonication, radiolysis, electrospinning, microwave, sol-gel technique, spray pyrolysis, inert condensation, and chemical reduction (Lahiri et al. 2021; Shah 2021a, b). These methodologies have their drawbacks, affecting high production rates and the use of poisonous chemicals during the manufacturing process. The need to replace poisonous solvents with environmentally secure strategies for synthesizing NPs leads researchers to offocus their attention on alternative nontoxic, eco-friendly, and budget-friendly strategies (Fang et al. 2019).

Nature has provided ways to manufacture advanced nanomaterials, and the biological system can act as a 'bio-laboratory'. Researchers are now focusing on employing biological entities to synthesize pure metal and metal oxide particles that can be identified and classified according to the production process.

Table 1. So far, several living organisms have been involved within NP production for potential applications, representing different domains, including bacteria, actinomycetes, fungi, yeast, and algae. Thus, biomimetic nanomaterial constructions are much 'cleaner' and 'greener' owing great appropriateness to the field of nanobiotechnology (Salvadori 2019).

Among various sorts of NPs, metal NPs have raised more attention over the last few decades because of their large surface area per weight or volume and many characteristic biological, physical, chemical, thermal, mechanical, optical, dielectric, electrical, electronic, and magnetic features, introducing them as attractive tools for research work (Khan et al. 2019). Notably, microbes could adapt to greater metal

Table 1 Nanomaterial classification according to synthesis strategy

Nanomaterials (NMs)	Bio-nanoreactors	References
Metal NPs		
Au	*Bacillus* sp. SDNS	(Abouelkheir et al. 2016)
Ag	*Streptomyces* sp. strain OSIP1& *Streptomyces* sp. strain OSNP14	(Bakhtiari-Sardari et al. 2020)
Cu	*Gelidium* sp.	(Rasool 2019)
Ni	*Alcaligenes faecalis*	(Spoorthy et al. 2017)
Carbon nanomaterials		
Carbon nanotubes (CNTs)	*Magnetospirillum magneticum* strain AMB-1	(Ozden et al. 2017)
Graphene oxide (GO) NPs	*Bacillus* sp., *Pseudomonas* sp.	(Balakrishnan et al. 2021)
Metal oxide NPs		
ZnO	*Aspergillus terreus*	(Baskar et al. 2015)
Fe2O3	*Proteus vulgaris* ATCC-29905	(Majeed et al. 2021)
Polymer NMs		
Bacterial extra polysaccharide stabilized NP	*Lactobacillus plantarum*	(Pradeepa et al. 2016)
Nanocellulose	*Bacillus velezensis* SMR	(Abouelkheir et al. 2020)
Nanocomposite		
Gold activated charcoal (Au-Ac)	*Bacillus* sp. SDNS	(Abouelkheir et al. 2018)
BMP-Au rods-folic acid	Magnetotactic bacteria	(Nima et al. 2019)
Bionanomaterials		
Plasmids (Arsenic sulfide NPs)	*Shewanella* sp. ANA-3 and *Salmonella enterica* genes into *Escherichia coli*	(Chellamuthu et al. 2019)
Protein functionalized bacterial magnetosome (BM)	*Magnetospirillum magneticum* AMB-1	(Kotelnikova et al. 2018)

concentrations and can convert inorganic elements into NPs via extracellular or intracellular pathways (Koul et al. 2021). This is happening when microbes take up the metal ions from their surroundings or media and convert them to elemental form through an enzymatic reduction process, as clearly illustrated in Fig. 1.

During the extracellular production of NPs, the broth supernatant containing microbial enzymes is subsequently utilized to synthesize NPs via the conversion of metal ions that are retained outside the cell by the extracellular enzymes (Koul et al. 2021). In other words, in a separate vessel, the reductase enzyme-containing supernatant is allowed to react with the metal ions. Metal ions are bio-reduced in a

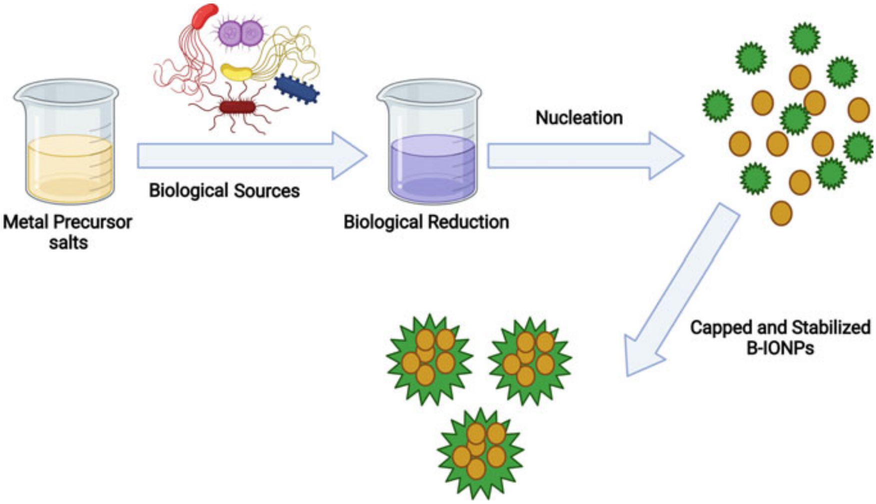

Fig. 1 Schematic drawing of the enzymatic bio-reduction process of metal ions into metal NPs (yellow balls), capped with a capping agent (green)

cell-free supernatant, resulting in the creation of NPs. Whereas in the intracellular process, the microbial cultures are kept in a suitable liquid medium, and the microbial biomass is rinsed with sterile-distilled water before being centrifuged to extract the biomass pellet (Koul et al. 2021). After that, the microbial biomass is allowed to react with a metal-aqueous solution. The microbial biomass and metal-containing solution are then cultured at the proper incubation conditions until a specified chromatic change is detected. The creation of NPs is indicated by the development of a certain hue. The formation of a whitish-yellow to yellow tint implies the production of zinc and manganese NPs. While a light yellow to pinkish tint signals the formation of gold NPs, a pale yellow to brownish color indicates the formation of AgNPs. The positively charged metal ions are then trapped within the negatively charged microbial cell. The production of nanoclusters arises from the bio-reduction of these metal ions within the cell by intracellular enzymes, and the NPs later diffuse from the cell to the solution (Marooufpour et al. 2019). Figure 2 illustrates the two different mechanisms in microbially mediated NP synthesis.

4.1 Bacteria-mediated Synthesis of Nanoparticles

Bacteria have been identified as possible bio-resources to produce nanoparticles such as silver, gold, palladium, magnetite, platinum, titanium dioxide, titanium, cadmium sulfide, and other metals (Kapoor et al. 2021). Under various physicochemical factors like exposure time, pH, temperature, bacterium concentration, and metal salts, bacteria can create NPs with metals and metalloids either intracellularly

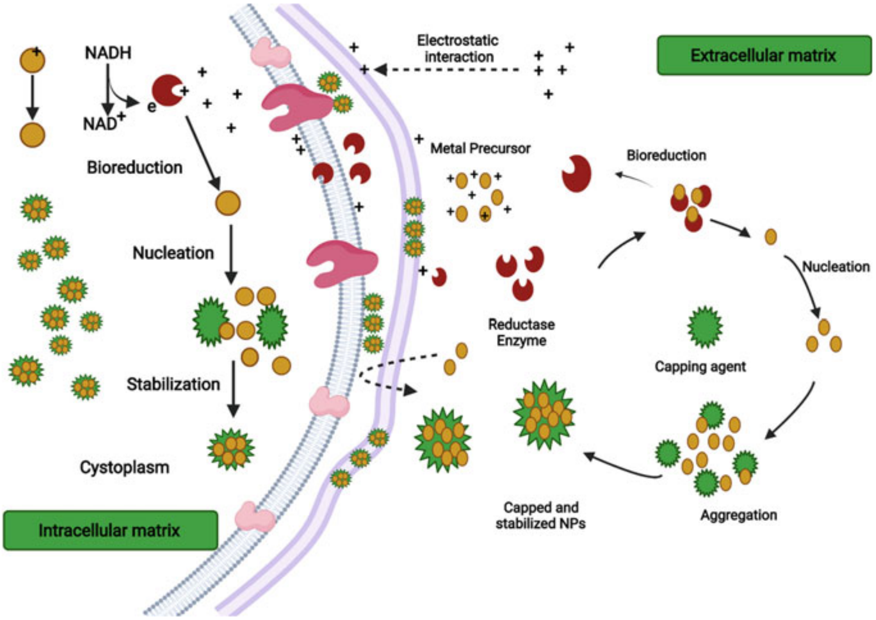

Fig. 2 Schematic illustration of intracellular and extracellular mechanisms for NP (yellow ball) production

or extracellularly. Extracellular reduction looks to be more beneficial than intracellular reduction as a consequence of the ease of extraction and high efficiency (Kapoor et al. 2021).

Bacteria may catalyze different processes and create inorganic NPs owing to their enzymes. These NPs may be used in numerous applications, including the invention of third-generation biosensors, labeling, cellimaging, biofilm formation, diagnostics, and sensoristic. Moreover, bio-fabricated NPs showed prospective uses as antitumor agents, antioxidants, anticoagulants, antimicrobials, antiproliferative, and antimigration agents (Koul et al. 2021).

4.2 Actinobacteria-mediated Synthesis of Nanoparticles

Actinobacteria are a phylum of bacteria found in both aquatic and terrestrial environments. These bacteria can produce various bioactive substances and extracellular enzymes, in addition to playing a substantial role in nanoparticle creation via extracellular or intracellular routes (Gahlawat and Choudhury 2019; Kapoor et al. 2021). Metal ions are reduced intracellularly on the surface of mycelia and cytoplasmic membranes in actinomycetes, leading to the creation of nanoparticles. For example, the enzymes found on the *Rhodococcus* strain NCIM 2891 cell wall help in the biomineralization of silver ions and the intracellular production of 10 nm Ag NPs (Ortega et al. 2015). However, extracellular synthesis is mostly desired for commercial uses. Therefore, Karthik et al. (2014) have focused their research on extracellular-mediated biosynthesis via NADH-dependent nitrate reductase for the production of stable AgNPs from *Streptomyces* sp. LK-3. Moreover, polydisperse AgNPs were synthesized in sizes ranging from 5 to 20 nm using *Streptomyces xinghaiensis* strain OF1 (Wypij et al. 2018).

4.3 Archaea-mediated Synthesis of Nanoparticles

However, almost all studies have concentrated on bacteria, fungi, and yeasts; archaea's hidden potential for nanomaterial formation has yet to be identified, as a few species from each have been used to make NPs (Dhanker et al. 2021). Archaeal species inhabiting a broader range of habitats are considered the best candidates for the field of nanobiotechnology owing to the S-layer glycoproteins present in their membrane (Tag et al. 2021). The unique property of haloarchaeal cells is that they rapidly lyse at low salt concentrations, resulting in the liberation of intracellular and membrane ingredients that act as building blocks for the creation of major biomolecules and are thus suitable for nanobiotechnology (Tag et al. 2021). Several processes have been used to demonstrate heavy metal resistance and detoxification in these extremophilic bacteria, including extracellular metal sequestration and intracellular enzymatic reduction (Voica et al. 2016). During these detoxification procedures, metallic ions are reduced to nanoparticles. However, because of the of natural mimicking condition in nature in which they exist, many of these species are difficult to grow in the laboratory. Despite much research on their biotechnological potential, halophilic prokaryotes' ability to produce NPs has remained understudied. Meanwhile, two intriguing halophilic archaea, *Haloferax* sp. and *Halogeometricum* sp., previously isolated from sun saltern, have been demonstrated to produce Ag and Se NPs with remarkable size homogeneity through both intracellular and extracellular metal ion reduction (Abdollahnia et al. 2020).

4.4 Mycogenic and Yeast-mediated Synthesis of Nanoparticles

Fungi are considered excellent candidates for producing metal NPs and metal sulfide NPs due to the presence of many enzymes in their cells and the ease of handling. Filamentous fungi are preferred for nanoparticle production due to their capability to secrete proteins, enzymes, and metabolites; their ease of scaling up, and downstream handling; their financial feasibility; their improved surface area due to the presence of mycelia; and their low-priced manufacturing processes (Spagnoletti et al. 2019).

Compared to bacteria, fungi produce NPs with a higher degree of monodispersity. The switch from bacteria to fungus for natural nanofactories has the added benefit of making downstream biomass processing and handling considerably easier (Ghosh et al. 2021). *Fusarium oxysporum* produces reducing agents in the solution via the NADH-enzyme-mediated process, which leads to the creation of AuNPs. Because of the protein-binding capability of cysteine and lysine residues, the produced NPs have long-term stability (Das et al. 2014). Yeast is not an exception for NP generation; it was used in the green production of NPs since it can endure high metal levels and can deposit metal ions (Koul et al. 2021). When yeast cells are exposed to metals, they adapt by using different detoxification mechanisms such as intracellular sequestration, chelation, and bio-precipitation (Gahlawat and Choudhury 2019).

4.5 Polymicrobial Communities-mediated Synthesis of Nanoparticles

Polymicrobial communities are microscopic organisms that have adapted to co-exist with others, forming complex communities that change dynamically depending on the quantity of each unique microbial kind. Most bacteria in nature do not exist in pure culture; however, they are found in heterogeneous and complex microbial communities (Kim et al. 2020). The dynamic interplay between these societies' co-existing associates is fascinating. The secretion of several bioactive chemicals, either directly into the surrounding environment or across the membrane, facilitates interaction between these communities. Biofilm is the most typical example of polymicrobial natural communities. Biofilms have some advantages over planktonic bacteria in nanoparticle syntheses, such as a bigger surface area and large-scale production with low precursor molecule requirements, higher accumulations of biomass, efficiency, and economics. For example, extrapolysaccharide Xanthan gum purified from biofilms successfully synthesizes Ag and AuNPs metals and shows a wide-range of applications (Xu et al. 2014). Dextran sulfate was used to stabilize AuNPs (Cakić et al. 2016). Furthermore, silver NPs encapsulated by dextran polysaccharide synthesized by *Leuconostoc mesenteroids* strain T3 have enhanced cysteine detection selectivity in addition to sensitivity in aqueous systems (Davidović et al. 2017). Similarly, for stabilizing Se and ZnNPs, pure carboxylic curdlan was employed (Yan et al. 2018).

5 Nanomaterials Biotransformation in the Environment

According to Abbas et al. (2020), engineered nanomaterials would eventually be dispersed in the environment, such as the air, soil, and water. Upon interaction with the environment, the nanomaterials may be subjected to change through various pathways, which may yield alterations to the nanomaterials on either the surface only or the entire particle. Biotransformation may determine the nanomaterials' behavior, and, subsequently, their fate in the environment. Despite being interconnected, biotransformation can be categorized into biological, physical, and chemical transformations. Briefly, chemical transformation results in the nanomaterials forming bonds with their environmental counterparts that involve factors like light, oxygen, and the nanomaterials' solubility in water. In physical transformation, nanomaterials tend to retain their chemical structure and get sorbed by other substances. Aggregation of nanomaterials may result in homoagglomeration or heteroagglomeration if it yields a complex formation. In biological transformation, alteration of the nanomaterials is caused by living organisms like plants, bacteria, and fungi.

The interconnectedness of nanoparticle transformations can be depicted in wastewater systems, such as in the study performed by Fernando et al. (2020). The transformation of AgNPs in wastewater treatment systems was shown to have been influenced by exopolysaccharides (EPS) and various types of cations. In the presence of $NaNO_3$ and at a low concentration of $Ca(NO_3)_2$, EPS was reported to have enhanced the colloidal stability of AgNPs. At higher $Ca(NO_3)_2$ concentrations, this enhanced the aggregation rate due to the interaction between the dissolved EPS and AgNPs through molecular bridging between them. Moreover, compared to SB-TBS and TB-EPS, LB-EPS showed an enhancement in AgNP stabilization, mainly due to the less hydrophilic constituents present therein (Fernando et al. 2020).

Meanwhile, NPs may also affect the composition of EPS from activated sludge containing microbial aggregates. In a bench-scale sequencing batch reactor experiment, Ma et al. (2018) reported an increase in total EPS production with the increase in Fe_3O_4NPs concentration because NPs shielded the bacteria against the entry of Fe_3O_4NPs and helped in the degradation of the NPs into the activated sludge. Therefore, compared to TB-EPS, higher susceptibility of LB-EPS towards Fe_3O_4NPs was deduced as greater production of LB-EPS was observed than that of TB-EPS at similar Fe_3O_4NPs concentrations. Moreover, while protein and polysaccharide amounts in both LB-EPS and TB-EPS increased with increased Fe_3O_4NPs concentration, a greater amount of protein than polysaccharide was generally observed.

In the study performed by Ye et al. (2021), increased EPS production under ZnO NPs stress was believed to have hampered the sludge cells from binding with each other, thus resulting in decreased sludge flocculation performance. Besides, the decreased zeta potential in the LB-EPS observed may be implicated by the mitigation of a tyrosine protein-like substance in the LB-EPS under the ZnO NPs stress.

6 Nano-enhanced Remediation

The application of nanotechnology in different remediation processes has improved remediation outcomes such as wastewater treatment, oil spill removal from the marine environment, or by increasing the biodegradability of plastic materials compared to non-biodegradable ones. It is also successful in preventing marine biofouling.

6.1 Nano-enhanced Wastewater Treatment

Water is very crucial for humans. Owing to the advancement in industrial and agricultural practices, water bodies receive various classes of contaminants because of anthropogenic activities, especially in undeveloped countries where environmental protection laws are not applied efficiently (Mandeep and Shukla, 2020). Wastewater is very complex; it has a municipal or industrial origin. For heavy metal removal, the carboxylation of multi-walled carbon nanotubes (MWCNTs) was very efficient in enhancing the adsorption of Mn (VII) and As (V) compared to their carboxylated counterparts (Egbosiuba et al. 2020). Moreover, coated magnetic NPs have increased the efficiency of the removal of pollutants in wastewater, such as COD and Pb (II), with the proficiency to reuse the NPs in Pb (II) up to six times, making it more cost-efficient and eco-friendly (Mohammadi et al. 2020; Najafpoor et al. 2020).

6.2 Nano-enhanced Metal and Nutrient Removal

According to Raj et al. (2016), the extracellular polymeric substances (EPS) present at the outer surface of microorganisms have the property to bind with several metal ions, besides functioning as the capping agent for NP synthesis. In the study, the metal adsorption effectiveness of the EPS extracted from the marine bacterium *Pseudomonas aeruginosa* strain JP-11 was subjected to functionalization by incorporating several types of functional groups, like sulfur. Hence, in a cadmium-polluted aqueous environment, incorporation of the sulfur group in the EPS might make it a good cadmium binding site and subsequently a cadmium sulfide NP (CdS NP) synthesis site. Integration of functionalized EPS with CdS NPs may result in a greater affinity for metal ion adsorption compared to only functionalized EPS.

Different bacteria have different EPS compositions, which are characterized by the properties of their elemental constituents (charge, molecular weight) and ultimately determine their mineral sorption capacity. As reported by Chen et al. (2021), two types of clay minerals (montmorillonite and kaolinite) and a type of iron oxyhydroxide (goethite) were preferentially sorbed by the aromatic fractions in the EPS of *B. subtilis* (Gram-positive) than the fractions in the EPS of *E. coli* (Gram-negative). Depending on the types of minerals, the formation of EPS-mineral complexes, in turn, enhanced the sorption distribution coefficient of phenanthrene, a type of hydrophobic organic contaminants, by up to five times more. This causes phenanthrene to be more strongly absorbed. It transpired that the EPS structures became more ordered upon being complexed with the minerals, thus becoming an enhanced phenanthrene sorption site.

6.3 Nano-enhanced Oil Spill Removal

Microorganisms are the best candidate for the degradation of oil spills to mitigate their effects on the environment. However, according to the complex nature of crude oil, each microorganism has a narrow spectrum in the degradation of it to simpler, eco-friendly compounds (Pete et al. 2021). The process of complete remediation requires the succession of different microbe' communities that start with alkane-degrading bacteria, followed by communities that can degrade polycyclic aromatic hydrocarbons (PAH) (Dubinsky et al. 2013). Moreover, in most cases, the indigenous microbial communities are not efficient in oil spill remediation and require external interventions either by bioaugmentation, biostimulation, or both. Nevertheless, oil spill bioremediation using either of these approaches might take years to complete, but it can be accelerated and improved by coupling with nanotechnology (Cheng et al. 2020; Omarova et al. 2018).

Emulsification of the oil inside the water will increase the surface ratio to the volume of the oil spill, which in turn will raise the bioavailability of bacteria to colonize the surface of the oil droplets in the water (Pete et al. 2021). This results in an improvement in the remediation process when clay NPs coupled with chitosan show improvement in the bioremediation of the oil spills by bacteria after the formation and stabilization of the oil droplets by the NPs (Omarova et al. 2018). Similarly, other NPs are employed to improve the bioremediation process's efficiency and can be used alone in optimum concentrations or along with biosurfactants. Another mechanism for improving the bioremediation process is the use of magnetoresponsive bacteria by cell surface modification (Pete et al. 2021). The modification of the bacterial cell's magnetic properties will make it attracted to magnetic NPs in the water and assist in its removal recovery from the system by the application of a magnetic field. Moreover, improving the hydrophobicity of the cell surfaces could boost the localization of bacteria on the oil or water surface, which helps in improving the remediation process. On the other hand, bio-stimulation is an efferent approach, enriching the indigenous microbes for the remediation of the oil spills; however, the nonspecific stimulation will result in promoting non-oil-degrading bacterial populations. Thus, the targeted stimulation approach is more appealing and can be accomplished through nanotechnology applications.

Bioremediation can be more efficient with the cooperation of remediators and NPs. In an oily water treatment laboratory experiment, Alabresm et al. (2018) reported 100% removal of (375 mg L^{-1}) oil within 48 hours of interactions between magnetite NPs coated with polyvinylpyrrolidone (PVP) and oil-degrading bacteria. On the other hand, only 70% of lower-chain alkanes and 65% of higher-chain alkanes were removed by using only NP and magnetic separation of NP, respectively. The removal of the oil particles was believed to occur by their sorption by the bacteria, and then the Fe and C were jointly utilized.

6.4 Nano-enhanced Plastic Biodegradation

Plastic materials have infiltrated every nook and cranny of the globe, resulting from significant growth in global plastic manufacturing. The photodegradation of plastics yields microplastics that change into nanoplastics. These potential threats lead to bioamplification. So, the plastic problem is a current and continuous occurrence that requires increased attention to its degradation. Plastic devastation caused by burying, burning, or any other means will pollute the ecosystem. The only solution to eliminate plastic trash is to adopt recyclable plastics with a thickness greater than 40 microns or to develop novel biodegradation technologies. The biodegradation of plastic materials happens at molecular, macroscopic, or microscopic scales (Singh 2016). For instance, microorganisms such as *Aspergillus niger*, *A. japonicus*, and *Pseudomonas* species, as well as the gut microbes that are present in the beetle *Tenebrio molitor* gut, found the polystyrene (PS), low-density polyethylene (LDPE), and high-density polyethylene (HDPE) to be the most borne plastic materials for biodegradation (Van Cauwenberghe and Janssen 2014). These procedures can break down plastic, but they are inconsistent, time-consuming, and even labour-intensive when it comes to achieving total biodegradation (Reisser et al. 2014). Thus, the use of enhancers, which are extra ingredients put into plastic films to speed up biodegradation, was advocated to improve the biodegradability of plastic materials. In the plastic degrading sector, this new technology is powerful and perceptive, but it also indirectly adds to the nanoplastic problem (Dawson et al. 2018). For instance, TiO_2 is regarded as one of the most exceptional catalysts and most popular semiconductors. Most of the current research is focused on biodegradable plastics using nanomaterials. The combination of PS and TiO_2 was proven to be an efficient degradation approach when compared to standard photolysis of plastics in nature (David et al. 2020). Moreover, Fullerene 60 nanoparticles accelerated the degradation of low-density polyethylene (LDPE) using a bacterial consortium (Sah 2010). Using nanobarium titanate (NBT) in LDPE degradation demonstrated NBT's ability to increase the bacterial consortium's exponential phase (David et al. 2020).

6.5 Nano-enhanced Dye Biodegradation

Water pollution that is very persistent and xenobiotic, such as azo dyes, acidic and cationic dyes, and dyes, must be remedied for future usage or before discharge into water bodies. These contaminants contribute to water contamination and harm aquatic life. Because NPs have a higher surface area-to-volume ratio, they can either serve as catalysts or absorb pollutants. Many studies have looked at the catalytic characteristics of various NPs combined with biological components to decrease harmful contaminants. According to Sharma et al. (2015), AgNPs successfully decolorize organic dyes when they are no longer catalytically active. They demonstrated that NPs may be employed as a high-efficiency catalyst in industries for the breakdown of organic dyes (Sharma et al. 2015). Silver (Ag) and gold (Au) NPs have been shown to have adequate catalytic activity in the removal of organic dyes. These NPs shorten the time it takes for dye to be removed and increase the pace of response (Panáček et al. 2014). According to Bhargava et al. (2016), gold NPs can also be employed as an organic dye adsorbent. It was reported that gold NPs containing *Cladosporium oxysporum* strain AJP03 fungal surface proteins efficiently improved Rhodamine-B adsorption (Bhargava et al. 2016).

6.6 Nano-enhanced Anti-fouling

Biofouling is a main challenge and concern for maritime industries because it continuously affects marine apparatus, including platforms, ships, and nets. This harmful phenomenon accelerates corrosion, decreases the buoyancy of ships, clogs pipes and membranes, and increases fuel consumption (Kumar et al. 2021). Biofouling of marine facilities costs the maritime sector and naval forces throughout the world billions of dollars (Aghajani and Esmaeili 2021). The ancient conventional anti-fouling procedures rely mostly on the use of poisonous biocidal substances that deplete or kill organisms in the area, which is not environmentally friendly. Novel nano-based anti-fouling coatings are under development in response to ecological concerns and regulations governing the use of existing deadly biocidal coatings. The finest anti-fouling coating should be simple to apply, substrate-independent, long-lasting, dependable, cost-effective, and environmentally benign. It is still quite difficult to comprehend all the necessary needs at the same time. No one chemical or procedure has yet been recognized as the worldwide anti-fouling approach. Rather, integrating several anti-fouling techniques into a single multifunctional coating with synergistic potential is indubitably the most excellent way to attain the goal. Hybrid nanocomposites consisting of organic-inorganic compounds, for example, allow the features of distinct materials to be combined, perhaps preventing biofouling (Nathanael and Kumar 2021). Metal and metal oxide NPs, such as silver NPs, titanium, and zinc oxides, have anti-fouling properties (Song et al. 2020). These nanostructured may absorb ultraviolet and visible light and limit microbial development due to the photocatalytic process that leads to redox reactions (Spirescu et al. 2021). During photocatalysis, producing reactive oxygen species (ROS) such as superoxide, peroxides, and hydroxyl radicals restricts the growth of microorganisms (Ganguly et al. 2018). It has been found that carbon nanotubes (CNTs) in coatings reduce macrofouling by preventing larvae from settling and sticking to the surfaces of the objects (Aghajani and Esmaeili 2021).

7 Nanomaterial Mechanisms and Interactions with Contaminants and Remediators

Interaction between the NPs and the microbial remediator and other environmental components happens via different mechanisms such as absorption, adsorption, enzymatic conversion, redox reaction, photocatalysis, and filtration.

7.1 Absorption

Nanosorbents are nanosized (organic or inorganic) substances with a high surface area that absorb toxins and efficiently restore damaged sites. Zeolites, a hydrated mineral comprising oxygen, silicon, and aluminium, are one of the most extensively utilized nanomaterials. They are crystalline and contain cations such as zinc and silver on their surface inside the electrostatic holes (Bingre et al. 2018). Silver ions with antibacterial and antiviral properties are integrated into the zeolite nanoholes and aid in the efficient elimination of pollutants (Azizi-Lalabadi et al. 2019). On the other hand, graphene and metal oxides are examples of diverse varieties dependent on the material employed in their creation. The best polymeric nanocomposites are dendrimer-based nanocomposites (Hussain and Mishra 2018). They eliminate organic contaminants via the core's intrinsic hydrophobic contacts. These polymer nanocomposites are recyclable and ecologically friendly.

7.2 Adsorption

Iron NPs are unique adsorbers. They have a good oxidizing capacity but a poor reducing one. Zerovalent iron (ZVI) is oxidized by a proton or water in the absence of oxygen, releasing divalent hydrogen and iron. Following reduction, a redox reaction occurs between the contaminant and nano-zero-valent iron, resulting in trivalent iron and iron hydroxide. The reduction of impurities is aided by iron hydroxide. Zerovalent iron NPs oxidize several organic pollutants, releasing hydroxyl radicals that help to lessen the extent of these chemicals in the ecosystem (Fu et al. 2014). Pollutants such as radioactive elements, heavy metals, dyes, and inorganic and organic compounds are sequestered using zero-valent iron NPs (Lu et al. 2016).

7.3 Enzymatic Conversion

Enzymes are at the heart of bioremediation programs, which aim to lessen the environmental impact of pesticides by decreasing and transforming contaminants into less hazardous forms (Wong et al. 2021). Nanozymes are novel nanomaterials that demonstrate enzyme-like characteristics, simulating natural enzymes. Nanozymes demonstrated higher structural stability, making them attractive candidates for pollutant clean-up in the environment. Nanozyme-enhanced degradation of phenolic compounds, for example, is a promising technology that offers substantial advantages over present methods (He and Liang 2020).

7.4 Redox Reaction

It is a sort of nanomaterial with evident catalytic capabilities, as the name indicates. They have a large surface-to-volume ratio, which makes them extremely reactive. They are used to remove radioactive materials and heavy metals from the environment. Nonmetals are the nanomaterials in question (zerovalent NPs and oxides such as nano-silver and titanium oxide, respectively). Some of these oxides are superparamagnetic nanoparticles, which can be easily isolated from contaminants using a low magnetic field, such as nano-magnetite (El-Sayed 2020). Iron NPs have a high oxidation capacity but a poor reduction capacity. Zero-valent iron is oxidized by a proton or water in the absence of oxygen, releasing divalent hydrogen and iron. Both demonstrate contaminant reduction and redox reactions, and nano-zero-valent iron is found, resulting in trivalent iron linked with iron hydroxide. Contaminants degrade more quickly when exposed to iron hydroxide. By unleashing hydroxyl radicals and aiding the breakdown of these compounds, zero-valent iron NPs oxidize a range of organic contaminants (Fu et al. 2014). Bokare et al. (2013) showed that zinc exhibited enhanced reduction potential, allowing it to reduce the pollutant more quickly than iron. However, the treatment capability of zero-valent zinc NPs is limited to halogenated organic compounds such as carbon tetrachloride (Lu et al. 2016).

7.5 Photocatalysis

Due to UV radiation, ROS are generated from metal oxide NPs, which react with the pollutants in a procedure called the photocatalytic process. A good example of this is titanium oxide, which has an especially high bandgap. As a result, UV photons that produce hydroxyl radicals are required to excite this metal oxide nanoparticle. The cells are harmed by these radicals. They wreak havoc on the structure of fungal, bacterial, and algal cells, preventing them from fulfilling a variety of tasks (Foster et al. 2011). Many pollutants, including PAHs, organic compounds including chlorine, pesticides, dyes, and heavy metals, were degraded by photocatalytic nanomaterials with high photocatalytic activity and photostability.

7.6 Filtration

Nanomaterials are used in the latest generation of membrane technologies for water treatment. This method is based on a membrane that functions as a picky wall between two homogeneous phases, separating feedwater flows into a retentate and permeate fraction. The pressure difference between the feed and permeate surfaces acts as a driving force for the membrane's function, allowing water to move through (Homaeigohar and Elbahri 2017). The size, charge, and shape of solutes and particles identify them. Nanomaterials can be used to build nanocomposite membranes in a range of forms and sizes. Anti-fouling substances such as Au, Zn, Ag, Cu, GO, and TiO_2 NPs have been used in membrane technology to address this issue (Homaeigohar et al. 2019) and carbon nanotubes (CNTs) (Ghadimi et al. 2020). A significant type of coatings that may dynamically filter water is nanocomposite membranes, which consist of a fine polymeric film coated with nanofillers. Composite materials maintain a promising set of characteristics. New properties and functions emerge, particularly when the filler's dimensions reach the nanoscale (Homaeigohar et al. 2020).

8 Conclusion, Challenges, and Future Perspective

Researchers are concerned about nanotechnology due to its beneficial impacts, such as the huge surface area it provides, the ability to employ it for numerous purposes, its resilience in difficult environments, enhanced contact, and easy and efficient manipulations. The proposed creation of nanomaterials from natural precursors, particularly microbes, offers improved options for cost-effective and long-term wastewater treatment that is biocompatible, low-toxic, and either compostable or eco-friendly. This interdisciplinary combination offers new opportunities and novel functionalities that can be proven as a potential game-changer strategy. Nano-enhanced bioremediation is a new field of research, and this multidisciplinary mixture provides new possibilities and novel features that can be demonstrated as a potential game-changer strategy. The introduction of new nanomaterials with unique optical and electrochemical properties improves pollutant removal performance and offers a promising technique for limiting the spread of environmental toxins. The interactive exploration of nanomaterials and emerging environmental pollutants will help us better understand how nanomaterials work together in the environment. The residues that remain are either biocompatible or easily separated using simple filtration and precipitation procedures.

The most difficult task is commercialization. Researchers are concerned about nanotechnology's positive effects, such as the large surface area it gives, the capacity to use it for a variety of purposes, its resistance in harsh conditions, enhanced interaction, and simple and efficient manipulations. A green bioremediation technique for industrial wastes has been developed using a blend of nanotechnology, enzymes, and microorganisms. The suggested production of NPs from natural precursors, notable microorganisms, provides more cost-effective and long-term wastewater treatment options that are biocompatible, low-toxic, compostable, or environmentally friendly. This interdisciplinary approach opens new possibilities and capabilities, and it has the potential to be a game-changing strategy. Nano-enhanced bioremediation is a new field of research, and this multidisciplinary approach opens new avenues and features that can be proven as a game-changing technique. These nanotechnological characteristics are only used in 1% of cases. As a result, the widespread use of these simple and successful microorganism-assisted nanotechnology procedures will serve as a steppingstone towards environmental bioremediation. This necessitates ongoing support and confirmation from researchers, as well as government financing, to cultivate nanotechnology's potential for long-term, cost-effective production. Because no cost-related data is currently available, a cost-benefit analysis should be done for its commercialization. Introducing nanomaterials into the marine environment has many limits and obstacles. Among these obstacles is the difficulty of synthesizing non-aggregating NPs. Also anticipating the optimal approach for boosting biodegradation due to diverse indigenous populations and environmental conditions. These circumstances result in a fundamental misunderstanding of how the characteristics of NPs govern their biodistribution, orientation, and separation from seawater. Furthermore, the challenges of implementing these CO_2 capture absorbents based on nanomaterials in the real world mean that efficient C-capture devices must be built swiftly and investigated further in the future.

Controlling microbial enzymes to produce NPs with a wide spectrum of organic functional groups for selective and multi-pollutant removal from wastewater is in high demand. Computational design, material genomics, and artificial intelligence are among the cutting-edge tools that may be combined with bioengineering procedures to find more effective and translational NPs of a certain form and size. Even though researchers may improve the nanomaterial world by controlling nanomaterial synthesis, manufacturing costs, and energy efficiency, the use of recombinant organisms is currently limited. However, the existing synthesis technique remains a bottleneck, preventing in-depth investigation of nanomaterial characteristics and applications. Furthermore, environmentally friendly nanoplastic degrading technologies must be devised to restrict the spread of this minute pollution. These particles end up in landfills and eventually flow out into the sea, contaminating marine life quickly. The accumulated nanoplastics in the water injured several marine organisms, such as the larvae of the blue mussel *Mytilus edulis*, which filter and accumulate the nanoplastics of sizes 100 nm to 2000 nm within 4 h of exposure. Finally, the dumping of such NPs into the environment should be regulated by tight rules and legislation. As a result, there is still a lot of space for improvement, and long-term studies of their potential environmental impact are needed.

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Cell Immobilization for the Fungal Bioremediation of Wastewater Contaminated with Heavy Metals

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1 Introduction

The use of water for human consumption and for industrial and agricultural activities has reached an important position on the rank of global concerns. Related to these conditions, the expansion of any nation's productive capabilities is closely linked to the supply of high-quality water.

On another level, the increase in urban occupations due to the swollen cities stands out. Such demographic dynamics overload water treatment systems, causing erroneous redundancies in the process. As a result, a high demand of supplies is required, which ultimately raises operating costs considerably.

Additionally, there are specific situations that require smart water solutions, such as in desertic and semi-arid regions. The Brazilian semi-arid region, for example, is characterized by a short rainy season, high temperatures, and high evaporation rates (Brasil 2012; Oliveira et al. 2014). The Mediterranean region faces climatic droughts with certain periodicity due to the high variability of rainfall throughout the year with extensive intervals of low precipitation (Lionello, 2012; Lloyd-Hughes and Saunders 2002; Gouveia et al. 2017). Therefore, in areas with these characteristics, the rational use of water should be a permanent concern.

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It is worth noting that water sources for agricultural and industrial production, as well as those intended for human consumption, must have minimum quality standards, especially related to physical–chemical and organoleptic characteristics, such as taste and odor. Therefore, as identified by Ribeiro et al. (2012), inorganic contaminants have gained greater visibility through toxicity studies of heavy metals and their detrimental impact on human health and aquatic biota. The excessive presence of metals in the human body often results in neurotoxicity and associated neurological disorders (Li et al. 2021).

That said, it is imperative to eliminate or mitigate the presence of harmful metallic elements on water sources. Conventional removal methods can be highly expensive and inefficient for low concentrations, such as in the case of lead (Pb II) removal, which could also cause secondary pollution during the process (Fomina et al. 2005; Miransari 2011; Wanget al. 2019; Xu et al. 2020). Hence, more technologically sophisticated strategies are necessary for the treatment of contaminated water, including ultrafiltration, nanofiltration, and reverse osmosis (Peavy et al. 1986; Mcghee 1991; WHO 2017; Siwila and Brink 2018; Shah 2020). However, several of these methods are also expensive and unavailable to low-income communities (Mcallister 2005; Siwila and Brink 2018).

Alternative approaches to water purification have inspired widespread scientific research. Among several other routes there is bioremediation, a technique that aims to decontaminate soil and water using living organisms, such as microorganisms and plants (Pires et al. 2003). Microorganisms have been used with great success to decontaminate areas affected by a variety of contaminating residues (Soares et al. 2011), including petroleum products such as benzene, toluene, and xylene (Rodrigues et al. 2012).

Fungi are potential candidates to be bioremediators of contaminated areas due to their biosorption versatility and survival capability under extreme conditions of pH, temperature, and nutrient availability, as well as under high metal concentrations (Anand et al. 2006; Iskandar et al. 2011). To benefit from the application of fungi-based removal, these techniques must be adapted to allow its use in several operation cycles and to remain active under extreme conditions for long periods of time.

To overcome adverse conditions, the immobilization of microorganisms could be a viable solution in the bioremediation of contaminated water. Encapsulated cells facilitate the separation and protection against adverse external conditions, while reducing the susceptibility of contamination by foreign organisms (Keskin et al. 2018). Thus, immobilized materials can promote a microenvironment favorable to cell maintenance and even decrease cell dispersion.

2 Heavy Metals

Harmful heavy metals can be classified as toxic metals (e.g., Cr, Zn, Cd, Hg, Cu, Sn, As, Ni), precious metals (e.g., Pt, Au, Pd, Ag), and radionuclides (e.g., U, Ra, Th, Am) (Singh et al. 2021). Those metals are necessary in very low concentration to the metabolism of several organisms, including humans. However, they become toxic when their concentration in the body exceeds certain well-known thresholds (Rehman et al. 2021).

The best known heavy metals are Cd, Zn, Cu, Pb, Cr, and Hg, whose presence in the environment is linked to pollution and toxicity (Moreira 2008; DAVI et al. 2020; Shah 2021a, b). According to Frois and Pereira (2020), toxicity is defined as the essential ability of a chemical agent to damage the health of living organisms. High levels of harmful elements and substances are a consequence, in many cases, of human action, predominantly in economic activities.

Solid waste from human activities constitutes an important source of contamination by heavy metals, affecting primarily soils and water reservoirs. This occurs, for example, in landfills, where high levels of zinc have been reported (Oliveira et al. 2019). Thus, the management of solid waste and any other contaminant source must be an object of constant concern, given that even groundwater reserves could be reached by those chemicals.

Many industrial effluents generate waste that contains heavy metals. Some of the main heavy metals and their associated industries can be highlighted: Zn from metallurgy, battery manufacture, galvanizing, and electroplating processes (Radhika et al. 2006); Cu from mining sites, landfills, domestic waste waters, and combustion of fossil fuels (Singh et al. 2021); Cr from metal plating tanneries, pigment oxidants, oil well drilling, sewage, and fertilizers (Ghani 2011); Cd from mining, tobacco smoking, incineration, and fertilizers manufacturing; Hg from industries involving paper and pulp preservation, medical waste, pharmaceuticals, and caustic soda production (Morais et al. 2012).

3 Bioremediation

Bioremediation is a naturally occurring process by which microorganisms immobilize or transform environmental contaminants into harmless products (Thassitou and Arvanitoyannis 2001).

Due to their ability to survive in inhospitable environments, particularly those contaminated by heavy metals, microorganisms have developed various detoxification mechanisms. Among many are biosorption, bioaccumulation, biotransformation, and biomineralization, all of which can be exploited for ex situ or in situ bioremediation (Gadd 2000; Lin and Lin 2005; Dixit et al. 2015). In situ bioremediation presumes that the treatment takes place at the site of contamination. In a case of ex situ bioremediation, the contaminated material is sent for treatment in a place other than its origin (Verma and Kuila 2019).

According to Lemos et al. (2008), biosorption is the adsorption of metallic species on the microorganism's cell surface via physical–chemical mechanisms. Biosorption mechanisms may involve adsorption, ion exchange, and covalent binding with the biosorptive sites of microorganisms, including carboxyl, hydroxyl, sulfhydryl, amino, and phosphate groups (Frurest and Volesky 1997; Say et al. 2001; Iskandar et al. 2011). In the fungal cell walls, there are polysaccharides, polypeptides, and proteins, which are equipped with negatively charged functional groups and show excellent binding properties (Fomina and Gadd 2014; Chen et al. 2019; Xu et al. 2020).

Bioaccumulation is a phenomenon caused by the microorganism's defense system, which occurs in reaction to toxic species in their environment (Pino 2005; Davi et al. 2020). The bioaccumulation also includes metabolism-dependent processes that lead to the transport of metal to fungal cells (Legorreta-Castañeda et al. 2020).

The biosorption process can be carried out with live, dead, or inactivated fungi. In the case of dead or inactivated fungi, there is no need for a continuous supply of nutrients. In addition, one could avoid concerns about cell toxicity in the face of high metal concentrations. It also enables the regeneration of biomass and its reuse in several cycles (Binupriya et al. 2007; Legorreta-Castañeda et al. 2020). These characteristics make the biosorption with non-viable cells process appear to be more interesting for large-scale application compared to bioaccumulation (Ajmal et al. 1996; Dilek et al. 1998; Dixit et al. 2015).

The immobilization of fungi for use in the removal of heavy metals is an interesting alternative for both biosorption and bioaccumulation. The encapsulation of non-viable fungal structures prevents their dispersion in the aqueous medium, while coated living microorganisms remain protected from the stress related to metal toxicity.

4 Bioremediation with Free Fungi

Among heavy metal removal techniques, biosorbents such as fungi and bacteria have replaced traditional effluent treatments. In addition, these biosorbents are considered the most economical and safe options, with the possibility of recovering the biomass at the end of the process (Basha and Jha 2008; Aftab et al. 2013). It is also worth noting that conventional processes can be ineffective and/or expensive, especially when the concentration of heavy metals in solution varies from 1–100 mg/l (Nourbakhsh et al. 1994; Kumar et al. 2014).

Fungi have a high potential in the removal of metals in contaminated water, and several studies have confirmed this claim. Table 1 presents several fungi involved in the bioremediation of heavy metals, as well as the main metals removed by these microorganisms.

In the report by Sabuda et al. (2020), the fungus of the phylum *Ascomycota* was used to remove selenite and selenate from sewage with great success. The biosorption removal of chromium (Cr(VI)) and cadmium (Cd(II)) using a consortium of fungi of the genus *Aspergillus* resulted in a reduction of around 70% of the species in liquid medium (Talukdar et al. 2020). In a study about the employment of *Rhizomucor pusillus* and *Aspergillus flavus* in the bioaccumulation of metals in aquatic environments, the strains were tolerant to levels up to 100 mg/l of Pb, Cr, and Cd (Qayyum

Table 1 List of recent studies regarding fungi in the bioremediation of heavy metals, as well as the main metals removed by these microorganisms

Fungi	Metal	References
Cladosporium halotolerans	Mn(II), Cd	Wang et al. (2022)
Trichoderma lixii	Cu^{+2}	Kumar and Dwivedi (2021)
Lecythophora sp.	Hg	Chang et al. (2019)
Ganoderma lucidum	Pb, Zn, Ni, Cu, Cd	Ipeaiyeda et al. (2020)
Talaromyces islandicus and *Aspergillus terreus*	Pb	Sharma et al. (2020)
Aspergillus flavus, A. gracilis, A. penicillioides (sp. 1), A. penicillioides (sp. 2), A. restrictus and *Sterigmatomyces halophilus*	Cd, Cu, Fe, Mn, Pb, Zn	Bano et al. (2018)
Penicillium piscarium	U	Coelho et al. (2020)
Penicillium citrinum and *Trichoderma viride* *Rhizopus sp* *Aspergillus flavus* CR500	Cr (VI)	Zapana-Huarache et al. (2020) Espinoza-Sánchez et al. (2019) Kumar and Dwivedi (2019)
Aspergillus niger (M1DGR), Aspergillus fumigatus (M3Ai) and *Penicillium rubens (M2Aii)*	Cd, Cr	Khan et al. (2019)
Penicillium spp.	Hg(II)	Chang et al. (2020)
Aspergillus penicillioides (F12)	Pb (II)	Paria et al. (2022)
Penicillium chrysogenum FMS2	Cd	Din et al. (2021)
Neurospora crassa and Aspergillus flavus	Ni(II)	Sharma et al. (2021)

et al. 2016). Bioaccumulation at these levels confirms the effectiveness of microorganisms in a critical concentration range for removal by conventional methods (0–100 mg/l) (Nourbakhsh et al. 1994; Kumar et al. 2014). Therefore, these findings demonstrate the effectiveness and high potential of fungi in the biosorption and bioaccumulation of heavy metals.

5 Bioremediation with Immobilized Fungi

Immobilization refers to the various forms in which cells can be trapped in matrices or supports (Elizei et al. 2014). The technique uses a series of substances that act as envelopes, which, depending on their conformation and composition, can considerably alter the environment of the once free cells.

Dias et al. (2001) noted that significant factors such as nutrient diffusion, oxygen transfer, physical, and chemical properties of the immobilizer, and the immobilization procedure highly affect microbial metabolism and system efficiency. According to Saifuddin and Raziah (2007) metabolically active cell immobilization is the first option when there is a need for cofactors in catalytic reactions, for example, and the technique excludes many restrictions typical of cell-free systems.

Microorganisms tend to naturally create protective mechanisms against adverse conditions. Biofilms production is an example of this behavior, as they are generally extracellular polymeric substances formed to protect against stressful situations (Chew and Yang 2016; Valdivia-Rivera et al. 2021). Analogously to biofilms, the cell immobilization method also preserves the desired catalytic activity for both laboratorial and industrial applications (Covizzi et al. 2007). For this purpose, the cell encapsulation technique employs a semi-permeable coating substance that enables the diffusion of nutrients and substrates to the microorganism (Kumar et al. 2016; Wang et al. 2020).

Several materials are used in the production of microcapsules for environmental applications, such as alginate, polyvinyl alcohol (PVA), silica gel, and biopolymers that include chitosan, starch, and carrageenan. These substances allow the entry of nutrients and the exit of metabolic residues (Majewsk et al. 2016 and Valdivia-Rivera et al. 2021). Table 2 presents some fungi, recovered metals and fungi immobilizing materials.

For example, Cai et al. (2016) reported the use of living *Penicillium janthinellum* conidia immobilized in polyvinyl alcohol (PVA)-sodium alginate granules. This system was applied to remove copper, lead, and cadmium from aqueous solutions, constituting an interesting low-cost alternative for the assimilation of heavy metals. In addition to the already established immobilizing materials, alternative materials have been used with considerable success, and their results reported in the literature. Sharma and Malaviya (2016) obtained promising results using immobilized fungal consortium on corn cob and coir to remove heavy metals in wastewater from

Table 2 List of recent studies regarding fungi, recovered metals, and immobilizing materials

Fungi	Metal	Materials	References
Pestalotiopsis sp	Cu, Cr, Pb, Zn	Alginate	Choo et al. (2019)
Aspergillus nidulans	Cu^{+2}	Cellulose Membrane	Toledo et al (2021)
Trichoderma harzianum	U	Ca-Alginate	Akhtar et al. (2009)
Aspergillus ustus, Fusarium verticillioides, and *Penicillium funiculosum*	Cr	Nanosilica	Mahmoud et al. (2015), Velkova et al (2018)
Phanerochaete chrysosporium	Hg	Carboxymethylcellulose	Saglam et al. (2002), Blaga et al (2021)
Ustilago maydis and U. digitariae	Cr, Cu, Cd, Ni, Zn	Chitosan	Sargin et al. (2016)
Aspergillus niger	Cu, Mn, Zn, Fe, Pb, Ni,Cd	PVA and Ca-alginate	Tsekova et al. (2010)
Penicillium janthinellum	Pb, Fe, Cu	PVA-sodium alginate	Wang et al. 2021

the tannery industry. In another study, Ahn et al. (2020) observed that fungal structures of *Aspergillus fumigatus* increased the assimilation capacity of heavy metals present in wastewater from the dye industry when externally surrounded by halloysite nanotubes, a mesoporous clay mineral.

Under certain conditions, the immobilizing support also acts as an adsorbent, intensifying the uptake of heavy metals (Costa and França 1996; Covizzi et al. 2007). Tan and Ting (2012) evaluated viable and non-viable cells of *Trichoderma asperellum* immobilized with alginate. The results indicated that the uptake of Cu (II) by immobilized viable cells was quantitatively similar to the one observed by the pure alginate control.

The effective action of free cells to remove harmful chemical species faces some operational limitations. Among such constraints, it is important to emphasize low cell density, reduced survival in the medium, and difficult separation of biomass from the analyzed effluent (Wang et al. 2007; Sharma and Malaviya 2016; Cai et al. 2016). Despite the deficiencies presented, the immobilization of fungal cells or their structures represents a promising alternative for the removal of heavy metals in contaminated water.

6 Conclusion

Fungi have great potential to assimilate heavy metals in contaminated water, since they can capture metallic species by several mechanisms. However, their action can be strengthened by cell immobilization. This process brings several benefits such as the maintenance of microorganisms in inhospitable environments, and the reduction of operating costs. In addition, cell immobilization can promote the system reuse in repeated operation cycles.

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Wastewater Treatment Technologies

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1 Introduction

Industrialization and urbanization are the backbone of economic growth in various countries of the world. This process, however, has led to the generation of wastewater in large quantities. The need for wastewater purification is paramount due to the scarcity of water, hence, the need to treat and re-use for human consumption. Wastewater generally refers to water which contains various pollutants generated from various anthropogenic activities such as commercial, agricultural, domestic,

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as well as runoff water from other sources (Edokpayi et al. 2017). Wastewater have varying physical and chemical properties which differ based on their chemical compositions as well as flow conditions as this is a feasible parameter for the design of specific waste treatment plants. The conditions of flow in wastewater differ depending on the specific seasons since it is during the wet season that there is inflow of run-offs. The inorganic and organic contaminants present in wastewater are employed and functional indicators of the physicochemical properties of the wastewater. Some of the paramount characteristics such water include pH, total phosphorus, total nitrogen, chemical oxygen demand, and biochemical oxygen demand (Englande et al. 2015).

The treatment of wastewater also known as sewage treatment involves the removal of various impurities from sewage or wastewater before it gets into natural bodies (oceans, lakes, river, or estuaries) or aquifers. It is a process employed for the removal of various pollutants from wastewater and the conversion into effluents which can be released back to the water cycle. When returned into the water cycle, the effluents have acceptable impacts on the ecosystems and entire environment and can be re-used for different purposes (Talabi and Kayode 2019; Shah 2020). Basically, water is considered to be polluted when it has high amount of impurities thereby making it unsuitable for a specific usage such as swimming, fishing, or drinking. Even though the quality of water is influenced by natural conditions, pollution is commonly used with regards to anthropogenic influence as pollution source. The pollution of water, therefore, is brought about by the draining of polluted wastewater into groundwater or surface water and treatment of wastewater is a prominent feature of water pollution monitoring and control. When trace quantity of sewage is released into a body of flowing water, a natural mechanism is involved in the self-cleansing of the water. However in populated areas, a large amount of sewage is generated thereby affecting the self-purification processes, hence, wastewater treatment becomes paramount prior to disposal or re-using. Most of these techniques for water treatment comprise basically of physical and chemical processes which have emerge over time (Patel et al. 2019).

There is pressing need for the development of novel methods for the mitigation of the effects of wastewater on the environment that is currently degrading. Fresh water has become a very scarce resource with high demand most especially in developing countries where contamination is high. Various researchers have documented numerous technologies for the treatment of wastewater. One of the prominent reason responsible for the emergence of new technologies in the treatment of wastewater is the hefty fines and legislation that go with the disposal of wastewater that do not meet the permissible limits based on standards. Such impact with its financial implication on the industry has given rise to the emergence of more improved technology for the treatment of wastewater (Ahmed et al. 2021; Shah 2021). There are different technologies available for the treatment of wastewater. The use of conventional approaches in the treatment of wastewater is highly limited due to the inherent limitations of some of these techniques. The current trend of emerging contaminants in wastewater further engenders many challenges. Various groups of contaminants are generated from anthropogenic activities such as agriculture, and industrial production

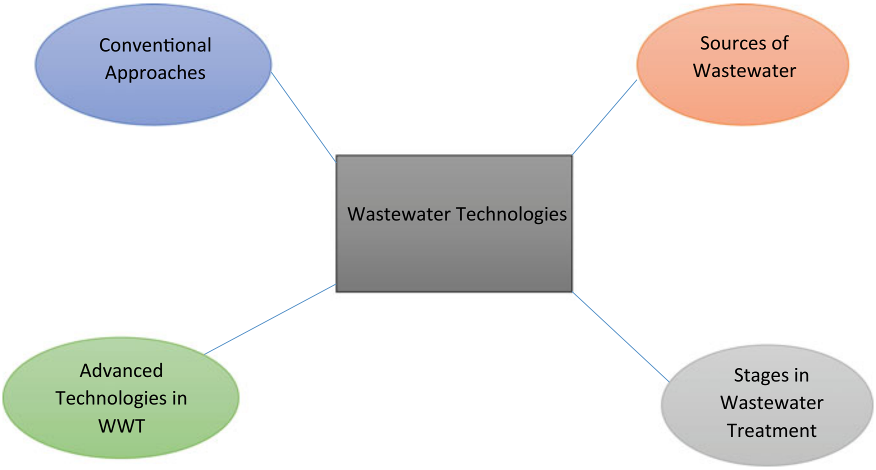

Fig. 1 Schematic representation of chapter

processes, among others (Bunce et al. 2018). Some of the existing techniques have evolved over time with the advent of new classes of environmental contaminants. The choice of technology to be adopted is determined by the nature and compositions of the wastewater. It is therefore paramount for efficient characterization of the wastewater prior to selection of appropriate technology for its treatment (Armah et al. 2020).

This chapter discusses the various technologies employed for the treatment of wastewater. It also highlights the sources, composition, and stages involved in the treatment of wastewater as schematically represent in Fig. 1.

2 Contaminants in Wastewater

2.1 Metals

There are various categories of metals in wastewater mainly from various activities such as mining, manufacturing, and textile industries, among others. Some of the metals present in wastewater include chromium, copper, lead, tin, arsenic, mercury, potassium nickel, aluminum, etc. They are generated from industries such as iron, steel, textiles, and micro-electronics. The presence of metals in wastewater brings about a rise in the cost of treatment (Hughes et al. 2020).

2.2 Nitrogen and Phosphorus Compounds

There are various nutrients of plants that are found in wastewater. These usually occur in the form of ammonia and nitrate from fertilizers producing companies. The total nitrogen is a combination of organic and inorganic nitrogen as well as ammonia present in wastewater. They exist as nitrite, nitrate, ammonium, and organic compounds that are dissolved in water.

2.3 Total Solids

This is made of organic and inorganic materials; settle-able substances dissolved and suspended matter, and volatile solids, among others. Although physical methods of separation are suitable for the removal of suspended solid materials, some of these particles still find their way into the environment. The total dissolved solids continue to increase as a result of chemicals from cleaning, washing, and processes of production (Jasim and Aziz 2019).

2.4 Microorganisms

There various groups of microorganisms that are found in wastewater. Various classes of bacteria, protozoa, and viruses have been documented in wastewater. Bacterial and viral infections due to water borne outbreaks are commonly linked with the areas with the release of wastewater. The presence of enteric viruses in wastewater has been associated with diseases such as hepatitis, respiratory tract infection, and gastroenteritis.

2.5 Pharmaceutical Compounds

There are also groups of emerging pollutants in wastewater which have long-term impacts on aquatic habitants and humans. Such compounds include antibiotics, analgesics, anti-cancer agents, lipid regulator, and hormones, among other. Their presence is also due to the fact that humans' drugs are released either in metabolized or their original forms. The various compositions of wastewater are presented (Fig. 2).

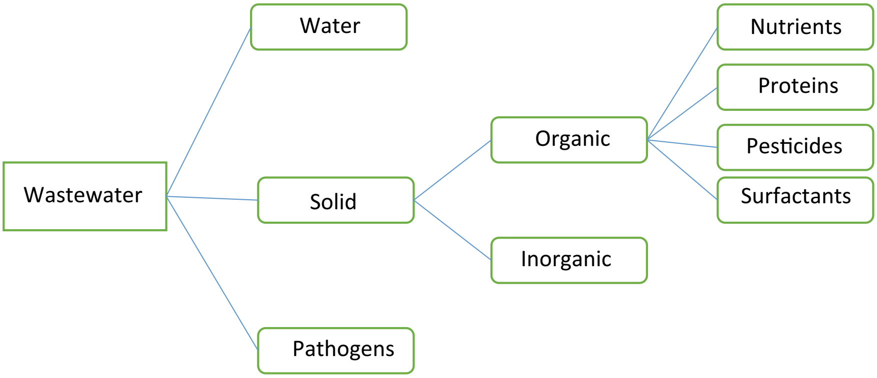

Fig. 2 Compositions of wastewater

3 Stages in the Treatment of Industrial Wastewater

Wastewater from industries contains various inorganic and organic materials in different concentrations depending on the activities within the industries. Some of the materials present are not readily acted upon by activities of microorganisms. The treatment of wastewater occurs in stages.

The traditional processes for the treatment of wastewater consist of preliminary steps, primary stage, secondary stage, and tertiary procedure which function on the basis of biological, chemical, and physical processes. The most prominent primary process involves the treatment with activated sludge. Wastewater treatment involves the conduction of the activated sludge stage such as the use of biofiltration. Such processes have proven effective in the removal of compounds. The efficiency the process in the removal of drugs varies within different investigations and is depended on the efficient construction and design of the wastewater facility for treatment. The conventional approaches are highly limited, hence, other processes such as ozonation, reverse osmosis, as well as advanced oxidative processes are more promising in the treatment of wastewater (Loucks and Beek 2017).

3.1 The Primary Stage

In the primary stage various solids matter, particulates, oils and other materials suspended are removed from the water. The primary stage basically involves the use of physical methods for the removal of these materials. It involves the use of oil separators for removing oil from the surface of the water, primary clarifiers for removing various materials and screens.

3.2 Secondary Stage

Secondary is considered as the heart of the process of wastewater treatment. It involves the biodegradation of various pollutants. Thus, biological processes involving the use of microorganisms such as bacteria are adopted. Activated sludge treatment using aerated system is also carried out here. The use of aerated activated sludge mechanism is highly promising and it is less complex, efficient in terms of cost, and highly efficient. The removal of many contaminants like organic pollutants and various soluble biodegradable substances is achieved through the use of aerobic and anaerobic treatment processes. There is also a rise in the use of membrane technologies for the treatment of wastewater. Various classical treatments involving the use of chemicals as well as the application of modern-advanced systems have been adopted in the efficient treatment of wastewater from industries.

3.3 Tertiary Stage

The tertiary stage in the treatment of wastewater involves the filter steps which is usually the final, finishing, and polishing stage such as the use of carbon filters made of activated materials. The tertiary stage is also referred to as the polishing stage during which the water is disinfected to the highest state. The stage is vital for the production of water of remarkable specification like the technical waters and for the treatment of wastewater use in public water system. The tertiary state of treatment includes chemical disinfection as well as UV disinfection. The disinfection using UV does not need the use of chemicals hence can be applied in the place of chemicals. The use of UV has no effect on the physical properties of the water such as smell, taste, appearance, and pH (Ahmed et al. 2021).

Stages	Activities	Processes involved	References
Primary	Various suspended solid materials are removed from the wastewater prior to passage to the other stage Removal of various activated sludge materials	Sedimentation and flocculation Smuts skimming Use of bioreactors, use of aerated ponds, biofillers, rotating biological contactors, and microbial induced oxidation ditches	Donald et al. (2022)
Secondary	Phosphate, nitrates, and other compounds are removed		Englande et al. (2015)
Tertiary	Other inorganic and organic nutrients are also removed	Use of chlorine, ozone, and other chemical substances Use of chloramines and chlorine dioxide	Milani and Bidhendi (2022)

3.4 Coagulation and Flocculation in Wastewater Treatment

Most industrial wastewater treatment plants consist of sedimentation in their treatment process. The sedimentation process also known as the clarification involves the lowering of the speed of the wastewater below the velocity of suspension, making it possible for the particles suspended to settle down as a result of the action of gravitational force. The wastewater then proceeds to the next stage of treatment after leaving the sedimentation tank. The efficiency of the overall performance of this stage is determined by temperature, details of tanks, and duration of retention, among others (Njewa et al. 2021). Coagulation is the process which involves the addition of various chemical compounds which speed up the sedimentation of some particles inside the tank. These coagulants consist of various inorganic and organic compounds such as aluminum hydroxide chloride, aluminum sulfate, and cationic polymers of high molecular weights. The primary essence of coagulant addition is for the removal of about 90% of the solids suspended in the wastewater in this stage.

4 Technologies for the Treatment of Wastewater

Technologies for the treatment of water have evolved over time in view to meeting the dynamic demands of this era. Several trends in water treatment techniques have been reported in different countries of the world. The production of wastewater is of environmental concern due to the presence of various classes of organic, inorganic, and biological contaminants in the water which poses health challenges to humans. Presence of new and emerging contaminants in wastewater has given rise to the need for advanced approaches to wastewater treatment. The traditional approaches to the treatment of wastewater are highly limited in the treatment and removal of some of these contaminants, and this could further worsen the existing challenges of water scarcity (Sathya et al. 2022).

4.1 Biotechnological Approaches in Wastewater Treatment

i. Microalgae technology
   This technique in the treatment of wastewater is autotrophic in nature with a remarkable potential of fixing the atmospheric carbon. This technology is commonly employed after the treatment of water in upstream as a secondary or in some other cases tertiary processes of treatment for various effluences containing organic materials and heavy metals contaminants in wastewater. It is an attractive dimension involved for the treatment of wastewater which also gives rise to the generation of biomass which has a high value that can be applied for various purposes. The application of microalgae in this regards gives rise to

highly minimal risk in terms of production of secondary contamination due to its potential of utilizing phosphorus and inorganic nitrogen for the purpose of growth and their unique ability for the removal of toxic organic substances and heavy metals.

ii. Microbial fuel cell technique
Microbial fuel cell (MFCs) this is one of the fastest growing technology involved in the treatment of wastewater. This approach makes use of the bioelectrical catalytic process of the microorganisms that are involved during the generation of electric power through the oxidation process of inorganic and organic material that are found in the wastewater. This technology offers two different goals since it permits the recovery of energy as well as the wastewater within a unit configuration.

iii. Aerobic and anaerobic-based technology
This approach has been recently adopted in the treatment of wastewater due to the fact that they are environmentally friendly and also cost effective. The anaerobic approach, however, has a clear cut above some of the other techniques most especially due to it lower energy demand. Various technologies based on the use of microorganisms have been employed for the treatment of wastewater. This has made it possible for the degradation of various organic contaminants under aerobic conditions. The biodegradation of organic contaminants under aerobic conditions is cheap, simple, and eco-friendly in the breakdown of waste. Some of the essential factors affecting the biodegradation of the various contaminants include moisture, nutrients, pH, temperature, rate of aeration, and aeration. Aerobic processes are basically employed as the primary means for the reduction of BOD in the wastewater due to the fastness of the anaerobic process of microbial reactions. Aerobic bioprocess, however, has its disadvantage in wastewater treatment which that it results to the production of large amount of sludge when compared to the anaerobic process (Chahal et al. 2016). A remarkably high concentration of biomass takes place in the aerobic bioreactor due to the yield of biomass for aerobic microbes is comparably high, about four times higher than that of anaerobic microorganisms. The sludge that is found inside the reactor effluents may have remaining BOD which makes it necessary to reduce them further in the subsequent processes.

4.1.1 Merits of Anaerobic Biodegradation of Contaminants

The anaerobic processes of microbial breakdown of contaminants in wastewater have the following merits: The byproducts generated are useful for various purposes such as generation of methane gas, it is relatively cheap and limited cost during the passage of oxygen into the reactor, it can be operated even at a high toxicity of contaminants and higher waste BOD, and it has a relatively lower formation rate of the sludge. Aerobic processes, however, have certain delimitations such as it high operating cost and restricted during the treatment of wastewater in streams of low rate of flow (Shevtsov et al. 2022).

4.2 Role Membrane Technology in Wastewater Treatment

This approach to wastewater treatment includes various interconnected scientific and engineering approaches for the movement of species, components, or materials through a membrane. This technology is used generally for the explanation of the mechanical phenomena for the separation of liquid or gases streams. Membranes are grouped as thin layers boundary for differential separation based on sizes of the substances. The membranes are mostly integrated with biological and chemical treatments or as a system standing-alone during the secondary treatment of contaminated water. In a typical system of membrane separation, a driving force exists such as a selectively permeable boundary which is capable of controlling the speed of movement of the various components through fractional permeation and also rejection through the varying sizes pores. The selective rejection and permeation are determined by the chemical affinity and pore sizes of the membrane (Naidoo and Olaniran 2013).

Some of the typical types of membrane technology are as follows:

i. Microfiltration (MF)
This makes use of a mechanism for sieving for the retention of macromolecules or some other particles that have sizes than are bigger than 0.1 micro meters, of specially, within the range of 0.1 to 10 μm. The transmembrane pressure in the both side of these membranes is low due to the retaining of the smaller particle components. Bigger pore sizes of the membrane filtration boundaries tend to limit the process of removing various suspended materials, viruses, bacteria, and organic colloids, among others that are within the region.

ii. Ultrafiltration (UF)
This approach to wastewater treatment has gained remarkable attention in recent times due to its application in bioreactors and desalination processes. It uses sieving process as its mechanisms of separation. The sizes of the pores used in UF range from 0.05 μm to 1 nm, the cutoff based on molecular weight varies from 1 to 500 kDa with an operational pressure of 1 to 7 bar. The use of UF has progressively gained popularity in industrial applications. It has been used as pretreatment process for nanofiltration and reverse osmosis technology. There is extreme distinguishing of fouling in UF utilization, as a result of the large molecular weight of the different fractions that are retained with respect to the small differential osmotic pressure. The configurations on the applications of UF are affected by cost implication (Inobeme et al. 2023).

iii. Ion exchange membrane

A membrane is said to be an anion exchange membrane if its polymeric matrix has a fixed charged group enclosed within it, and the other way round for a cation exchange membrane where there is permeation of cations/anions and also the rejection of anions/cations. There are various processes that involve the exchange of ionic particles across membrane between solutions, such as diffusion dialysis, reverse electrodialysis, and Donnan membrane technique.

iv. Reverse and forward osmotic process

This is commonly referred to as a membrane that is tight and has been broadly employed in the treatment of wastewater. It has also been reported to be vital for the desalination of water with better results when compared to the traditional thermal flashing. Hypertonic feed is employed for the generation of high pressure from the external region, within range of 15 to 150 bars, and this external pressure is higher than the osmotic pressure. This pressure is applied for the retaining of the dissolved solute which prevent and permit for the permeation of solvent. Reverse osmosis has certain advantages which include simplified configuration, low energy consumption, low membrane fouling occurrence, and high rejection of various ranges of pollutants (Nasir et al. 2022).

v. Electrodialysis

This is an approach that combines the various principles of generation of electricity and ion permeable boundary for the separation of dissolved ions present in water. The differences in the potential are responsible for the movement of ions into a concentrated solution from a dilute solution through a membrane that is permeable to ions.

4.2.1 Advantages of Membrane Technologies

Membrane technology has several merits in its application in wastewater treatment which include: energy saving potential, its clean and environmentally friendly nature, its potential of replacing some of the existing approaches such as ion exchange, filtration, chemical treatment and distillation, it unique ability of giving rise to products that are of high quality, and its remarkable flexibility with respect to design of the system. Due to its high flexibility and its multidisciplinary potential of applications, it has been adopted in various industries (metallurgy, biotechnology, chemical, and pharmaceutical) for the treatment of sewage water (Tetteh et al. 2018).

4.3 Chemical Oxidation Processes in Wastewater Treatment

Oxidation is a process during which electrons are shifted from a substance to another resulting to a potential commonly expressed as volt. The use of chemical oxidation seems to be a potential solution which complies with wastewater policies and legislations. This is commonly used after the secondary stage of wastewater treatment for the breakdown of various compounds that cannot be acted upon by microorganism. A primary parameter of reference in the oxidation processes for wastewater treatment is the chemical oxygen demand (COD). Wastewater that contains a small COD can be easily treated using these processes because a higher COD contents would need the utilization of high quantity of reactants that are expensive (Armah et al. 2020).

4.4 Advanced Oxidation Process (AOPs)

AOPs refer to the processes of treating wastewater that involve the formation of highly reactive intermediates such as free radicals and hydroxyl species under ambient pressure and temperature. The free radicals are generated in such concentrations that they can induce the purification of the contaminated water. This approach has been found highly promising approach in the remediation of contaminated ground water, wastewater, and surface water that contain a high amount on organic contaminants that are non-biodegradable. The hydroxyl radicals formed are very reactive and capable of attacking most of the organic compounds present in the wastewater resulting to their breakdown.

The use of AOPs, however, has certain limitations. It is difficult applying AOPs in permanent operations and also difficult applying it in a whole site. In some big critical treatment sectors, AOPs are needed for the purpose of dealing with peak COD so as to meet the treatment levels. AOPs are also efficient during the conversion of recalcitrant substances into intermediate species that can easily be acted upon by microorganism, through their transfer into the biological units where they are mineralized. Among the various types of AOPs, Fenton has been reported to be highly effective for the efficient treatment of wastewater breaking down pesticide residues, surfactants, dyes, and aromatic amines. The Fenton reagents have the advantage that it does not require energy input for the activation of the peroxide (Kesari et al. 2021).

5 Conclusion and Future Trends

Wastewater treatment is an issue of global concern. Wastewater is a serious challenge for different countries of the world as a result of the rising contents of unknown and undesired contaminants which are toxic to human health. It has been generally recognized that the challenges associated with the treatment of wastewater have serious effect on several other sectors such as energy, water, and food. In this work, we have highlighted the various technologies for the treatment of wastewater.

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Genetically Modified Microbe Mediated Metal Bioaccumulation: A Sustainable Effluent Treatment Strategy

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1 Introduction

The increasing demand for metal supplies in various industries at a global scale has resulted in large-scale mining which is associated with release of heavy metals in the environment. Several industries associated to electronics, metal plating, paper, tanneries, paint and pesticides, tanneries discharge large volumes of metal contaminated effluent into the environment (Mathivanan et al. 2021). Heavy metals are non-biodegradable and toxic to the flora and fauna. Thus, cadmium, copper, cobalt, chromium, mercury, lead, zinc and others metal contamination through industrial effluents poses a dangerous threat to human and environmental health (Diep et al. 2018; Ghosh 2020).

Several conventional methods of wastewater treatment methods focus on heavy metal removal some of which includes chemical precipitation, coagulation/flocculation, membrane filtration, ion exchange method, photocatalysis as well as adsorption (Ghosh et al. 2021d; Carolin et al. 2017). Although several all of these techniques are useful in heavy metal removal, they do have certain drawbacks such as toxic by-product formation, energy intensive, utilization of unsustainable sources like coal and oil (Ghosh et al. 2021e; Mahapatra et al. 2020). Therefore, extensive research on sustainable effluent treatment strategies having an eco-friendly approach is going on for effective heavy metal removal.

Biology-mediated heavy metal removal is one such potential approach that involve cost-effective and eco-friendly heavy metal bioremediation using biomass of plants,

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animals and microorganisms as well (Ghosh and Webster 2021). Biological heavy metal removal is primarily carried out either through biosorption or bioaccumulation (Priyadarshanee and Das 2020). Although several microbial biosorbents have been demonstrated to have excellent heavy metal removal ability, they certainly have some limitations which are similar to that of conventional adsorption (Sharma et al. 2021; Ghosh et al. 2021b). Since biosorption is a surface phenomenon, it is susceptible to pH, temperature and ionic strength variations of heterogeneous water effluent samples (Ghosh et al. 2021a). Moreover, majority of biosorbents are composed of dead biomass that gradually degrades which results in fouling of the biosorbent followed by reduction in metal adsorption efficiency (Fomina and Gadd 2014).

Unlike biosorption, bioaccumulation is a metabolically-driven process which is commonly observed in plants and microorganisms (Ghosh et al. 2021c). Several microbes have also been reported to accumulate heavy metals in their intracellular space through multiple translocation pathways (Tarekegn et al. 2020). Since bioaccumulation is performed by live cells, the process is entirely dependent on the genetic expression levels of the cell in response to heavy metal stress. Thus, genetic modification of bacteria for enhanced uptake and tolerance against these heavy metals is in this chapter that and summarized in Table 1. Implementation of genetic engineering for introducing various metal ion import and storage systems has been explained in this chapter that can improve metal uptake ability of the engineered cells. Further exploration and detailed studies to alleviate decreased cellular viability, excessive protein aggregation, loss-of-phenotype due to competition along with other drawbacks of these genetically modified bacteria could potentially prove to be useful for making bioaccumulation an amicable wastewater treatment strategy.

2 Bioaccumulation by Genetically Modified Bacteria

2.1 Arsenic

Arsenic (As) is one of the most hazardous elements that can cause severe arsenicosis which is associated with skin lesions, diabetes, reproductive impairment, high blood pressure and cancers of the skin, bladder, kidney and lungs (Ghosh et al. 2021a). Several microbe-associated bioremediation processes are developed for its effective removal. Villadangos et al. (2014) reported genetic engineering of As resistant strain of *Corynebacterium glutamicum* for enhanced accumulation of inorganic As species. Double mutation in arsenate reductases of *C. glutamicum* namely, ArsC1 and ArsC2 resulted in 28-fold increase in As(V) accumulation as compared to control strain. Similar results were obtained for *C. glutamicum* 2Δars mutant which lacked *ars1* and *ars2* operons and accumulated 28.80 nmol/mg of As(V) as well. The As(V) uptake in ArsC1-C2 mutant was not affected in presence of As(III). Moreover, mycothiol (MSH) mutants namely MshA and MshC mutant strains also demonstrated enhanced arsenic accumulation of 27.50 and 30.00 nmol/mg, respectively. As(III) accumulation was also enhanced through mutation in arsenite permeases Acr3-1 and Acr3-2 wherein, 2Acr3 and 2Δars mutants accumulated 3.60 and 1.94 nmol/mg of As(III), respectively. In presence of As(V), 2Acr3 mutants displayed further increase in As(III) accumulation to 7.44 nmol/mg. Overexpression of aquaglyceroporin genes (*glpF*) from two actinobacteria namely, *C. diphtheriae* NCTC 13129 (*CdglpF*) and *Streptomyces coelicolor* A3(2) (*ScglpF*) demonstrated further improvement in As(III) uptake by 2.6 and 2.1 fold, respectively. Maximum accumulation of As(III) was observed in 2Acr3 mutant harbouring pEglpCd plasmid. The proposed mechanism of bioaccumulation of As can be seen in Fig. 1.

In another study, *Escherichia coli* JM109 strain was genetically modified by Su et al. (2009) for improved As bioaccumulation. Heterologous expression of human

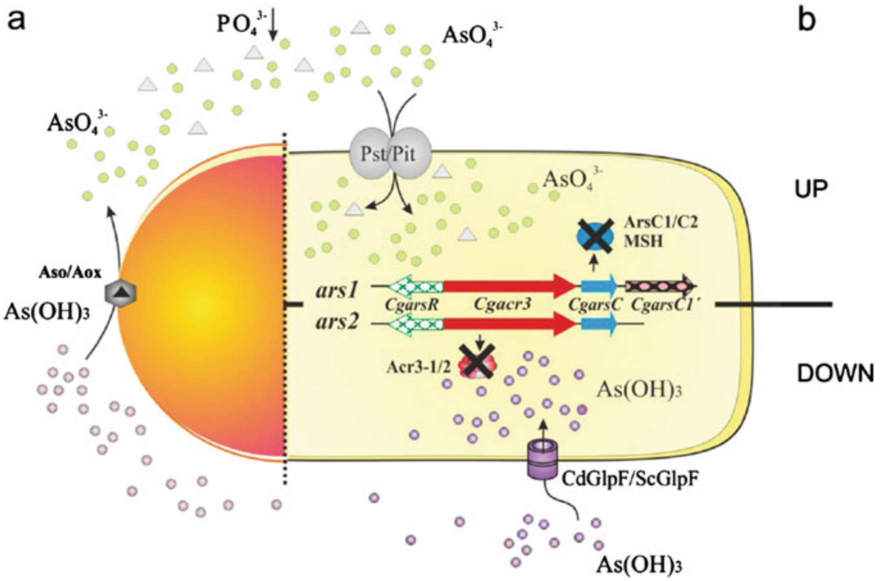

Fig. 1 Proposed microbial consortium system for the bioremediation of inorganic arsenic species. (**a**) In aquatic environments and at neutral pH, some species of bacteria (e.g. *Ochrobactrum tritici*) use periplasmic arsenite oxidases (Aso/Aox proteins) to oxidize arsenite [$As(OH)_3$; As^{III}] to arsenate (AsO_4^{3-}; As^V), which provides an energy source. (**b**) When phosphate levels (PO_4^{3-}) in the environment are low, engineered *C. glutamicum* strains can use the Pst/Pit system to incorporate As^V. Strains lacking functional arsenate reductases (ArsC1/C2; involved in biochemical conversion of As^V to As^{III}) or unable to synthesize MSH (UP) accumulate As^V; mutant strains lacking arsenite permeases activities (Acr3-1/2; involved in cellular extrusion of As^{III}) and expressing heterologous GlpF proteins (CdGlpF/ScGlpF) (DOWN) incorporate both arsenic species, but only As^{III} is finally accumulated. The gene structure of the *ars1* and *ars2* operons of *C. glutamicum* is indicated: *CgarsRs* encode the repressor proteins; *CgarsC1'* encodes the thioredoxin-dependent arsenate reductase. Reprinted with permission from Villadangos AF, Ordóñez E, Pedre B, Messens J, Gil JA, Mateos LM (2014) Engineered coryneform bacteria as a bio-tool for arsenic remediation. Appl Microbiol Biotechnol 98:10143–10152. Copyright © 2014 Springer-Verlag Berlin Heidelberg

metallothionein_IA (hMT_IA) in *E. coli* JM109 strain was facilitated by pGEX-4T-1 plasmid wherein, commercially synthesized hMT_IA gene sequence was inserted to construct pGHM recombinant vector that was confirmed through agarose gel electrophoresis which demonstrated presence of 230 bp inserted DNA sequence. After successful transformation of pGHM plasmid in *E. coli* JM109 strain, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis further revealed presence of an extra band with a molecular weight of 33 kDa. This fusion protein was made up of hMT_IA and glutathione-S-transferase (GST) with protein sizes of 7 and 26 kDa, respectively. As(III) bioaccumulation of *E. coli* (pGHM) was increased up to threefold from 76.3 to 319.6 μg/g of dry cells when compared with control. The optimal As(III) concentration of 50 μmol/L resulted in maximum bioaccumulation by *E. coli* (pGHM) cells. The contact time required for As(III) bioaccumulation by recombinant *E. coli* (pGHM) cells was also analysed that revealed 70% and 93% accumulation within 1 and 2 h, respectively. The optimal pH 3 further showed 400 μg of As(III) bioaccumulation per g of dry cells.

Yang et al. (2013) performed genetic engineering in *E. coli* cells for expression of ArsR that had high selectivity as well as affinity towards arsenic. *E. coli* BL21 (DE3) cells were used for expression of the metalloregulatory protein wherein, pET30a-ArsR recombinant plasmid was used for insertion of *arsR* gene from *Bacillus subtilis*. Polymerase chain reaction (PCR) products of positive clones revealed a prominent band of around 250 bp which verified proper insertion of 210 bp-*arsR* gene segment in the *E. coli* BL21 (DE3) cells. Further SDS-PAGE analysis of intracellular protein content of *E. coli* BL21 (DE3) cells harbouring pET30a-ArsR plasmid demonstrated a protein band with a molecular weight of 10.3 Da when the cells were induced with isopropyl β-D-1-thiogalactopyranoside (IPTG). This indicated the presence of recombinant ArsR protein fused with 6His tag that could be purified through Ni-Nitriloacetic acid (Ni-NTA) affinity chromatography. Moreover, supplementation of 200 mg/L of arsenite displayed almost 30% reduction in *E. coli* cell density in absence of ArsR expression whereas only 10% cell density was decreased in recombinant *E. coli* cells that expressed ArsR protein. Hence, As resistance was enhanced in cells that expressed *arsR* gene. Adsorption capacity of *E. coli-ArsR* cells against four different species of As namely As(V), As(III), methylarsonic acid (MMA) and dimethylarsinic acid (DMA) was also evaluated wherein, the binding capacities were 0.37 ± 0.02, 0.95 ± 0.02, 5.24 ± 0.66, and 3.92 ± 0.06 mg/g, respectively. Hence, recombinant *E. coli-ArsR* cells showed 1.3, 2.6, 5.6 and 3.4 fold improvement in As(V), As(III), MMA and DMA adsorption, respectively, as compared to adsorption by *E. coli* cells without any ArsR expression. Alteration in ionic strength up to 2 g/L of Na^+ ions displayed no change in MMA and DMA adsorption capacity of *E. coli* cells expressing ArsR protein. Thus, a wide range of pH (4–11) was suitable for selective sorption of methylated As species from water samples heavily contaminated with salts. Amount of biomass also modulated As removal efficiency. Around 82.4% and 96.3% of MMA and DMA (with initial concentration of 50 μg/L) were removed by 12 g/L dry weight of *E. coli-ArsR*. Binding selectivity of genetically engineered *E. coli* cells was also investigated in which 1.4, 3.1, 4.9 and 2.2 fold improvements in As(V), As(III), MMA and DMA adsorption capacities were observed after *AsrR* expression, respectively, while no improvement in Cr(III), Cu(II), Cd(II) and Pb(II) sorption was observed.

In another study, Singh et al. (2008) also reported improvement in As removal by metabolically engineered *E. coli* cells through expression of MT derived from *Fucus vesiculosus.* The fMT gene responsible for As chelation was cloned into pUC18 plasmid vector to be overexpressed as a fusion protein with MBP for enhanced stability in *E. coli* JM109 strain. Whole-cell binding experiments displayed 30 and 26 fold increase in As(III) and As(V) accumulation by recombinant cells, respectively, as compared to the wild type control strain. However, fMT mediated As(V) binding experiments in *E. coli* AW10 strain was tenfold lower due to absence of arsenate reductase which suggested reduction of As(V) to As(III) followed by interaction with fMT. Additionally, co-expression of MT with As(III) transporter GlpF further resulted in a three-fold enhancement in As(III) accumulation. Meanwhile, presence of other heavy metal ions such as Cd^{2+} showed 56% reduction in As(III) accumulation. Moreover, the ability of recombinant *E. coli* resting cells that co-expressed fMT and GlpF to act as biosorbents was also investigated which showed complete removal of trace amounts (35 ppb) of As(III) from water samples within 20 min of incubation, hence, highlighting its potential as a promising tool for heavy metal removal from potable water.

2.2 *Cadmium*

Cadmium is immensely hazardous which can cause cancer, bronchiolitis, emphysema, fibrosis, skeletal and kidney damage due to long-term exposure (Ghosh et al. 2021b). Hence, removal of this hazardous metal is of utmost significance. Ma et al. (2011) demonstrated enhanced Cd(II) and As(III) bioaccumulation by genetically engineered strain of *E. coli* BL21 that expressed oligomeric hMT-1A gene. pGEX expression vector was used for transformation of the cells with varied copies of amplified hMT-1A gene. SDS-PAGE analysis then displayed protein bands with a molecular weight of 32, 38, 44 or 50 kDa in *E. coli* transformants harbouring pGEX-MT, pGEX-2MT, pGEX-3MT or pGEX-4MT, respectively. Moreover, inclusion bodies were formed in cells harbouring pGEX-2MT, pGEX-3MT or pGEX-4MT plasmids which could be due to the intramolecular reaction between the sulfhydryl groups. However, Cd(II) and As(III) bioaccumulation capacity of *E. coli* cells having pGEX-3MT was 1.4 and 1.7 times higher than transformants with pGEX-MT, respectively. Moreover, maximum As(III) accumulation of 7.59 mg per g of dry cells was obtained with *E. coli* BL21 transformants with pGEX-3MT plasmid.

In another study, He et al. (2014) demonstrated heterologous expression of MT from *Sinopotamon henanense* (freshwater crab) in *E. coli* BL21 (DE3) strain for enhanced tolerance and uptake of heavy metals such as Zn, Cu and Cd. In order to have a stable overexpression of freshwater crab MT, a fusion protein was prepared using a small ubiquitin-related modifier (SUMO) which is a ubiquitin-related protein. A recombinant pET-28a-6His-SUMO-MT plasmid vector was then prepared and transformed into the bacterial cells. The confirmation of fusion protein overexpression was obtained through SDS-PAGE analysis wherein, a highly soluble protein with an apparent weight of 26 kDa was observed only after IPTG induction in the transformants. Western blot analysis revealed similar results wherein, a 26 kDa band of purified SUMO-MT fusion protein having the 6X histidine tag as well as another 7 kDa purified MT band that were obtained after digestion of the fusion protein as evident from Fig. 2. Thereafter, the three recombinant *E. coli* strains which harboured SUMO-MT and two mutants of the fusion protein namely SUMO-MTt1 and SUMO-MTt2 were investigated for its metal tolerance ability. In comparison with control cells containing empty pET-28a plasmid vector, all three recombinant strains showed significant tolerance towards Zn^{2+}, Cu^{2+} and Cd^{2+} ions. Likewise, accumulation of these heavy metal ions was improved in recombinant strains as compared to the control strain with maximum accumulation of Cd^{2+} ions. Cells harbouring the SUMO-MTt1 construct exhibited Cd accumulation of about 4.67×10^{-5} mol per g of cellular dry weight which was fourfold and twofold higher than cells expressing SUMO-MT and SUMO-MTt2, respectively.

Deng et al. (2007) demonstrated efficient Cd bioaccumulation by genetically modified *E. coli* JM109 strain that had heterologous expression of a Cd transport system along with metallothionein (MT). The pCLG2 and pGPMT plasmids were used for construction of recombinant *E. coli* cells as they were compatible with each

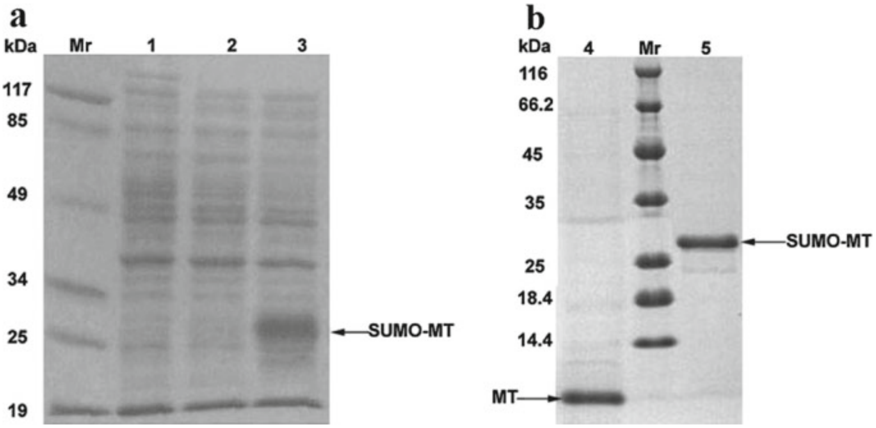

Fig. 2 Expressing of SUMO-MT fusion protein in *E. coli* induced by IPTG (1 mM) and isolated on 12% SDS-PAGE. *Mr* protein molecular-weight markers; *lane 1* total protein extracts of the control bacterial culture (including PET-28a vector); *lane 2* total protein extracts of the bacterial culture expressing SUMO-MT without IPTG; *lane 3* total protein extracts of induced culture expressing SUMO-MT with 1 mM IPTG; *lane 4* MTs in the supernatant after protease cleavage (about 7 kDa); *lane 5* purified SUMO-MT after Ni-NTA affinity chromatography. Reprinted with permission from He Y, Ma W, Li Y, Liu J, Jing W, Wang L (2014) Expression of metallothionein of freshwater crab (*Sinopotamon henanense*) in *Escherichia coli* enhances tolerance and accumulation of zinc, copper and cadmium. Ecotoxicology 23:56–64. Copyright © 2013 Springer Science+ Business Media New York

other and expressed CdtB and fusion protein of *Pisum sativum* MT along with GST (GST-MT), respectively. The cells were then cultured in Luria-Bertani (LB) medium supplemented with 30 mg/L of spectinomycin and 50 mg/L of ampicillin followed by overnight incubation at 37 °C under shaking conditions at 180 rpm. Cells expressing both CdtB and GST-MT demonstrated considerable Cd^{2+} resistance with only 20% reduction in optical density value at 600 nm (OD_{600}) in presence of 65 mg/L of Cd salt. Bioaccumulation ability of *E. coli* JM109 strain was enhanced from 30.2 mg/g (in the wild type strain) to 63.26 mg/g in the recombinant strain expressing both CdtB and GST-MT. It was suggested that such enhanced Cd accumulation by the recombinant strain was facilitated by Cd transport system that interacted with the metal ions and transported them to the interior of the cells which was further enhanced by MT overexpression. Maximum reduction in bioaccumulation of around 35% was observed at a pH value of 4.0 in case of the recombinant strain while 63% reduction was obtained in the wild type strain which highlighted the resistance of recombinant strain to pH variation. Cd bioaccumulation ability of recombinant *E. coli* cells was further evaluated in presence of other heavy metals such as Pb^{2+}, Ni^{2+}, Cu^{2+} and Mn^{2+} wherein, around 60% of Cd^{2+} binding capacity was retained in presence of 1000 mg/L of Ni^{2+} and Mn^{2+}, respectively. However, 50 mg/L of Pb^{2+} and Cu^{2+} reduced the uptake process by 67% and 74%, respectively. Influence of chelators such as 50 mg/L of ethylenediamine tetra acetic acid (EDTA) and 500 mg/L of citrate drastically reduced Cd bioaccumulation capacity by 90% and 60%, respectively.

Likewise, Kang et al. (2007) metabolically engineered *E. coli* JM109 cells for improved Cd accumulation. In this study, phytochelatin synthase obtained from *Schizosaccharomyces pombe* (SpPCS) was expressed in *E. coli* cells under the control of T5 *tac* promoter along with a 6X histidine tag added to the C-terminus of the enzyme. The transformed *E. coli* cells demonstrated an eightfold increase in Cd accumulation as compared to the control strain. Moreover, intracellular thiol contents in recombinant *E. coli* cells that expressed SpPCS was also evaluated in which four different forms of phytochelatins was synthesized. It is interesting to note that the intracellular glutathione (GSH) levels were decreased due to its utilization in phytochelatins synthesis. In addition, γ-glutamylcysteine (γ-EC) levels were increased after overexpression of SpPCS. In order to improve the synthesis of phytochelatins, a variant of GshI enzyme (GshI*) that was insensitive to feedback inhibition by GSH was expressed along with SpPCS. The introduction of GshI* in SpPCS overexpressing recombinant cells showed a tenfold improvement in phytochelatin-2 content while a 200 fold increase in γ-EC content was also obtained. Thereafter, a Cd transporter (MntA) was also overexpressed. The cells that co-expressed MntA, GshI* and SpPCS revealed 1.5 fold increase in intracellular Cd accumulation. Hence, a maximum accumulation of 31.6 μmol of Cd^{2+} per g of dry weight of cells was achieved that was 25 fold higher than the parent strain.

Zagorski and Wilson (2004) also showed heavy metal accumulation in *E. coli* JM109 strains because of heterologous expression of *MntA* and *copA* genes obtained from *Lactobacillus plantarum* and *Enterococcus hirae* that are heavy metal-transporting bacterial P-type adenosine triphosphatases (ATPases) which acted as transporters for Mn(II) and Cu(I), respectively. The Cd(II) and Ag(I) bioaccumulation rate of recombinant strains expressing MntA and CopA transporters along with MT were increased by 6-folds and 3-folds, respectively. More than 60% of the total metal bioaccumulation was observed within the initial 10 min wherein, MntA transporter showed effective accumulation of 8.56 μmol of Cd per g of cellular dry weight in presence of 1 μM of metal ions while CopA transporter accumulated 4.74 μmol of Cu per g of cellular dry weight under similar conditions. The heavy metal saturation level for both the recombinant strains was around 50 μmol per g of cellular dry weight. Moreover, the rate of accumulation was reduced under low temperature of 20 °C while no heavy metal uptake was carried out at 4 °C. It was proposed that such drastic temperature decrease may affect the fluidity of the cell membrane, enzyme activity as well as diffusion kinetics which may further regulate the metal accumulation activity. Alkaline metals such as 50 mM of NaCl and 1 mM of $MgCl_2$ reduced the Cd bioaccumulation activity by 50% and 80%, respectively. Similarly, other heavy metals such as Ag and Mn also reduced both CopA and MntA mediated bioaccumulation of Cu and Cd by 50%, respectively. Chelating agents such as EDTA and citrate also demonstrated considerable reduction in heavy metal accumulation in both recombinant strains. Moreover, MntA transporter displayed a broad pH range of 3.0–11.0 for maximum bioaccumulation activity whereas CopA transporter had a narrow pH range of 6.0–8.0 for heavy metal uptake.

Yoshida et al. (2002) also reported improved Cd uptake by recombinant *E. coli* JM109 cells that harboured pZMT001 plasmid vector for heterologous expression of human metallothionein II (hMT-II) gene fused with a β-galactosidase gene. SDS-PAGE analysis of *E. coli* transformants harbouring the pZMT001 plasmid showed presence of a 122 kDa band that corresponded with the 116 kDa and 6 kDa protein size of β-galactosidase and hMT-II, respectively. Densitometric analysis demonstrated that the fusion protein concentration contributed to more than 24% of the total protein content. Hence, the fusion protein was produced at a rate of 2 g/L of culture broth. Thereafter, heavy metal absorption ability of the recombinant cells was investigated that displayed effective removal of 10.5 μg of Cd^{2+} per mg of dry cells as compared to 3.11 μg of Cd^{2+} being removed per mg of dry cells that lacked hMT-II gene. Moreover, 1.63 mg of Cd^{2+} was accumulated by the recombinant cells after two days of incubation which resulted in 74% reduction of Cd^{2+} ions from the culture broth. The rate of Cd^{2+} uptake by *E. coli* cells was 310 ng/mg dry cell/h. Transmission electron micrographs (TEM) further revealed presence of Cd ions in the cytoplasm and cell membrane of the recombinant cells which expressed hMT-II fusion protein.

In another study, Sriprang et al. (2003) expressed phytochelatin synthase gene from *Arabidopsis thaliana* in a legume microsymbiont *Mesorhizobium huakuii* subsp. *rengei* B3 strain. The PCS_{At} gene was cloned into pBlueScriptKS plasmid followed by sub-cloning in pBBR1MCS-2 and pMP220 expression vectors after which the constructs were designated as pBBRnifHPCS and pMPnifHPCS, respectively. Expression of transformants harbouring these plasmids were carried out under microaerobic conditions since *nifA* gene expression require low oxygen levels that was confirmed using reverse transcription and PCR wherein, a single 120-bp cDNA fragment was obtained when PCS_{At} gene specific primers were used. Phytochelatins were produced in recombinant B3 cells after treatment with 30 μM $CdCl_2$ for 40 h. However, no phytochelatins were obtained in case of B3 cells that harboured control pMP220 plasmids. High-performance liquid chromatography (HPLC) results further demonstrated the presence of phytochelatins (PC) complexes such as $PC_2[(\gamma\text{-Glu-Cys})_2\text{-Gly}]$ and $PC_7[(\gamma\text{-Glu-Cys})_2\text{-Gly}]$ along with other PCs having sulfhydryl groups as well. Moreover, the GSH content decreased from 1.0 ± 0.49 to 0.27 ± 0.09 nmol per mg of cellular dry weight after Cd^{2+} ions treatment. Hence, GSH was suggested to act as the substrate for phytochelatin synthesis. Evaluation of Cd^{2+} accumulation also revealed 9 to 19 fold increase in Cd ions uptake in recombinant B3 cells containing pBBRnifHPCS and pMPnifHPCS plasmids with 35.52 ± 3.16 and 36.41 ± 4.03 nmol of Cd^{2+} accumulated per mg of cellular dry weight, respectively. Further increase in Cd concentration to 50 μM resulted in accumulation of 43.4 ± 11.43 nmol of Cd^{2+} accumulated per mg of cellular dry weight in B3 cells containing pMPnifHPCS plasmid.

2.3 Mercury

In a similar study, Shahpiri and Mohammadzadeh (2018) effectively showed accumulation of Hg^{2+} ions by genetically engineered *E. coli* cells with heterologous expression of *Oryza sativa* L. (rice) MTs. Four different isoforms of MTs namely OsMT1, OsMT2, OsMT3 and OsMT4 were fused with GST and individually cloned into pET41a plasmid vector. The OD values of the recombinant strains R-MT1, R-MT2, R-MT3 and R-MT4 were higher than that of the control strain in presence of 30 μM of mercury nitrate which indicated improvement in the heavy metal tolerance in strains that expressed isoforms of rice MTs. Flame atomic absorption spectroscopy results after 6 h of incubation showed 20, 13.7, 10 and 7 nmol of Hg accumulation per mg of cellular dry weight by R-MT1, R-MT2, R-MT3 and R-MT4 strains, respectively. In vitro metal accumulation ability of the recombinant GST-OsMTs was also evaluated using 5,5-dithio-bis-(2-nitrobenzoic) acid (DTNB) assay wherein, high Hg^{2+} binding strength was observed for all isoforms of the fusion protein.

Zhao et al. (2005) also reported Hg bioaccumulation using genetically engineered *E. coli* JM109 cells wherein, expression of mercury transport system (*merT-merP*) along with MT was carried out using compatible plasmids pSUTP and pGPMT. The bacterial growth inhibition due to heavy metal stress was alleviated in genetically modified strain as they were able to grow in presence of up to 7.4 mg/L of initial Hg^{2+} concentration whereas the control strain growth was severely inhibited in presence of 1.0 mg/L of Hg^{2+} ions. Moreover, the lag phase was extended in presence of high concentrations of Hg. Bioaccumulation of the heavy metal increased from 50 to 120 μg/L when the initial Hg^{2+} ion concentration was subsequently increased from 1.63 to 3.70 mg/L with a maximum accumulation of 180 μg/L at an initial concentration of 7.40 mg/L. Thus, over 96% of the heavy metal from the culture medium was effectively bioaccumulated even in the absence of IPTG inducer. Addition of IPTG demonstrated inhibition in growth of recombinant cells. However, Hg uptake ability was enhanced to 98.7% when the initial Hg concentration was 3.65 mg/L. Around 6.86 mg of Hg^{2+} ions were bound per mg of induced cells that was almost twice as compared to non-induced cells.

Ruiz et al. (2011) also improved Hg resistance and accumulation in *E. coli* JM109 strain with heterologous expression of *Mus musculus* (mouse) MT (*mt-1*) and polyphosphate kinase (*ppk*) proteins. Enhanced expression vectors namely pBSK-P16S-mt1-rpsT and pBSK-P16S-g10-ppk-rpsT were constructed for transgenic expression of the two genes wherein, P16S acted as a powerful promoter, g10 behaved as a transcriptional enhanced element and rpsT was the terminator element. Hg resistance of transformants containing pBSK-P16S-mt1-rpsT and pBSK-P16S-g10-ppk-rpsT constructs were increased up to 20 μM after 16 h of incubation in comparison with 5 μM Hg resistance exhibited by the wild type *E. coli* strain. Moreover, bacteria harbouring pBSK-P16S-g10-mt1-rpsT and pBSK-P16S-g10-ppk-rpsT constructs effectively exhibited significant growth after 120 h of incubation in presence of 120 and 80 μM of Hg, respectively. Bioaccumulation ability of *mt-1* gene was also demonstrated with uptake of 51.6 ± 14.1 μM of Hg in the first 72 h followed by 100.2 ± 17.6 μM by 120 h of incubation.

2.4 *Other Metals*

Several other metals have also been reported to be bioaccumulated using genetically modified bacteria. For instance, *E. coli* BL21 expressing vanabin genes were reported by Ueki et al. (2003) to effectively bioaccumulate Cu^{2+} ions as well. Two different genes obtained from vanadium-rich ascidian *Ascidia sydneiensis samea* that encoded vanadium binding proteins namely vanabin1 and vanabin2 were fused with maltose binding protein (*malE* gene) and then cloned into pMALp2 vector. Moreover, the transformants expressed majority of the fusion protein in the periplasm that was further evaluated for its metal bioaccumulation ability. Cu(II) ion accumulation up to 876 ± 215 and 882 ± 136 ng/mg of cellular dry weight was achieved when the recombinant cells expressing MBP-vanabin1 and MBP-vanabin2 fusion proteins, respectively. The cells were cultured at 37 °C for 6 h with supplementation of 10 μM of Cu ions and 0.5 mM of IPTG. Similar bioaccumulation behaviour was observed in presence of 100 μM of Cu^{2+} ions as well which suggested the maximum threshold of Cu bioaccumulation to be 10 μM only. Additionally, homogenates of MBP-vanabin 1 and MBP-vanabin 2 were purified after 6 h of incubation in medium supplemented with 10 μM of Cu ions wherein, one molecule of MBP-vanabin 1 or MBP-vanabin 2 was directly bound to eight or five Cu(II) ions, respectively.

Raghu et al. (2008) showed Co removal ability by genetically engineered *E. coli* cells that harboured NiCoT genes of *Rhodopseudomonas palustris CGA009* (RP) and *Novosphingobium aromaticivorans F-199* (NA) strains. Two different vectors namely pET28a and pUC plasmids were used for cloning of NiCoT genes whose expression was induced by 0.5 mM of IPTG which resulted in a NiCoT protein from RP and NA with a molecular weight of 38 and 40 kDa, respectively. Three different strains of *E. coli* namely MC4100, ARY023 and BL21-DE3 were used for expression of NiCoT genes followed by evaluation of ^{60}Co-tagged carrier cobalt (*Co) removal efficiency from simulated effluent. The optimal pH for genetically engineered *E. coli* *Co removal ability was 5.8 with 20% removal at OD value of 0.5 units that was further increased to 60–70% at OD value 2.0 units. *E. coli* ARY023 cells that expressed NiCoT gene of RP strain showed more than 75% *Co removal. Moreover, maximum *Co removal activity of more than 55% was obtained after 1 h of incubation with around 25% cell lysis that was further increased with subsequent increase in incubation time. Therefore, under optimal conditions with OD of 1.0 unit, pH value of 5.8 and incubation for 60 min, maximum *Co removal of 60% was achieved wherein, after addition of fresh batch of engineered culture, additional 25% of *Co was also removed. Thus, a cumulative removal of 85% of *Co from simulated effluent was obtained in a two-cycle treatment with the genetically modified bacteria expressing NiCoT genes.

E. coli Rosetta (DE3) strain was genetically modified by Li et al. (2015) for transgenic expression of phytochelatins synthase gene of *Pyrus calleryana* (*PcPCS 1*) in order to enhance heavy metal tolerance as well as accumulation. The pET22b(+) plasmid vector was used for cloning of *PcPCS 1* gene whose expression was induced in *E. coli* transformants through addition of 0.5 mM IPTG followed by incubation for 6 h. SDS-PAGE results revealed presence of a 56 kDa protein band that was comparable to the His-PcPCS1 fusion protein. Thereafter, heavy metal ion tolerance of the recombinant bacteria was investigated by supplementation of 1.5 mM of Cd(II), 2.5 mM of Cu(II), 100 μM of Hg(II) or Ag(I). The growth rate of transformed cells was higher in presence of all the heavy metals as compared to the control strain thus, highlighting enhanced metal tolerance. Moreover, cells expressing *PcPCS1* gene demonstrated a 2.0 to 2.22 fold increase in Cd^{2+} accumulation as compared to cells containing control pET22(b)+ plasmid vector. Likewise, accumulation of Cu^{2+} and Hg^{2+} was also enhanced in presence of transgenic phytochelatin synthase.

Likewise, Sauge-Merle et al. (2012) showed heavy metal accumulation by recombinant *E. coli* TB1 strains which expressed a fusion protein composed of sheep *MTII* and *malE* genes. Two varied constructs were prepared for cloning and expression of the mammalian metallothionein gene wherein, one construct produced MBT-MT fusion protein in the cytoplasm of *E. coli* TB1 (MBP-MTc) while other construct exhibited periplasmic production of MBT-MT in the recombinant cells (MBP-MTp). SDS-PAGE analysis revealed that MBP-MTc attributed to 15% of the total protein content with more than 90% recovery of the fusion protein from the soluble fraction. Bioaccumulation studies further demonstrated 5.5, 8.4 and 55 fold increase in Zn(II), Cd(II) and As(III) uptake by *E. coli* cells expressing MBP-MTc as compared to control strain while MBP-MTp expressing cells exhibited 1, 3.1 and 7.5 fold increase in bioaccumulation of the respective heavy metals. The lower amount of bioaccumulation in MBP-MTp was speculated to be a result of oxidation of cys residues which hindered metal uptake. In addition, *E. coli* (ZntA-MTc) strain that had disruption in the zinc transporter gene *zntA* further showed improvement in metal uptake ability of Zn(II), Cd(II), Hg(II) and Pb(II) by 1.2, 1.6, 2.0 and 3.5 folds, respectively. Arsenic bioaccumulation of MTc expressing strain was 55 folds higher as compared to the control strain. On the contrary, Cu(II) accumulation was 1.2 folds higher in the periplasm section as compared to the cytoplasm. Therefore, such genetically modified strains that overexpressed MBP-MT in their cytoplasmic fraction along with disrupted metal export system could potentially be used for effective heavy metal bioaccumulation (Table 1).

Table 1 Genetically modified bacteria for enhanced heavy metal bioaccumulation

Bacterial species	Gene introduced	Metal removed	References
C. glutamicum ArsC1-2, 2Δars and 2Acr3	*CdglpF* and *ScglpF*	As	Villadangos et al. (2014)
E. coli (pGHM)	hMT_IA	As	Su et al. (2009)
E. coli BL21 (DE3)	*arsR*	As	Yang et al. (2013)
E. coli JM109, *E. coli* AW10	fMT, GlpF	As	Singh et al. (2008)
E. coli BL21	hMT-1A	Cd and As	Ma et al. (2011)
E. coli BL21 (DE3)	SUMO-MT	Cd	He et al. (2014)
E. coli JM109	*cdtB* and GST-MT	Cd	Deng et al. (2007)
E. coli JM109	*mntA*, GshI* and SpPCS	Cd	Kang et al. (2007)
E. coli JM109	MntA	Cd	Zagorski and Wilson (2004)
E. coli JM109	hMT-II	Cd	Yoshida et al. (2002)
Mesorhizobium huakuii subsp. *rengei* B3	PCS_{At}	Cd	Sriprang et al. (2003)
E. coli	OsMT1, OsMT2, OsMT3 and OsMT4	Hg	Shahpiri and Mohammadzadeh (2018)
E. coli JM109	*merT-merP*	Hg	Zhao et al. (2005)
E. coli JM109	*mt-1*	Hg	Ruiz et al. (2011)
E. coli BL21	*malE*-vanabin 1 and *malE*-vanabin 2	Cu	Ueki et al. (2003)
E. coli MC4100, ARY023, and BL21-DE3	NiCoT	*Co	Raghu et al. (2008)
E. coli Rosetta (DE3)	*PcPCS-1*	Cd, Cu and Hg	Li et al. (2015)
E. coli TB1	*MTII* and *malE*	Zn, Cd, Hg, Pb and Cu	Sauge-Merle et al. (2012)

3 Conclusions and Future Perspectives

Genetically engineered microbes provide a new alternative for rapid, efficient, cost-effective green approach for removal of heavy metals from the water and the environment. Biomolecules such as arsenate reductases, mycothiol, arsenite permeases, aquaglyceroporin, metallothionein, and glutathione-S-transferase are responsible for efficient bioaccumulation of As. Similarly, cellular uptake of Cd and accumulation is regulated by the expression of phytochelatin synthase, P-type adenosine triphosphatases (ATPases), MntA and CopA transporters. Highly toxic Hg is removed from the environment and immobilized in the microbial cells by mercury transport system (*merT-merP*) and polyphosphate kinase (*ppk*) proteins. Vanabin1 and vanabin2 play a critical role in Cu bioaccumulation while expression of NiCoT gene is specific to Co uptake. Likewise, Zn bioaccumulation is mediated by zinc transporter gene *zntA*. Hence, these genes can be introduced within microbial cells using suitable vector to induce metal resistance in microbes and thereby facilitating metal uptake and bioaccumulation.

Further, various parameters like cell density, initial metal concentration, duration, temperature and pH can be optimized in order to achieve maximum metal bioaccumulation. The mechanism behind the process should be thoroughly investigated using omics such as metabolomics, proteomics and genomics. Metal chelating proteins can be immobilized, encapsulated or entrapped in polymeric materials like alginate, chitosan, collagen or others so that they can be recycled and reused. Also, the recovery of the metals from the microbial cells should be standardized as it can help to collect economically significant metals from the effluents and the environment.

In view of the background, it can be concluded that genetically engineered microbes with metal bioaccumulating potential can revolutionize the wastewater treatment strategy and ensure clean environment.

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Nano-Remediation: Ecofriendly Approach in Pollutants Removal

Anu Kumar, Bhanu Krishan, Sunny Dhiman, Shivani, and Gunjan Mukherjee

1 Introduction

Since the nineteenth century industrial revolution has brought global economic and technological advancements. However, these advancements have elevated the course of humanity with high throughput production of resources, vast distribution of the product but an extensive generation of waste material and in addition greater disposal of generated waste with assiduous disposal (Cecchin et al. 2017; Tripathi et al. 2022). Assiduous disposal of waste has led to serious contamination affecting the environment, social and economic aspects of the world at a greater concern. Thus, the contamination of physical and biological aspects of the environment can be defined as environmental pollution (Suzuki et al. 2020). Any substance which is divulging toxicity into the environment upon excess accumulation of natural level is referred to as a pollutant (Suzuki et al. 2020; Tyler Miller and Spoolman 2018). The form of pollution and type of pollutant is entirely dependent upon the human activities yielding pernicious by-products which directly or indirectly interfere with the environment leading to natural imbalance. Involvement of human activities such as deforestation, agricultural waste, household waste disposal and dumping, industrialization, population growth, mining, the maneuver of chemicals, illegitimate disposal of electronic waste contributes to land, air and water pollution (Ukaogo et al. 2020). According to USEPA, these human exploitations can divide the source of pollution into two specific terms namely nonpoint and point sources. Nonpoint sources (NPS) are the repercussion of land run-off, atmospheric deposition, drainage and hydromodification. NPS culminates surplus fertilizers, insecticides and herbicides which are disposed from

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the agricultural site and residential land, chemical toxins, oils, metals from urban run-off, atmospheric deposition and hydro modification. The deposition of NPS into water bodies namely lakes, rivers and groundwater is convicted by rainfall or run-off which carries the pollutants into the water bodies and finally its deposition (Basic Information and about Nonpoint Source (NPS) Pollution 2021; Xepapadeas 2011). Point sources on the other hand are detectable, single sources of pollution habitually discharged from the industrial wastes to the water bodies (EPA 2021). The release of pollutants can be classified into various groups but their effects are limited and adverse. The impact of these harmful pollutants is restricted not only toward humans but also has shown an adverse effect on the flora and fauna.

Any polluted body alone contains some of the toxic, non-toxic and microbial contamination that can cause various infectious diseases and harmful effects to the human as well as to the plant body. Table 1 summarizes different pollutants present in the environment with their sources and effect on living bodies.

As discussed, these pollutants arise from different sources and are localized to the specific environment type. However, in order to facilitate the removal of these unwanted toxins, various NGOs and Government bodies have drafted laws and awareness programs; various instruments are designed and implemented to degrade and eliminate these toxins without the release of any lethal by-product. The conventional treatment methods for pollutants removal involve the use of adsorbents, filtration and coagulation, advanced oxidation processes and microbial treatment technology. These methods are studied for the removal of toxic metals, organic pollutants and certain radioactive contaminants with a high-efficiency rate of up to 90% (Crini et al. 2019). These technologies overcome the problem of In situ remediation by injecting the NMs into the contaminated sites. The employment of these methods has provided a space for effective treatment but due to their limitations such as high cost, poor recyclability, toxicity, low biodegradability and poor scale-up process; these methods are unsafe to be regarded for universal applications (Crini et al. 2019; Shahid et al. 2020).

2 Application of Nanotechnology and Nanobioremediation in Pollutant Degradation

Nanotechnology has always given us tremendous applications in terms of every field, and the use of nanomaterials to outcast the use of conventional techniques in pollutants removal is of significant attention these days. The classical definition of nanotechnology states the application and manipulation of nanomaterials (NMs) having one dimension at least less than 100 nm for the treatment, diagnostic, construction and therapeutic purposes (Saleh 2020). The extensive interest in nanotechnology is due to the solitary properties of NMs which includes smaller size, high stability and specificity, physical, optical, magnetic and chemical properties with large surface area to volume ratio (Saleh 2020; Khan et al. 2019). These NMs can be divided into two

Table 1 The major pollutant sources and their effect of humans present in environment

Type	Major pollutants	Sources	Effect on humans	Findings
Water	Toxic metals (Arsenic, Barium, Chromium, Copper, Lead, Mercury Nickel and Zinc)	Glass, Metal, Tannery, Plumbing, Cement and electroplating industries, pesticides, mining, dyes, paints and can be found in industrial effluents if left untreated	Cardiac abnormalities, poisoning, intestinal diseases, impairment of vision and speech and carcinogenic effects	Kumar et al. (2020), Manasa and Mehta (2020), and Hu et al. (2014)
	Ammonia	Food processing and Agricultural waste	Cellular toxicity	Kumar et al. (2020)
	Organic toxins (N-nitrosodimethylamine, Perchlorate, Polybrominated diphenyl ethers, Naphthenic acids, phenols, dyes, polycyclic aromatic hydrocarbons (PAH), polycarbonates (PCB) and certain heterocyclic compounds	Industrial processes, flame retardants, food and chemical industries	Neurodegenerative disorders, neurological impairment, impaired cognitive function in elder beings and carcinogenesis	Kumar et al. (2020), Zheng et al. (2013), and Coxon et al. (2019)
	Antibiotics and Pharmaceuticals	Pharmaceutical wastewater	Allergic reaction and organ disorder	Phoon et al. (2020)
	Microorganisms (Bacteria, protozoa, parasites, fungi and algae)	Municipal wastewater, animal waste and food industry	Enteric diseases and infections	Kumar et al. (2020)
Air	Bioaerosols	Airborne particles such as seeds, spores, pollens and danders	Infectious and can cause allergic reactions	Walther (1972)

(continued)

Table 1 (continued)

Type	Major pollutants	Sources	Effect on humans	Findings
	Particulates (PM_{10} and $PM_{2.5}$)	Emission from industries, volcanic ash, powdered pesticides, bioaerosols, construction and demolition	Lung infection, Nausea and increased heart rate	Walther (1972)
	Ozone (O_3)	Lightning, electronic devices	Toxicity to human including cancers	Walther (1972)
	Volatile organic solvents, pesticides and methane	Paints, Aerosol sprays, Disinfectants, pesticides and herbicides sprays	Shortness of breath, fatigue and skin problems	Walther (1972)
	Secondary pollutants (NO, SO_2, CO, PMs)	Volcanic ash, biomass burning, industrial smoke	Asthma, bronchitis, emphysema and breathing problems	Guo et al. (2018)
Soil	Solid waste	Municipal, industrial, agriculture and commercial waste	Enteric diseases and health hazard	Boyd et al. (1990)
	Heavy metals	Anthropogenic activities which involves mining, electroplating, smelting, extraction of metals	Various diseases involving organ failure and cancer	Havugimana et al. (2015)
	Pesticides, DDT sprays and fertilizers	Agricultural activities	Cancer and toxicity	Boyd et al. (1990)
Radioactive	Actinide compounds and radioactive elements such as U^{235}, U^{238}, Np^{237} and Cs^{137}	Radiowaste from Nuclear power Industries, medical and instrument manufacturing industries	Diarrhea, nausea, cancer and mutations	Natarajan et al. (2020)

groups, either organic or inorganic and the synthesis can occur using two standards approaches, namely top-down and bottom-up approaches. The conventional methods for the synthesis of NMs involve the use of hazardous chemicals using physical techniques. These techniques exploit the use of high energy and increased production costs due to the involvement of specialized instruments (Khan et al. 2019). The green synthesis of NMs from the biomolecules available in microorganisms and plants is much of study interest these days. The plant-based synthesis of NPs is gauged as a cost-efficient, superficial, expeditious and easy process in terms of scale-up and production. These NMs are synthesized using extracellular and intracellular plant materials. The synthesis of NMs from microbial consortia depends upon the maintenance of the microbial culture which can produce also toxic components and can be toxic for human health. Further, the NMs can be characterized into nanoparticles, carbon nanotubes, fullerenes, nano-scaled zero-valent ions, nanofilters and nanoadsorbers (Fig. 1).

In recent years, nanobioremediation (NBT) has been employed for the remediation of soil, water and air pollutants into non-toxic and eco-friendly components. The allocation of NBT stated by Carata et al. is gradually based upon sensing and detection, treatment and bioremediation and prevention of pollution (Carata et al. 2017). This technique has served potential benefits in the removal of pollutants, organic compounds and toxic metals which serves as threat to the environment. Also, the integration of nanotechnology with phytoremediation and bioremediation has proven to neutralize certain elements which involve pesticides, herbicides, toxic

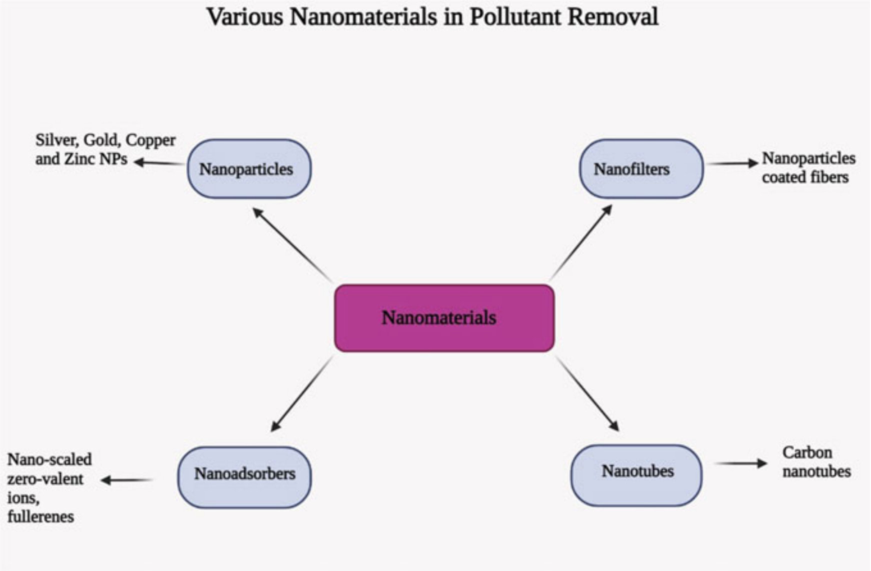

Fig. 1 Application of nanotechnology in pollutant removal

heavy metals and organic compounds. The unification of NMs with phytoremediation has proven to induce degradability of PCB and PAHs, living organisms that serve as health hazards and extraction of toxic components and xenobiotic.

3 Removal of Pollutants from Water Sources Through NBT

The unique property of NMs has allowed its greater advantage over conventional wastewater and pollutant removal technologies. Nanoparticles (NPs) have the ability to penetrate deeply due to high sorption and reactivity and are explored for application in various operations related to wastewater treatment. NMs have efficiently reported for the development of the cost-effective filtration processes, production of membranes with controlled permeability and fouling resistant structure. The manufacturing of inorganic and organic membranes involves the assembling or blending of nanoparticles with the subjected membrane. Example of such membranes includes TiO_2-coated membranes and carbon nanotubes which are studied for improved permeability and antimicrobial activities (Kim et al. 2008; Chae et al. 2009).

3.1 Removal of Microbial Contamination

Metal-oxide nanoparticles such as Silver NPs, Gold NPs, Cu NPs and others are most widely used for the inactivation of microbes in waster because of their low toxicity and high specificity (Saleh 2020). Silver NPs are derived from silver salts, with their size ranging from 8 to 23 nm. The mechanism involved for the antimicrobial effect of AgNPs is based upon the formation of free radicals which disintegrates the bacterial cell wall integrity, interacts with the genetic molecule and adhesion to the cell surface antigens disrupting cell wall properties and enzyme degradation (Liau et al. 1997; Danilczuk et al. 2006). A study involving the use of immobilized Ag nanoparticles has shown effective bactericidal activity against Gram-positive and Gram-negative bacteria (Savage and Diallo 2005). The incorporation of AgNPs with cellulose acetate fibers and the production of antimicrobial nanofibers and nanocomposites have also shown greater antimicrobial properties against microbes such as *Escherichia coli*, *Pseudomonas, Bacillus subtilis* and other pathogenic microbes (Botes and Eugene Cloete 2010; Lala et al. 2007). Similarly, the use of filtration membrane composed of CNT, TiO_2 nanoparticles and Fe_3O_4 NPs have been studied for effectively removing bacteria and viruses from water in a very short duration. CNT have high bactericidal property because of the production of oxidative stress and disruption of the cell wall (Rao et al. 2007). Thus, CNTs act as an efficient nanostructure for the removal of microbial contaminants from water sources.

3.2 Removal of Heavy Metals

NMs such as carbon nanotubes (CNT), zeolites and dendrimers have an excellent adsorption property and are being utilized for the removal of toxic heavy metals (Savage and Diallo 2005). The ability of CNT to absorb toxic metals has been studied by various scientists, the experimental shreds of evidences showed the high efficiency of CNT to absorb metals such as Cadmium, Chromium, lead and zinc. CNTs have a fast adsorption mechanism due to numerous accessible adsorption sites and minimum diffusion distance between intraparticle (Rao et al. 2007). This property of CNTs enables its use for the removal of toxic metalloids such as arsenic. A study based on the use of CNTs composites with Fe and CeO_2 showed complete removal of arsenic and other metals from water/wastewater (Sharma et al. 2009). Similarly, the use of TiO_2 NPs anchored on graphene sheet has been reported to reduce Cr(IV) to Cr (III) under sunlight (Zhang et al. 2012). Fungal-based nanotechnology that includes biological synthesis of NP using fungus such as *Fusarium oxysporum, Rhodobacter sphaeroides, Schizo saccharomyces pombe* has been tested to convert toxic metals into elemental metal nanoparticles because of the enzymes generated by cellular activities.

3.3 Removal of Organic Pollutants in Water

Organic waste in water consists of PCBs, PAH, dye and phenols, these compounds are generated by various industrial processes and due to their non-biodegradable nature, the conventional technologies fail to degrade completely (Kumar et al. 2020; Coxon et al. 2019). However, nanostructures such as CNTs and TiO_2 NPs have shown an excellent absorption toward these organic pollutants. Nanoporous-activated carbon fibers produced by CNT electrospinning has shown to exhibit high absorption capacity for organic pollutants such as benzene, toluene, xylenes and ethylbenzene present in wastewater. Multi walled CNTs have also been studied for high absorption of chlorophenols, herbicides, DDTs and PAHs. TiO_2 NPs, on the other hand, has been shown to completely degrade organic pollutants such as PCBs, chlorinated alkenes and aromatic compounds. Amin et al., has summarized different studies based upon the removal of organic pollutants from wastewater and water bodies (Amin et al. 2014).

4 Removal of Air Pollutants by NMs

Volatile gases and aerosols have been potentially toxin to the environment with the most challenging operation of their disposal. These gases involve SO_x, NO_x and other secondary pollutants (Walther 1972) which directly or indirectly cause harmful effects to humans and the environment as well. The conventional methods for the removal of such gases include the use of air purifiers, electrostatic precipitators and scrubbers, these methods although have high efficiency for the removal of such harmful gases but are higher in cost and are not universally applicable. The novel properties of NPs have enabled us to involve their application in the removal of these gases. The basic mechanism by which NMs remediate these toxic pollutants is by adsorption of the contaminants by nano-adsorptive material, degradation of the contaminants by nanocatalysts and the involvement of nanofiltration for the separation of contaminants (Hussain et al. 2021).

4.1 Nano-Adsorptive Material in Air Pollution

Nano-adsorbents such as CNTs, graphene and graphite are extensively employed in cleaning air pollutants because of their high absorption and high selectivity. Su et al. conducted a study based on the coating of CNTs with various amine groups which provided multiple adsorption sites for CO_2; this enabled the adsorption of CO_2 at the low-temperature range of 20–100 °C. Thus, this study concluded that the addition of amino groups enhances the structural bonds of CNTs for adsorption, and therefore, the process becomes more efficient for the removal of greenhouse gases as compared to the conventional strategies (Hussain et al. 2021; Su et al. 2009; Muralikrishnan et al. 2014).

4.2 Nanocatalysts

Photocatalytic degradation of pollutants by the use of TiO_2 semiconductors have been a study of interest in the removal of toxic metals, organic components and other volatile components. Atmospheric contaminants such as NO_x and SO_x are proven to be effectively removed by the use of TiO_2 semiconductors and converting them into less toxic specimens. Various nanocatalysts such as ZnO, nano-gold-based semiconductors and Bismuth oxy bromide nanoplate catalysts are reported to exhibit effective degradation against various air pollutants namely NO, CO_2 and other greenhouse gases (Hussain et al. 2021; Ai et al. 2009; Tandon 2015; Yadav et al. 2017; Muralikrishnan et al. 2014).

4.3 Nanofiltration

The dust arising from an industrial plant can be captured by the use of novel nanofilters which involve the integration of nanostructured sheets consisting of small pores. These filters are capable of separating minute contaminants from the air. NPs such as AgNPs and Gold NPs having high antibacterial activity are being exploited as air filters for the purification and removal of bioaerosols namely viruses, bacteria and fungi. Nanofiber-coated filter media are also a study of interest these days for the removal of industrial dust (Hussain et al. 2021; Muralikrishnan et al. 2014).

5 Nanobioremediation

The nanobioremediation (NBT) is a new method that includes the unification of nanotechnology and bioremediation for an efficient degradation in minimum time. This unification has been found useful and promising technology for the removal of various contaminants this is because bioremediation alone is a time-consuming process. With recent advancements, nanotechnology is integrated with three main approaches which include microorganisms, plants and enzymes. The physical and chemical interactions between the NMs and microorganisms depends upon various factors such as pH, temperature, type of microorganism and contaminants; these parameters are responsible for the specialized interactions between NMs and the contaminants. The degradation of contaminants is carried out by displayed events of synergistic effect after the stable interactions between NMs and microorganism; these include dissolution, adsorption and biotransformation (Vázquez-Núñez et al. 2020). Sorption is the most important process in NBT; it includes adsorption and absorption of contaminants. During adsorption, the interaction between the pollutant and sorbent takes place at the surface level whereas in absorption pollutants get penetrated inside the deepest layer to form a solution (Vieira and Volesky 2000). Another phenomenon of degradation involves photocatalysis depending on the nature of NMs, further; the resulting products produced after the photocatalytic degradation is bio-transformed by the biotic system and reduce the overall concentration of the pollutant.

Biotransformation involves the enzymatic degradation by the enzymes produces by the microorganisms (Vázquez-Núñez et al. 2020). This synergistic effect serves a greater advantage over the degradation of pollutants by bioremediation alone. Table 2 summarizes the different NMs used in the integration of microorganisms to degrade the pollutants in different sources.

Various studies are being reported these days which includes utilization of NBT for treatment of dyes in textile effluents, removal of heavy metals, organic pollutants, micro-pollutants and halogenated compounds and soil reclamation. The development of immobilized NPs for wastewater treatment is of great study as well. Studies include the development of microbe isolated enzyme immobilized NP such as synthesis of the Peroxidase enzyme immobilized magnetic nanoparticles. In this study, Peroxidase enzyme was isolated from *Pseudomonas aeruginosa* strain OS and was used for bioremediation of blue azo dye in textile wastewater. Fe_2O_3 NPs were synthesized using the co-precipitation method and by surface modification of the synthesized NPs, the immobilization was carried out. These immobilized NPs were then subjected to different pH and temperatures and were reported to show greater advantages over the enzyme alone. The immobilized NPs successfully degraded different dyes in

Table 2 Various nanomaterials used in conjugation with bioremediation

Nanomaterial	Microorganism Involved	Pollutant degraded	Source	Degradation efficiency	Findings
Nano-scale zero-valent iron, Ti, Mn and Ag	*Sphingomonas sp.*	Chlorinated hydrocarbons and pathogens	Water	>76.8%	Vázquez-Núñez et al. (2020) and Kim et al. (2012)
Microorganism-immobilized nanocellulose composites	*Arthrobacter globifornis*D47	Herbicide	Industrial wastewater	>90%	Vázquez-Núñez et al. (2020) and Liu et al. (2018)
Nanomembranes	Biological extract of *Cynomorium coccineum* L	Cyanide	Industrial wastewater	20%	Sebeia et al. (2020)
Iron oxide nanopowder	Soil Microorganism	Azo dye direct red 23	Water	98%	Liu et al. (2018)

four hours which was conveyed by the absence of reactive azo dye in the textile wastewater (Sebeia et al. 2020).

Single enzyme nanoparticle (SEN) in which each enzyme is contained by a hybrid polymer of the inorganic or organic material network is also reported advantageous in terms of degradation of organic contaminants and xenobiotic compounds. In SEN, each enzyme is modified with a porous structure of less than nanometer-thick dimension which further changes the stability of the enzyme and forms a new nanostructure molecule (Tripathi et al. 2022). This special characteristic of SEN has been studied for the removal of xenobiotic with the potential of binding with the targeted compound and transforming it into a less toxic form (Tripathi et al. 2022; Hussain et al. 2021). Nano-sized zero-valent ions have also been studied for the degradation of organic contaminants such as atrazine, chlorpyrifos and molinate. These enzyme-based nanoparticles are also studied for synergistic effects with phytoremediation which have proven to degrade the plants and microbes resistant long-chain hydrocarbons and organochlorines (Tripathi et al. 2022).

6 Nano-Phytoremediation

The consolidation of phytoremediation with the application of nanotechnology has shown a greater effect on soil decontamination. Nano-phytoremediation (NPT) has been recently studied for a greater potential against pollutants that are poorly degraded by phytoremediation. NPT is effectively utilized in the fields of textiles, paints and cosmetics for the treatment of contaminants that includes heavy metals, organic and inorganic pollutants such as atrazine, PCBs, PAHs and organic solvents. Despite such great efficiency of nanomaterials with plants, certain factors affect the degradation potential of NPT. Similar to NBT, the parameters which influence this process is the type of contaminant, environmental factors (pH, temperature and organic matter), characteristics of plant such as root system and the type of enzyme involved (Srivastav et al. 2018). The uptake of nanomaterials by the plant is restricted upon the physical characteristics of the NPs, considering the size, shape and chemical composition of the NPs involved. The size of the NP is considered as the important factor for the penetration of the NPs into the plant and translocate from roots to other parts of the plant. This penetration is also an important factor to consider in how efficiently the plant will take up the NP. The ideal characteristics of choosing a plant for this process are also dependent upon various processes. Generally, the requirement is the use of rapidly growing plants with high productivity, well-developed roots, high tolerance against contaminants and ease to harvest. The selection of NPs is also based upon certain characteristics which include the selection of NPs which are non-toxic, which can increase the germination, phytoenzyme production of plants, ability to enhance plant growth hormones and increased bioavailability for plants (Srivastav et al. 2018).

The interaction mechanism involves the uptake of NPs by the plant roots, after entering the plant roots, the transport of NPs takes place in two ways: (a) Apoplastic

Table 3 Nanomaterials studied with various plants for nano-phytoremediation

Nanomaterial	Plant species	Pollutant	Removal efficiency	Findings
Nano-scaled zero-valent ions	*Alpinia calcarate*	Endosulfan	100%	Srivastav et al. (2018) and Pillai and Kottekottil (2016)
Nano-scaled zero-valent ions	*Ocimum sanctum*	Endosufna	76.28%	Srivastav et al. (2018) and Pillai and Kottekottil (2016)
Fullerene	*Populus deltoides*	Trichloroethylene	86.16%	Srivastav et al. (2018) and Ma and Wang (2010)
Salicylic acid NPs	*Isatiscappadocica*	Arsenic	705 ppm and 1188 ppm accumulation of metal in roots and shoots of plant	Srivastav et al. (2018) and Souri et al. (2017)
Nano-scaled zero-valent ions	*Panicum maximum*	TNT contaminated soil	Removal of TNT from soil from 100 mg/kg to 1/10 mg/kg	Jiamjitrpanich and Parkpian (2012)

transport; which takes place outside the plasma membrane and xylem vessels, (b) Symplastic transport; involves the water movement between cytoplasm and sieve tubes (Srivastav et al. 2018; Sattelmacher 2001; Roberts and Oparka 2003). This interaction is studied to stimulate various beneficiary effects to the plant, silver nanoparticles have been reported to increase ABA and GA phytohormones, and certain MnNPs have shown to improve the nitrogen assimilation and improvement in plant metabolism. Also, the NPs has been shown to increase the chlorophyll amount and the seed germination ability of the plant (Srivastav et al. 2018).

Recent studies involving plant-based remediation assisted by NPs includes the use of fullerenes, nano-scaled zero-valent ions and metal oxide NPs, some of these are summarized in Table 3 (Srivastav et al. 2018).

7 Challenges in Nanobioremediation and Nano-Phytoremediation

The synergistic effect has no doubt shown an effective result in the control and degradation of the pollutants with minimum to zero release of any toxic compound. The negative effect of NPs on certain microbial species (*E. coli*, and *Staphylocoides sp.*) has been reported. The formation of reactive oxygen species due to the NPs inside the microbial cell is reported to cause damage in cellular structure and inhibit the overall growth. Thus, this factor limits the use of several microbial communities in association with nanotechnology. In the case of nano-phytoremediation, long-term experimentation is required for an effective degradation. This technique is only suitable for a site with a moderate level of contamination. Also, there is a need for the determination of release of any toxic product by this process, as the released toxin can enter the food chain or groundwater and can also be toxic to the microorganisms involved with the process. These toxins can also accumulate in plant parts such as leaves, stems and litter which can ultimately affect humans and herbivores (Nwadinigwe and Ugwu 2018).

8 Conclusion

As discussed earlier, the synergistic effect of nanotechnology with bioremediation and phytoremediation is an emerging technology with greater efficiency to remove the pollutants. Nanotechnology is a promising field for various applications due to its unique properties. These properties, hence, increase the accumulation and degradation capability of certain microorganisms, fungi and plants against several pollutants. Various studies have been included above which shows the degradation of organic pollutants, heavy metals, herbicides and xenobiotics. These compounds are proven to be non-biodegradable and are difficult to remove from the environment. The use of nanobioremediation under control parameters suitable to the choice of microorganism and plant has shown from complete to 20% removal of compounds such as Arsenic, atrazine, azo dyes, chlorinated hydrocarbons, chromium, cyanide, PCBs and other toxic components present in the soil, wastewater and other water sources. These compounds are toxic when present in excess and cause serious concerns to human health. Thus, this integrated approach can prevent the toxicity of these compounds under low capital cost compared to the conventional strategies and bioremediation alone. However, certain criteria should be considered before such operation, these include the selection of suitable nanomaterials, microorganism and the plant, determination of any toxicity of selected nanomaterials to the microorganism or plant, maintenance of controlled environment and nutrition for the effective synergistic remediation. The overall process is easier and can be operated in situ without any limitation, this flexibility of the mentioned operation allows the application of such methods to a larger extent with the exertion of flair supplication of nanotechnology.

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Bioremediation of Soils Polluted with Hexavalent Chromium Using Bacteria

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1 Introduction

Release of untreated industrial wastewater has a significant impact on the environment. Unchecked release of heavy metals because of industrial and sewage/sludge applications has resulted in a widespread contamination of these elements in the terrestrial environment (Viti and Giovannetti 2007). These are found in soil primarily as a result of natural weathering of metal-rich parent material and human activities such as industrial, mining, and agricultural activities (Abdu et al. 2017). Despite being aware of the toxicity of heavy metals, industries continue to employ huge amounts of these metals like hexavalent chromium into the ecosystem. Chromium (Cr) enters the food chain through plant material consumption and concentrations Cr over a certain level have been demonstrated to be hazardous to flora. As the concentration of Cr in plants rises, it has an unfavourable effect on a number of biological parameters, finally causing the soil to become barren. Naturally, Cr is present in rocks, soil, volcanic dust, plants etc., in trivalent +3 oxidation state referred as Cr(III). Hexavalent chromium is a form of Cr metal having +6 oxidation state usually generated by the industries and has various impacts on different organisms. Electroplating, stainless steel fabrication, leather tanning, textile manufacturing, and wood preservation all involve Cr compounds like Cr(VI). Hexavalent chromium, Cr(VI), due

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to its high water solubility in broader pH range, mobility and ability to get easily reduced makes it 100-fold more toxic than Cr(III) for both acute and chronic exposures (Saha et al. 2011). US Environmental Protection Agency (EPA) has classified Cr(VI) under Group "A" carcinogen and as one of the main pollutants (Dhal et al. 2013). Hexavalent chromium is reported to cause lung cancer in both smokers and non-smokers; certain malignancy of the gastrointestinal and central nervous systems (Costa and Klein 2006). The United States is a major producer of chromium across the world. Despite reports on chromium toxicity in acute and chronic exposures, there is a knowledge gap regarding the mechanism of action (Pavesi and Moreira 2020). Currently, the physical or chemical remediation strategies employed for Cr remediation in landfills are adsorption, electro-dialysis, ion exchange, reduction, precipitation and reverse osmosis (Dhal et al. 2013). But since, these methods are non-eco-friendly and non-sustainable, practice of remediation mediated by microbes, known as bioremediation is gaining popularity among researchers. Microorganisms can survive in conditions where there is high levels of heavy metal contamination. These microorganisms can be isolated from such regions and used in the remediation of water or soil contaminated with heavy metals like Cr(VI). In countries like South Africa and India where there are large number of tanneries, microbial remediation has become a preferred method for the treatment of Cr(VI) (Tang et al. 2021; Shah 2020). Although the microbial remediation studies are being conducted in depth, these are being confined to laboratory experiments and though the organisms are screened from soil, they are mostly used in the remediation of polluted soil rather than heavy metals polluted soils.

This chapter is an informative resource to learn about the bioremediation of hexavalent chromium metal that is a potential pollutant leading to various health hazards.

2 Sources of Chromium in the Environment

2.1 Occurrence in Soil

Cr is abundantly found in the earth's crust with oxidation state ranging from Cr $^{+2}$ to Cr $^{+6}$. It never occurs in the nature in its elemental form, rather it occurs in its chromite form ($FeOCr_2O_3$) belonging to the spinel group, and is a major chromium commercial mineral. Chromite ore is mined in more than 20 countries, of which, India, South Africa, Turkey and Kazakhstan together accounts for over 80% of global production (Koleli and Demir 2016). It can be large, in the form of lenses and tabular formations, or distributed as granules. It can also be found in diamonds as a crystalline inclusion along magnesium and aluminium.

Chromite as accessory mineral in the form of layers is found commonly in iron- and magnesium-rich igneous rocks and sediments. Similar layers of almost pure chromite may be discovered in sedimentary rocks. When sedimentary rocks transform into serpentinite, the strata are maintained. These rocks, known as chromitites, are the most significant chromium ores. Weathering of chromite ore bodies can result in concentrations of the mineral in placer deposits.

The amount of chromium in soil varies depending on the contamination of soil with chromium by anthropogenic sources. The organic material in soil reduces Cr(VI) to Cr(III). Cr is also found in clay, shales and various other types of soil.

2.2 Occurrence in Air

As chromium is ubiquitous in nature, it can enter air through wind erosion of clay, shales and various other soil types. Mining processes of chromite ores also play a major role in introducing chromium into the air. The endpoint production of chromium compounds is perhaps the most important contributors of chromium-contaminated air in the Europe. Chromium in the aerosols is a result of emission from natural resources especially the windblown dust and volcanoes. However, data about occurrence of chromium in air is limited.

2.3 Occurrence in Water

Chromium is the second most abundant inorganic groundwater contaminant and a common soil contaminant, particularly in industrial and landfill areas. In both freshwater and marine environments, hydrolysis and precipitation determine the environmental fate of chromium, whereas sorption and bioaccumulation are relatively minor (Sueker 1964).

2.4 Chromium Cycle

Soil composition, physical conditions, texture and flora are the major aspects influencing the Cr mobility (Banks et al. 2006; Shah 2021). In aquatic conditions, Cr has oxidation states of +2, +3, and +6, with +3 and +6 being the most prevalent. Aqueous Cr(VI) is the most poisonous form, due to its easy oxidation, mobility, reactivity, in comparison with Cr(III), with no sorption in most sediment at $pH > 7$. The abundant available forms of Cr(III) occurring in aqueous and solid forms is $Cr(OH)_3$ and in solutions are $Cr(OH)_4^-$[$pH > 9$], $CrOH^{2+}$, $Cr(OH)^{2+}$, and $Cr_3(OH)_4^{5+}$.

Hydrolysis, oxidation-reduction, and precipitation are the three main chemical reactions that lead to chromium cycling. In partial equilibrium with oxygen soils and sediments contain Mn-oxides and carbon, which also play an important role in redox reactions with Cr, such reactions are thermodynamically spontaneous (James 2001).

The ability of soil to absorb the toxic Cr(VI) tends to limit the input of the metal from atmosphere. Cr(VI) is also introduced into soil via industrial effluents that increases the concentration of chromium in soil further leading to groundwater contamination. Hexavalent chromium is said to remain persistent in soil and water unless it is eliminated through leaching, adsorption, precipitation, uptake by living organisms or by reduction to trivalent form. Although the method of leaching has not been explored in depth, the occurrence of Cr(VI) in groundwater provides proof that it occurs. The minute levels of chromium present in plants are most likely absorbed by the root via hexavalent ion absorption. Although these seem to be the most important form of chromium available to plants, higher concentrations prove lethal to both plants and microorganisms.

The transport of chromium in natural waters, like rivers, lakes oceans, can occurs mostly through adsorbed suspended particles. Precipitated and solubilised chromium are in equilibrium state in ocean and sea waters. Dissolved chromium in marine water is removed by its integration into biological material and adsorption onto the soil particles. This integrated chromium dissolves in the water and at the sediment-water interface, resulting in dissolved chromium enrichment in deep and bottom water. These enriched sediments of anoxic basins and oxygen-free zones, in the bottom carries out the chemical reduction of hexavalent chromium. In partial equilibrium with oxygen soils/sediments contains carbon and Mn-oxides, which undergoes thermodynamically spontaneous redox reactions of chromium (James 2001). Mn-oxides possess higher surface area because of its tunnel structure which enables them to possess a high cation exchange capacity and as strong heavy metals scavengers under neutral pH condition. Thus, Cr(III) oxidation in soil is directly proportional to Mn(IV) oxides in the soil (Apte et al. 2005).

Metal transport can also be aided by living creatures. Plankton along the coast may have an impact on chromium transfer in the water. Marine flora and fauna also may absorb this metal. Also, instead of being transported, chromium can also form into small clumps or masses along with faecal pellets or dead organisms and absorbed into sediment. Since hexavalent anionic form is more bioavailable to living organisms in comparison with Cr(III), its removal from water and soil systems also occurs through living system (Kotaś and Stasicka 2000). The chromium cycle in polluted environment is depicted in Fig. 1.

3 Toxic Effects of Hexavalent Chromium

The biological activity of chromium depends on its oxidation state. The reduction of Cr(VI) into lower oxidation states, not necessarily to Cr(III), influences the chromium toxicity (Katz and Salem 1993). Hexavalent chromium is more poisonous and carcinogenic due to its strong oxidising properties, ability to permeate through biological membrane, reactivity with proteins and nucleic acids (Shekhawat et al. 2015), whereas Cr(III) is relatively innocuous (Pavesi and Moreira 2020). Further, the toxic effect of chromium in different living systems is discussed below.

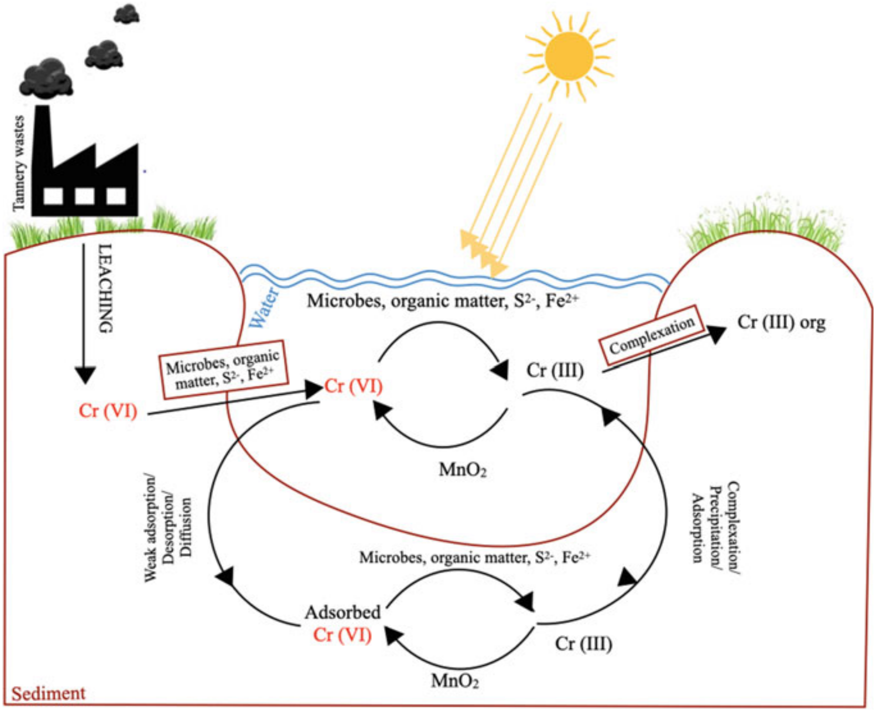

Fig. 1 Chromium cycle

3.1 Microbes

Toxicity of hexavalent chromium negatively impacts the soil-microbial diversity. According to a study conducted to unravel the soil-microbial activity under exposure to different dosage of hexavalent chromium, it was found that chromium is inversely proportional to soil-microbial load. Since, as the concentration of ($K_2Cr_2O_7$) increased, toxicity of hexavalent chromium drastically decreased microbial activity (Yao et al. 2008). In another study, increase in chromium concentration on the planktonic community of a coastal site revealed that viable bacterial density decreased. The results disclosed that even a minute increment in Cr(VI) levels drastically reduced phytoplankton and bacterioplankton population. Such effects pose challenge not only at the microbial population but also to higher trophic levels and to the entire community (Sathicq and Gómez 2018). Some microbes possess ability to convert hexavalent chromium into less toxic reduced form. Majority of them are facultative anaerobes that are commonly found in nature (Wang and Shen 1995). Bacterial chromate resistance determinants are commonly carried by plasmids. This field has, therefore, sparked attention of the researchers to explore such strains having chromate resistance and chromate reduction potential for effective detoxification of chromate-polluted wastewaters.

3.2 Plants

Chromium leads to the formation of ROS which induces oxidative stress conditions in plants. Chromium toxicity hampers plant's activities such as photosynthesis, transpiration, mineral intake, germination, growth of roots, stems, and leaves, thereby reducing the total dry matter and yield. Thus, exposure to chromium contamination in the plants challenges its metabolic pathway, either on enzymes or on other metabolites. However, in recent years, there has been increased interest in the potential of plants that can accumulate or stable Cr compounds for bioremediation of Cr pollution (Shanker et al. 2005).

3.3 Humans

Cr(VI) makes its entry into the body by the air we breathe, the food we eat, or the water we drink. Workers of chromium industry are exposed to mixed Cr(III) and Cr(VI) which gets absorbed in lung tissues. Even house dust and dirt contain Cr(VI), which can be swallowed or inhaled. Although the mechanism of how the hexavalent chromium elicit pathogenesis is not fully understood, some reports point that primary target organ for hexavalent chromium is the respiratory system. Exposure to the skin can cause skin cancer, irritate the skin or produce allergic response. Cr(VI) is one of the metals with the highest allergenic potential (Pellerin and Booker 2000). Though minute amount of chromium is needed for protein and lipid metabolism, higher doses cause oral toxicity in human. Intracellular reduction of Cr(VI) is mediated by glutathione and cysteine (Shekhawat et al. 2015). Exposure to relatively high levels of Cr(VI) might induce a runny nose in certain people, sneezing, itching, nosebleeds, ulcers, nose, as well as a hole in the nasal septum. Short-term exposure at high levels can result in harmful consequences at the point of touch, such as ulcers, nasal mucosal irritation, and perforations in the septum of the nose. Taking in a lot of sugar Cr(VI) in excessive amounts can induce renal and liver damage, nausea, gastrointestinal discomfort, stomach ulcers, seizures, and death are all possible side effects. Chronic exposures lead to perforations, ulceration of the septum, bronchitis, pneumonia, nasal itching, discomfort and impaired pulmonary functions (Saha et al. 2011).

4 Remediation Strategies

4.1 *Biological Detoxification of Cr(VI)*

Amid chemical and physical remediation of pollutant, biological means of degradation is a green technology that minimise the effect of toxic compounds. Bacteria-mediated remediation under the biological ways is the most effective one as it is rapid and requires minimum energy and chemicals for the process. Bacteria degrade heavy metals by utilising them to produce carbon dioxide, water, methane, mineral salt and gases (Ayele and Godeto 2021; Tirkey et al. 2021).

4.2 *SWOT Analysis of Bioremediation*

Strengths, weakness, opportunities and threats (SWOT) of bacterial remediation is presented in Fig. 2.

STRENGHTS

- Wide application in treatment of a diverse range of pollutants/contaminants
- Eco-friendly solutions
- By-products are non-toxic
- Since the process can be both aerobic as well as anaerobic thus a diverse variety of microbial groups are available for usage
- Operational stability is enhanced due to microbial methods even in in varied temperature, pH, salinity and storage conditions
- Enzyme mediated process provides higher specificity, sensitivity and selectivity
- Strain improvement is easier due to the use of microbes
- Other Industries can utilize Intermediates as precursors
- Ease in scaling up the operations for practical applicability
- Recyclability and reusability ensure that overall costs are low

WEAKNESS

- Research is in relatively nascent stages thus technical knowledge is limited
- Limited large scale deployment of existing technology
- Operational bottlenecks in maintaining culture conditions
- Assurance of absence of any toxic intermediates

OPPORTUNITIES

- Can promote global awareness of sustainable options for environmental preservation
- Improvement of soil quality and rejuvenation of soil polluted due to use of harmful chemicals in agriculture
- Opportunity for Transnational institutes and companies to partner in utilization of by-products
- Use of genetic engineering to modify microbes for industry relevant solutions
- Greater stress on efficient and ecofriendly solutions for the present market

THREATS

- Mutated microbes can pose a threat for natural ecosystems if unregulated
- Nascent microbial bioremediation technologies may posit an import-export barrier in future
- Current cultivation and maintenance costs are higher than alternatives
- Contemporary reluctance of markets to adopt microbial products
- Resistance from established physical and chemical treatment process lobbies
- Expensive raw material
- High rates of patent filing can counterproductive increase competition and create unnecessary regulatory bottlenecks

Fig. 2 SWOT analysis of bioremediation

5 Bacterial Remediation of Hexavalent Chromium

5.1 Bacteria-Mediated Cr(VI) Reduction

First report of Hexavalent chromium reduction to trivalent chromium was studied in *Pseudomonas* sp. (Mishra and Bharagava 2016). Rahman et al. (2015) reported a bacterium B2-DHA identified as *Enterobacter cloacae* which reduced chromium concentration >81% and can be used for chromium removal from contaminated area. Tolerance capacity of Cr(VI) by gram-positive bacteria was significantly better than gram-negative bacteria (Ayele and Godeto 2021). *Providencia* species are reported to remediate 100% and 99.31% chromate at a concentration of 100 to 300 mg/L and 400 mg/L, respectively. Under aerobic and anaerobic conditions, *Achromobacter* sp., *Bacillus* sp., *Brucella* sp. and *Enterobacter cloacae* HO1 are reported to reduce Cr(VI) (Laxmi and Kaushik 2020). Rahman and Singh (2014) reported 79% reduction of 50 mg L_1 of Cr(VI) by *Enterobacter* sp. DU17. Zhao et al (2016) isolated Fifty-five Cr(VI) tolerating strains from sediment samples and found isolate *Sporosarcina saromensis* M52 to degrade 100% Cr(VI) at 24 h. *Klebsiella* sp. had shown 95% and 63.08% Cr(VI) degradation in Luria–Bertani broth and tannery effluent, respectively (Hossan et al. 2020). Dead cells of Bacillus sphaericus were 13 to 20% more effective than the living cells and dead biomass showed 45% whereas 32% uptake by living biomass of B. sphaericus IV(4)10 on uptaking Cr(VI) (Arishi and Mashhour 2021). Qu et al. (2018) used a consortium of two-strain G1 *Geotrichum* sp. and B2 *Bacillus* sp. for the bioremediation of Cr(VI). The result indicated 91.67% reduction of Cr(VI) in 7 days. *Bacillus* sp. ltds1 was reported to reduce 95.24 ± 2.08% Cr(VI) at a concentration of 40 mg/L within 24 h (Seragadam et al. 2021).

5.2 Mechanism of Cr(VI) Remediation

Living and dead bacterial biomass has been employed to remove chromium from industrial effluents by following remediation techniques Biosorption, Bioaccumulation, Biotransformation and Bioleaching (Ayele and Godeto 2021). Different bacterial species employed for chromium bioremediation are presented in Table 1.

5.2.1 Biosorption

It is a metabolically passive technique where the metal ions can be trapped by microorganism on its cell walls extracellularly. Also the biosorption mechanism does not require energy for metal uptake. Biosorption can be done by active or inactive bacterial biomass that can be used as biosorbents containing amide, carboxyl, hydroxyl, phosphates and amino groups on bacterial cell walls (Guo et al. 2021; Arishi and Mashhour 2021; Ayele and Godeto 2021). Bacterial cell surface contain

Table 1 Bacterial species and their reduction mechanism for Cr(VI)

Bacterial species	Reduction method	References
Pediococcus acidilactici	Biotransformation	Lytras et al. (2017)
Pseudomonas gessardii	Biotransformation	Huang et al. (2016)
Sporosarcina saromensis M52	Bioremediation	Zhao et al. (2016)
Escherichia coli	Biosorption	Liu et al. (2015)
Streptomyces werraensis LD22	Biosorption	Latha et al. (2015)
Arthrobacter sp.	Bioremediation	Bhattacharya et al. (2015
Bacillus methylotrophicus	Bioremediation	Mala et al. (2015)
Serratia sp.	Biotransformation	Deng et al. (2015)
Ochrobactrum sp.	Biotransformation	Hora and Shetty (2015)
Arthrobacter ps-5	Biosorption	Shuhong et al. (2014)
Bacillus subtilis SS-1	Biosorption	Sukumar et al. (2014)
Bacillus amyloliquefaciens	Biosorption/Bioreduction	Rath et al. (2014)
Leucobacter sp. G161	Biotransformation	Ge et al. (2014)
Pantoea sp.	Biosorption	Ontañon et al. (2014)
Pseudomonas putida V1	Biosorption	Cabral et al. (2014)
Acinetobacter haemolyticus	Biotransformation	Ahmad et al. (2013)
Bacillus cereus	Biosorption	Naik et al. (2012)
Mesorhizobium amorphae	Biosorption	Xie et al. (2013)
Pseudomonas aeruginosa	Biosorption	Kang et al. (2007)
Halomonas sp. TA-04	Bioremediation	Focardi et al. (2012)

Adapted from Fernández et al. (2018) and Laxmi and Kaushik (2020)

anionic lipopolysaccharide, phospholipid and outer membrane protein and peptidoglycan which makes them negatively charged that helps the binding of positively charged metals. Studies have shown that exopolysaccharides produced by *Pseudomonas aeruginosa* increased with an increase in Cr(VI) concentration indicating an increase in the adsorption of Cr(VI) on the cell surface, thus preventing it to enter the cell. Reports have shown the adsorption of Cr(VI) caused due to presence of some functional group on the cell wall of *Aspergillus niger*, *Aspergillus oryzae* and *Fusarium oxysporum* (Guo et al. 2021; Bhattacharya et al. 2019).

5.2.2 Bioaccumulation

A technique where uptake of metal ions is metabolically done by living bacterial cells and requires energy for the transportation of Cr(VI) across the membrane. Intracellular reduction of Cr(VI) is performed by Cr-reducing bacteria (CRB) using Cr(VI) reductase (*chrR*). The structure of Cr(VI) ions is similar to that of tetrahedral sulphate (SO42-) and so it enters the bacterial cell membrane through SO42-transport channel (Arishi and Mashhour 2021; Guo et al. 2021). Bioaccumulation is a two-step process. An initial step involves, rapid adsorption at the cell surface and later slower metabolic transport of the metal ion inside the bacterial cell where it gets bind intracellularly (Guo et al. 2021; Anju 2017; Narayani and Shetty 2013). The Cr(VI) receives NADH molecules and reduces to Cr(V) by chrR followed by receiving two-electron to form Cr(III) (Shahid et al. 2017). An indigenous strain *Klebsiella pneumoniae* MB361 was able to accumulate an 83.51% of Cr(VI) at a concentration between 500 and 1000 ug/ml (Arishi and Mashhour 2021).

5.2.3 Biotransformation

It is a detoxification process that involves reduction of highly toxic Cr(VI) to less toxic Cr(III) by CRB through enzymatic reaction by chrR present in the cytoplasmic fraction (Ayele and Godeto 2021; Fernández et al. 2018) and then effluxed from the cells by chromate transporter gene (Arishi and Mashhour 2021). Various chromate reductases like NemA, LpDH and YieF were found in the cytoplasmic fraction catalysing the reaction under aerobic and anaerobic condition (Fernández et al. 2018). Gu et al. (2015) reported chrR found in the soluble fraction of *Aspergillus niger*. Biotransformation of Cr(VI) to Cr(III) generates intermediate oxidation states of Cr(V) and Cr(IV) which may have more toxic effect than Cr(III). But CRB have chromate resistant plasmid and iron efflux system to solve this problem (Dhal et al. 2013).

5.2.4 Bioleaching

Conversion of an insoluble metal into soluble form (metal sulphide to metal sulphate) by means of biological method is defined as bioleaching and in this process the metal is extracted into aqueous solution. Bioleaching bacteria are mostly from the following genera *Acidithiobacillus*, *Thiobacillus*, *Thermithiobacillus*, *Sulfolobus*, *Halothiobacillus* and *Leptospirillum*. But extensively employed bacteria for bioleaching of heavy metals are *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Bioleaching can be achieved by two mechanisms: direct leaching and indirect leaching.

Direct Leaching

It is related to the direct action of the bacteria. Bacteria through its extracellular polymer come in contact with the metal sulphide and oxidise it using intracellular oxidase system by producing H+ which lowers the pH of the sludge and produces soluble sulphate.

Indirect Leaching

It is related to indirect effect of low pH. The metabolites of *Thiobacillus* sp. are used in indirect leaching to solubilise metals. When oxidation of metal ions from low valency to high valency takes place, the high-valent metal ions oxidise sulphur compounds of low-valent which leads to low pH and form of heavy metal changes and extracted from the sludge. However, direct and indirect bioleaching may be involved at a same time (Yang et al. 2020; Gu et al. 2017). Ghavidel et al. (2018) studied the metal removal through bioleaching and chemical leaching. They found that bacteria and chemical leaching could dissolve Cd, Mn and Zn at 71.90%, 92.50%, 89.14% and 22.03%, 25.06%, 14.23%, respectively.

6 Improvement in Bioremediation Strategies for Cr(VI) Reduction

6.1 *Optimisation of Physicochemical Factors*

Effect of pH, temperature, inoculum size, initial metal concentration and electron donor are the basic and essential physicochemical factors to be considered for optimising Cr^{6+} reduction efficiently.

6.1.1 Effect of pH

pH plays an important role in the desorption of Cr(VI) from the contaminated soil (Qu et al. 2018). In general, Cr(VI) are found in the form of HCrO4 and CrO4 2 under acidic and alkaline environments, respectively. Soil particles having negative charge absorbs positive charged ions and can, therefore, easily desorp CrO4 2 than HCrO4 and the concentration of HCrO4 also decreases when pH is increased (Krishna and Philip 2005). Qu et al. (2018) had reported maximum desorption (81.26%) was achieved at pH 10 and the efficiency to reduce to 24.13% at pH 2 indicating alkaline condition was favourable for desorption of Cr(VI) from the soil. At pH 7.5, the reduction of Cr(VI) by *Sporosarcina saromensis* M52 was 100% (Ran et al. 2016). The amino, carboxylic and phosphate groups are deprotonated at pH values higher than pKa which leads to their binding with positively charged metal ions. The metal ion does not bind to the biomass once these functional groups become protonated at pH less than pKa. Therefore, the reduction of Cr(VI) generally occurs at low pH (Ayele and Godeto 2021). Gu et al. (2017) reported that the preacidification of the sludge at pH value 4.0 was not required for iron-based or sulphur-based bioleaching using *Acidithiobacillus*. A neutral pH was found to be most efficient for the removal of Cr(VI) from industrial wastewater by *Bacillus* sp. ltds1 for 24 h (Seragadam et al.

2021). Karthik et al. (2016) also found maximum Cr(VI) removal at pH 7.0 by *Cellulosimicrobium funkei* strain AR6. Thus it can be concluded that optimal initial pH for bioremediation depends on type of bacteria and their different binding sites.

6.1.2 Effect of Temperature

Temperature below and above the optimum level affects the cell growth and enzymatic activities essential for Cr(VI) reduction (Ayele and Godeto 2021; Bhattacharya et al. 2019; Ran et al. 2016). Joutey et al. (2014) had shown optimum reduction of Cr(VI) at 30 °C by *Serratia proteamaculans*. Ran et al. (2016) has reported 100% degradation of Cr(VI) at a temperature of 30 °C or higher by *Sporosarcina saromensis* M52. Studies on effect of temperature range (7–42 °C) had shown that low temperature resulted in slow process but when the temperature was raised from 7–28 °C, the time to acidify the pH decreased from 14 to 5 days. Also at 42 °C, there was no bacterial growth (Gu et al. 2017). Bacterial growth and Cr(VI) reduction are negatively affected by high temperature as it causes protein and DNA denaturation along with structural change of membrane (Ayele and Godeto 2021). Vijayaraghavan and Yun (2008) reported the decrease in membrane fluidity caused dysfunction of the transport system at low temperature.

6.1.3 Effect of Inoculum Concentration

An increase in cell density increases the rate of biosorption and reduction of Cr(VI) (Narayani and Shetty 2013) due to an increase in the surface area of biosorbent and their binding sites (Vijayaraghavan and Yun 2008). Ran et al. (2016) studied the effect of inoculum concentration using the range from 1 to 10%. With the increase in inoculum concentration, the efficiency of Cr removal increased to 100% after 24 h by *Sporosarcina saromensis* M52. Seragadam et al. (2021) used 1 to 5% of inoculum concentration and showed an increase from 51.3 ± 2.56% to 69.7 ± 2.85% removal of Cr(VI) after 24 h. Hence, 4% was selected for efficient degradation, whereas Li et al. (2021) reported 75.69% degradation using 10% inoculum of *Stenotrophomonas acidaminiphila* 4-1.

6.1.4 Effect of Initial Metal Concentration

Usually, an increase in initial chromate concentration leads to decrease in its biosorption and reduction (Wani et al. 2007). An initial metal ion at higher concentration causes more chromium ions unabsorbed due to saturation of binding sites (Pun et al. 2013). Jobby et al. (2019) reported a decrease in chromium removal (99.88% to 83.69%) when the concentration of Cr(VI) was increased from 100 mg/L to 500 mg/L. Similar results were also reported by Ran et al. (2016) where they found the maximum degradation (82.5%) at 50 mg/L Cr(VI) but no degradation was recorded at 500 mg/L after 12 h. A driving force is generated to overcome metals mass transfer resistance between the aqueous and solid phases (Shamim 2018). Toxicity generated by high Cr(VI) concentration affects the bacterial growth, metabolism and enzymatic activities which leads to lower adsorption for metal binding. There are also reports that showed the degradation capacity and tolerance level were not related to each other However, different bacterial species react on chromate concentration according to their resistance ability (Bhattacharya et al. 2019; Ran et al. 2016).

6.1.5 Effect of Electron Donor

Electron donor (organic or inorganic reductant) is an excellent strategy to catalyse bacterial chromate reduction. CRB use a variety of compounds an electron donor for the reduction of Cr(VI) to Cr(III). Biotransformation process depends on an electron donor (Arishi and Mashhour 2021). Electron donor in the form of amino acids, fatty acids, carbohydrates, NADPH and zero-valent iron is added to the contaminated medium (Bhattacharya et al. 2019). Enhanced chromate reduction was achieved after the addition of tryptic soy broth to the soil contaminated with Cr(VI) (Tokunaga et al. 2003). Losi et al. (1994) added organic manure as reductant to the contaminated medium.

6.2 Bacterial Immobilisation

In order to minimise the toxic effect and to protect the bacterial cells from direct damage of Cr(VI), CRB can be immobilised into various support materials/matrices (Bhattacharya et al. 2019; Joutey et al. 2015). Various support matrices such as alginate–carboxymethyl cellulose, calcium alginate gel matrix, biofilm supported on granular activated carbon, agar layers on the surface of synthetic membrane, untreated rubber wood sawdust, wood-husk, a natural cellulose-based support material, etc. are used to immobilise bacterial cells for the reduction of Cr(VI) (Narayani and Shetty 2013). Immobilisation of whole cell is more effective over free cells in terms of stability, high biomass loading, reduced nutrient depletion and reuse. Immobilised cells are used in designing bioreactors for heavy metal degradation. Bacterial immobilisation helps in mitigate Cr-contaminated soil (Guo et al. 2021).

6.3 Genetic Engineering

Genetic engineering is used to enhance the bacterial tolerance and removal abilities of CRB for reduction of Cr(VI). Certain bacteria are able to survive in highly toxic environment due to their genetic adaptation in harsh condition. Chromate resistance and reduction were plasmid mediated (Joutey et al. 2015). Gene *nemA and* gene *phaC* of *E. coli* and *Ralstonia eutropha*, respectively, were combined to develop and express as a Cr-reducing enzyme able to reduce Cr (200-fold higher) (Arishi and Mashhour 2021). An open reading frame, yieF of *E. coli*, was found to resemble with *chrR* was cloned and showed efficient Cr reduction (Ackerley et al. 2004; Park et al. 2002). Genetically engineered bacteria by Frederick et al. (2013) to produce trehalose reduced 1 mM Cr(VI) to Cr(III). To enhance bioaccumulation, bacteria have been engineered to express import-storage systems. This has led to the production of cytoplasmic metal-binding entities which are mostly metal-binding proteins and enzymes that can bind to heavy metals (Diep et al. 2018).

7 Concluding Remarks

Hexavalent chromium occurring naturally and through anthropogenic activities is a threat to the environment as it causes various toxic effect to living organism including microbes, plants and humans. It is widespread in nature thus polluting the soil, air and water resources. Remediation technologies including chemical and physical involve chemicals and expensive methods. Bacterial remediation stands out most effective green degradation process as their by and end products are less harmful compared to chemical and physical remediation. CRB through their unique characteristics are employed in Cr removal from the environment. Bacteria degrade Cr and transform highly toxic Cr(VI) to low toxic Cr(III) level through their various direct and indirect mechanism particularly as biosorption, bioaccumulation, biotransformation and bioleaching. These remediation methods in natural surroundings require and are influenced by physicochemical factors such as pH, temperature, bacterial density, metal concentration and electron donors in an optimum amount for effective removal of Cr(VI). Strategies like immobilisation and genetic engineering are being applied to enhance the Cr removal abilities of CRB. However, there is a constant need to isolate and develop improved bacteria for efficient remediation of Cr from the contaminated areas.

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New Bioremediation Technologies to Remove Heavy Metals and Radionuclides

Jaidev Bhatt, Snehal Desai, Nilesh S. Wagh, and Jaya Lakkakula

1 Introduction

Bioremediation, the term 'bio' refers to biological material and remediation, as in correction or breakdown. The focus here would be on bioremediation, this technology has made it possible to decontaminate groundwater soil and has assisted us in cleaning up our oceans after massive oil spills and other environmental disasters.

Its contaminants are limited, the timeframes involved are lengthy, and the contamination of residual levels achieved is not always appropriate. Even though the methods used are not technically complex, designing and implementing a successful bioremediation programme may necessitate a significant amount of experience and expertise due to the need to thoroughly assess a site for suitability and optimize conditions in order to achieve a satisfactory result (Azubuike et al. 2016).

There are three generic approaches used in bioremediation. The first method is to keep the contaminant contained within the affected area. This, however, does not reduce total pollutant concentrations in soil, but rather aims to manage pollutant exposure by reducing pollutant mobility and bioavailability. The second method is to treat the contaminants in place. The third option is to treat them ex-situ. The second and third approaches use passive or active bio/remediation technologies to

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reduce the total concentration of pollutants in soil to the maximum allowable total concentration (Length 2007).

Bioremediation is a natural solution that uses natural biological activity to destroy or render various contaminants harmless. As a result, the use of low-tech, cost-effective approaches that are widely accepted by the public and can often be carried out on-site has increased. Thus, the growth rate of microorganisms, number and type of microorganisms, bioavailability of contaminants, biodegradability of contaminants, nutrients, oxygen, hydrogen peroxide, electron acceptors, metal ions, temperature, pH, moisture content, redox characteristics, site hydrogeologic characteristics are some factors on which the rate of bioremediation shall be dependent on.

The introduction of industries has resulted in a growth in industrialization, which has contributed significantly to the formation of pollutants (heavy metals, radionuclides, and other pollutants) in the environment. Heavy metal-containing trash is discharged directly into rivers and drains by industries. Silver (Ag), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), zinc (Zn), and other metals and metalloids having a density more than 5 g/cm^3 are classified as ‘heavy metals’. Heavy metals can build up in the human body, posing health risks. Many significant diseases are linked to toxic metal exposure, including autoimmune disorders, cardiac disorders, digestive problems, kidney, liver, lung, and stomach cancer. Heavy metals must be removed from wastewater or changed into less hazardous forms before it is dumped to the environment or used for irrigation. Heavy metals were traditionally removed using chemical and physical methods. Similarly, there is a significant amount of radioactive wastes generated by nuclear power plants, which accounts for 95% of all radiation generated from all sources (Ahier and Tracy 1995).

The naturally occurring radioactive materials may also be exposed to personnel at any stage during processing of these materials (Krieger 2005). There are comparable effects in between radioactive waste and nuclear fallout which cause, enhanced risk of cancer, neurological disorders, infertility, birth defects, etc., are the repercussions caused by nuclear radiation generated by radioactive waste (Perspectives 2003). Numerous types of cancer like colon, breast, lung, ovarian, etc. have been reported after exposure to radiation from nuclear waste (Reza Najem and Voyce 1990).

Our focus on this book chapter is to highlight about methods of tackling heavy metals and radionuclides pollution through various methods of bioremediation, by process of bioaccumulation, biosorption, phytoaccumulation, microbial bioremediation, microbial reduction, response surface methodology (RSM), biotransformation. This segregation of the materials used for the bioremediation has been ordered as follows bacteria, fungi, algae, and plant. Although there is enough literature around bioremediation of heavy metals, there has also been a considerable amount of work around bioremediation of radionuclide; these studies would help us understand how well the bioremediation processes could work to reduce these harmful elements and compounds to lesser toxic levels.

2 New Bioremediation Technologies to Remove Heavy Metals and Radionuclides

Development of industrial activity produces a large volume of contaminated effluents rich in radionuclides and toxic metals posing a major environmental hazard. Heavy metals like Cd, As, Cu, Cr, Hg, Pb, Co (Cobalt) and some radionuclides, such as U(VI) (Hexavalent Uranium), ^{60}C (Cobalt–60), Th (Thorium), Se (Selenium), can have adverse effects on the environment over the permissible limits. For heavy metal remediation, chemical precipitation in combination with some other methodologies such as (e.g. precipitation of sulphide with nanofiltration) has being used. In chelation, for instance, in heavy metals chelation, a chelating agent such as 1,3-benzenediamidoethanethiol (BDET^2) dianion was utilized. Then, a procedure known as ion exchange has a great capacity and efficiency in treatment. For this reason, synthetic resins are being employed. The other expensive traditional techniques, like oxidation–reduction and ion exchange, result in harmful sludge as a secondary contaminant.

In our surroundings, naturally occurring radionuclides are found in either cosmogenic or terrestrial forms. The primary radioisotopes produced by the interaction of various gases with cosmic rays include ^{3}H (Tritium), ^{7,10}Be (Beryllium—7, 10), ^{14}C (Carbon—14), ^{26}Al (Aluminium—26), and ^{39}Ar (Argon–argon dating). The presence of (NORM) Naturally Occurring Radioactive Material in rocks, minerals, and soil is distinguished by longer half-life time span. Radionuclides have been discovered in variety of seafoods in India, a wide range of foods in the Balkans and in drinking water and food in Switzerland. Biological methods use natural organisms such as algae, bacteria, plants, and fungi to eradicate contaminants, or to mineralize metallic contaminants and their radioactive constituents, enclosing them inside a defined perimeter. For example, microbes such as *Desulfuromusa ferrireducens*, *Rhodanobacter* sp., etc. showed capability of removing these pollutants. Bacterial contact increases the solubility of modified radionuclides with electron addition or removal, boosting radioactive pollutants' mobility and allowing them to be rapidly removed from our environment. As a result, bioremediation has arisen as an appealing, cost-effective, and ecologically sound solution.

2.1 Bioremediation Using Bacteria for Removal of Heavy Metals and Radionuclides

2.1.1 Bioremediation Using Bacteria for Removal of Heavy Metals

Bioremediation strategies like bioaccumulation, biocoagulation, biosorbents, bioleaching and immobilization resulted in remediating heavy metals with few alterations in natural environmental constituents are relatively cheaper and more efficient. These studies pave way for the synthesis of metal-mineral forms/composites which are sustainable and might be useful in the recovery of these heavy metals from toxic systems.

Varied microorganisms are being used as tools in remediation of heavy metals which include many mechanisms like volatilization, valence transformations and extracellular chemical precipitations. *Rhodobacter sphaeroides* is one such example studied by Li. et al. which is the bioremediation of lead-contaminated soil. Isolation of desired strain was obtained from the oil field injection water in Daqing. Bacterial sampling was successfully achieved during the stationary and logarithmic phases (24 h–48 h) in the purview of conducting the subsequent experimental analysis. The optimum levels of culturing conditions for this strain were $T = 30–35\ ^\circ C$, $pH = 7$ and size of inoculum around 2×10^8 m/L^{-1}. Besides, various concentrations of $PbNO_3$ solution (0, 20, 50, 100, 150 mg/L) were followed, to study the levels of tolerance of the strain to Pb also under anaerobic environment for 5 days.

The performance of bioremediation on *R. sphaeroides* was examined with the help of wheat seedling growing experiments which showed reduced phytoavailability in the soils which were amended. The HNO_3 and $HClO_4$ (3:2 v/v) at a liquid/ solid ratio were used along with acid digestion of 10 mL g^{-1}. Each fraction of soil containing Pb and the overall concentration in wheat and soil was analysed using ICP-AES (IRIS Intrepid-II). The detection limit of Pb was noted at 0.02 ug m/L^{-1}. The findings showed that *R. sphaeroides* was unable to eliminate Pb from the soil, but rather modified its speciation. However, *R. sphaeroides* treated Pb contaminated soil by the method of precipitation of the inert compounds, which includes lead sulphate and lead sulphide. Even though Pb efficiency of bioremediation seemed to be low on wheat (14.78% root and 24.01% in leaf), *R. sphaeroides* is considered as an alternative which is favourable for lead bioremediation in the contaminated soils (Li et al. 2016b).

Another instance of same species is studied by Peng et al., reveals how microorganisms can act as challenging technique in remediation of soils contaminated with toxic pollutants especially heavy metals. A bioremediation agent, namely *Rhodobacter sphaeroides,* stabilized the Zn (Zinc) and Cd (Cadmium) contaminants in soil. This treatment helped in reducing the bioavailable fractions like exchangeable (30.7% reduction of Cd and Zn of about 100%) and phases of Cd and Zn bounded by carbonates. More stable fractions like organic bound, Fe–Mn Oxide and residual phases were higher after the process of bioremediation in the case of Zn.

One-way ANOVA (LSD method) Analysis of variance (Least Significant Difference) was used in identification of the differences between Cd and Zn in the soils (0 d, 14 d and 50 d), its geochemical fractions and the total content. Direct evidence for Cd bioremediation was given by wheat seedling experiment, as phytoavailability of Cd was seen to be reduced. Also, examining the Cd levels concluded that wheat root and leaf followed reductions of about 47.2% and 62.3%, respectively. This research also revealed that bioremediating *R. sphaeroides* in the soil environment enhances the ratio of Zn/Cd in the harvested wheat leaf and root. Thus, indicating *R. sphaeroides* as a potential applicant in biofortification and in agronomic practices (Peng et al. 2018).

Various industries release heavy metals as effluents and nuclear radiation into the environment, which accumulates consequently in the soil. This accumulation of noxious metals in the ecosystem is detrimental to biodiversity and mankind. Thus, generating heavy metal bioremediation techniques for soil is of paramount importance. The study of Audu et al. similarly focuses on the bioremediation of the noxious metals (Fe, Cu, and Pb) from the contaminated soil near the site of mining located at Zamfara State, Nigeria using the microorganism, *Pantoea agglomerans.* Using the spread plate method, isolation of the bacteria was performed. The confirmation of the desired bacterium *Pantoea agglomerans* was performed using Microgen GNA-ID system which denoted 84% of probability. The cell wall of the bacterium would have a peptidoglycan consisting of a thick layer making the bacteria thrive in metal-contaminated environments.

The concentration of the heavy metals was determined with the help of Atomic Absorption Spectrophotometer (AAS) wherein Lead (Pb) was around 1.328 ± 0.493 to 2.326 ± 2.093 mg/L, Copper (Cu) 0.234 ± 0.117 to 1.054 ± 1.486 mg/L & Iron (Fe) 18.498 ± 11.462 to 27.754 ± 57.510 mg/L. *P. agglomerans* perceived biosorption in the following trend Pb > Fe > Cu. The key factor affecting the microbial biosorption is the temperature wherein the optimal temperature was kept at 35 °C and ideal pH was at 7. The uptake of metals with the process of biosorption revealed that the bacterium had absorbed around 96% of Fe, 99% of Pb and 60% of Cu. This connotes the potentiality of *P. agglomerans* in eliminating these toxic metals from the metal-contaminated soils (Audu et al. 2020).

Another approach by P. Anusha and D. Nataranjan shows the potency of native bacteria *Bacillus cereus* (A1-4), being multi-metal tolerant isolated from bauxite mines of Kolli Hills, Tamil Nadu. The major aim was the determination of the remediation of metal and its efficiency for this strain in the field remediation study and the laboratory. *Bacillus cereus* (A1-4) strain was rod shaped, gram positive, spore producer, and showed motility. The colonies when cultured were characterized as smooth, large, round, and flat as shown in Fig. 1.

The characterization on molecular level of this isolate was performed using 16 rRNA sequence analysis. The metal tolerance efficiency was determined of this selective bacterium for Cr, Cu, Pb, Fe (Iron), Zn and Mn (Manganese). The ability of resistance of this metal-tolerant bacteria was checked by MIC (Minimum Inhibitory Concentration) test, which noted that the bacterial isolate showed high resistance towards lead ions followed by the other different test heavy metals. The efficiency of bioremediation by the method of batch by this bacterium noted to be 97.17% (Pb), 77.44% (Zn), 91.98% (Cu), 60.92% (Mg) Magnesium, 79.9% (Cr), 81.6% (Fe), 62.8% (Mn). The analysis by FT-IR showed C–C stretch, N–H primary amine and the presence of N–O aliphatic nitro compound in the Pb being treated by bacterium *B. cereus.* Some functional group alterations were seen in the treated sample in comparison with the control (–C=C– alkyne and C–N amine groups).

This study was highlighted to be novel when field-based metal remediation by *B. cereus* was carried out, wherein the strain showed satisfactory % age of in situ bioremediation in 8 weeks i.e. 71.8% (Pb), 41.4% (Cr), and 53% (Cu). In this in situ bioremediation, *B. cereus* showed notable capacity of remediation, particularly lead

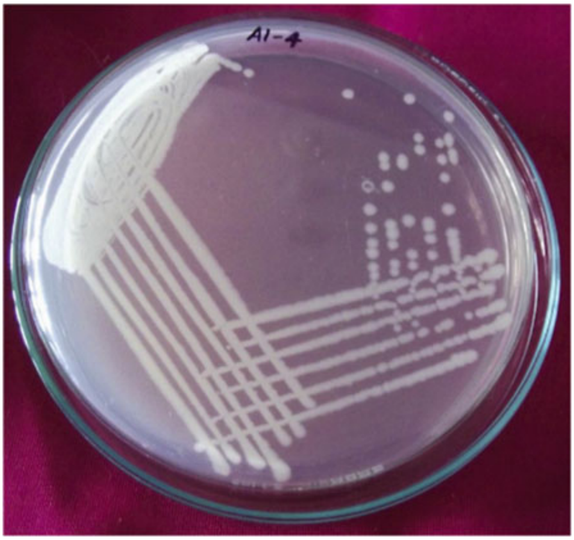

Fig. 1 *Bacillus cereus* (A1-4)—pure culture. Reprinted with permission from Anusha, P., Natarajan, D., 2020. Bioremediation potency of multi-metal-tolerant native bacteria Bacillus cereus isolated from bauxite mines, Kolli hills, Tamil Nadu. A lab-to-land approach. Biocatalysis and Agricultural Biotechnology 25, 101581. https://doi.org/10.1016/j.bcab.2020.10158

followed by other metals. The overall outcome strongly suggested that *B. cereus* can be used as a potential strain in treatment of heavy metals polluted soil on a large scale (Anusha and Natarajan 2020).

A vital trace element, copper, found in plants and animals, but it is a heavy metal with certain drawbacks on the environment and humans at the larger concentrations. Excessive copper consumption causes capillary damage, severe mucosal irritation and its corrosion, renal and hepatic damage, irritation of CNS followed by depression, gastrointestinal irritation, necrotic changes in the liver, kidney and Wilson's disease, which results in liver and brain malfunctions. There is a necessity for novel, low-cost technology that can remove copper from aqueous solutions while being environmentally safe.

Investigation for bioremediation of Cu with the help of Cu-resistant bacteria, *Stenotrophomonas maltophilia* PD2 was performed by Ghosh et al. Isolation of the soil, bacterial samples and its cultivation from the Dhapa area (solid waste disposal site at Kolkata, India) were carried out successfully. Biochemical tests gave the colony morphology and nature of biochemical test results. Bacterial characterization was completed based on nucleotide homology and phylogenetic analysis. Using the stock solution of Cu, (which was then prepared using Copper Sulphate Pentahydrate), different copper concentration solutions were formed. Optimization of bioremediation was performed by Design Expert Version 7.0.0 (Stat Ease, USA).

Response surface methodology (RSM) also called as Central Composite Design (CCD) had been applied for processing the parameters of optimization, which in turn these parameters were held accountable for bioremediation of copper ion effect. The optimum conditions found were pH 5.50, Cu concentration of 50.00 mg/L, contact time 26.00 h and around 90% of the removal of Cu. Thus, this study showed close interaction for optimization of bioremediation between experimental and simulated values concluding it as a potential agent for removing Copper Cu (II) from aquatic environments (Ghosh and Saha 2013).

Mercury polluted soils have proven to be expensive and logistically complex to remediate them. One such microbial strategy devised by Mccarthy et al. involves the intracellular volatilization and uptake of mercuric ions. In this research, immobilization of *Pseudomonas veronii* cells was taken place in xanthan gum-based biopolymer enrouting encapsulation method and then those cells were grown to stationary phase. Coating of *P. veronii* biopolymer was performed onto the natural granules of zeolite.

Moreover, when these immobilized cells were transported from Australia to the USA, they showed the retaining capability of the viability and Hg volatilization functionality. The maximum flux rates raised 10 ug Hg m^2 h^{-1} from the mine's tailings (=7 mg kg^{-1} Hg with 50% v/v water). Four orders of magnitude above the background flux levels envisioned that the GEM (Gaseous Elementary Mercury) emitted can be captured and transformed into the metallic Hg, it further be stored or recycled.

The Hg biogeochemical cycle, which might result in very hazardous compounds, must be disrupted by not isolating but also removing Hg. Devoid of GEM translocation back into the atmosphere breaks the Hg cycle. The overarching goal was boosting the GEM emissions from soil which was contaminated in order to facilitate bioremediation of metal-contaminated soils and mining waste streams. Furthermore, the studies are essential for identification of an appropriate emitted GEM capture technology that breaks the Hg biogeochemical cycle (McCarthy et al. 2017).

Ganga River, (which flows from the Himalayas) located in Bihar's Gangetic plain, is a natural source of arsenic. However, no confirmation of natural arsenic emission has been obtained in the Ganga plain. However, due to the natural processes in groundwater from the holocene sediments, arsenic is released in the Gangetic plain of Bihar. These holocene sediments comprised of clay and silt. Around 10 ppb (more than the permissible levels) the concentration of arsenic in the Bihar's Gangetic Plain was obtained. As a result, these hazardous places require immediate attention.

High arsenic concentrations are prevalent in the central Gangetic plain, posing a considerable health danger. This research by Satyapal et al. involves (48 morphologically distinct) arsenite-resistant bacterial isolation from the region of middle Gangetic plain for possible bioremediation of arsenic toxicity. The MIC values of arsenite ranged from 1 to 15 mM of the bacterium isolates. Thus, based on the MIC values AK1 (KY569423) and AK9 (KY569424) were selected for further studies.

The genus was confirmed as *Pseudomonas* by 16 S rRNA gene sequencing and retrieval of similar sequences from NCBI database was performed and then were aligned using CLUSTAL W. The biochemical (in terms of production of enzymes such as carbohydrate utilization, urease, β-galactosidase and nitrate reductase) and characterization of physiology hence was determined based on optimum pH 7.0 and optimum temperature 30 °C. The phylogenetic analysis was studied using the neighbour-joining method.

In the estimation for efficiency of arsenic transformation by the arsenic-resistant strains, i.e. from As III to As V and As V to As III, $AgNO_3$ test-based microplate screening assay was used. The presence of aoxR, aoxB, and aoxC genes was confirmed which played a pivotal role in bioremediating arsenic by the arsenite oxidation. Thus, this work is the first evidence of transformation of arsenic from *Pseudomonas* genus. Besides, these isolated strains portraited heavy metal resistance against Cr (IV), Ni (II) (Nickel), Co (II), Pb (II), Cu (II), Hg (II), Ag (I) (Silver), and Cd (II) (Satyapal et al. 2018).

This study facilitates another example of sustainable bioremediation, in which *Enterobacter cloacae and Serratia marcescens* were evaluated for bioremediation of Cd (II). The ureolysis-induced calcium carbonate precipitation methodology was used in this study. The technique of co-precipitation of Cd (II) and Ca (II) removed cadmium. At 96 h of incubation, *S. marcescens* and *E. cloacae* EMB19 removed 96% and 98% of the initial 5.0 mg/L soluble Cd (II) from the urea and $CaCl_2$ laden medium, respectively. *E. cloacae* showed higher cadmium removal efficiency in comparison with *S. marcescens.* In-vitro Cd (II) remediation studies were undertaken using urease-containing cell-free culture supernatant of *S. marcescens* and *E. cloacae* depicted respective 98% and 53% removal of initial 50 mg/L Cd (II) from these reaction mixtures in the copresence of Ca (II).

Only 16% and 8% removal of Cd (II) were detected for *S. marcescens* and *E. cloacae* EMB19, respectively, in the sole presence of Cd (II). The elemental analysis was carried out using Energy-Dispersive X-Ray Spectroscopy (EDX) described the prevalent Cd and Ca ions. Overall, it can be derived that in order to control Cd and other metal contaminations, urease-mediated remediation of Cd (II) could be effectively used (Bhattacharya et al. 2018).

2.1.2 Bioremediation Using Bacteria for Removal of Radionuclides

Currently, most of the radioactive waste is buried in a deep geological deposit. Radionuclide-tolerant microorganisms thrive in such contaminated surroundings. Soil samples were taken from sites around nuclear power plants that had been contaminated to radionuclides and heavy metals, and then tested for thorium-tolerant bacteria.

Because it could flourish at high levels of Th (Thorium) (1000 mg/L), the novel thorium-tolerant *Ochrobactrum intermedium* AM7 was chosen. The strain AM7 was found to be 99.88% like type strain to Ochrobactrum intermedium CGUG24694T using the BLASTn method, hence the sequence was submitted to GenBank as *Ochrobactrum intermedium* AM7. The strain exhibited production of exopolysaccharide (EPS) and biofilm. FTIR spectroscopy revealed the functional groups and their active participation of EPS involved in EPS-Th binding. FTIR spectra of the unloaded control (EPS) and loaded test (EPS Th^+) EPS of AM7 were measured at the range 4000–600/cm as shown in Fig. 2.

The primary functional groups in EPS are ascribed to characteristic broad and strong peaks at 3261 per cm (O–H hydroxyl stretching) and 2930 per cm (C–O carboxyl bonds stretching) according to IR spectra analyses. The effects of EPS medium ingredients on EPS yield by O. intermedium AM7 were investigated using a one-factor component in this study.

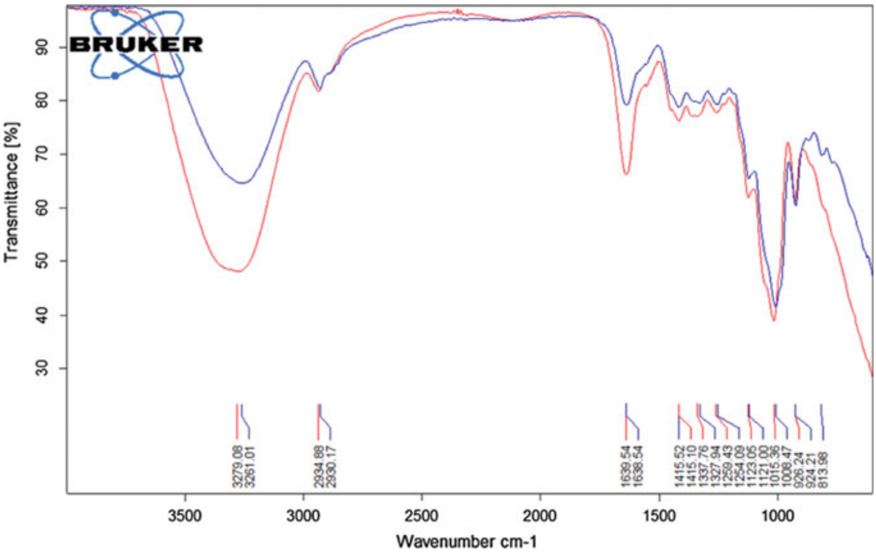

Fig. 2 FTIR analysis of *O. intermedium* EPS (blue) and EPS Th^+ (red). Reprinted with the permission from Shukla, A., Parmar, P., Goswami, D., Patel, B., Saraf, M., 2020. Characterization of novel thorium-tolerant *Ochrobactrum intermedium* AM7 in consort with assessing its EPS-Thorium binding. Journal of Hazardous Materials 388, 122,047. https://doi.org/10.1016/j.jhazmat.2020.122047

The optimum medium concentration of NB components for facilitating maximum AM7 growth was investigated using response surface methodology with central composite design (RSM-CCD) in the context of AM7 physio-chemical characterization. On MPE medium, the strain was also submitted to an OFAT study for EPS generation, which yielded the same amount of EPS. It's possible that Th didn't interact with the key enzymes involved in EPS production. This innovative study found that any bacterium can tolerate Th up to 1000 mg/L, and the findings can be used to optimize EPS production, allowing it to be employed in successful Th bioremediation (Shukla et al. 2020a).

Before being released into the environment, (U) Uranium-containing effluents produced by the nuclear energy sector must be effectively remediated. Sanchez-Castro et al. investigated the bacterial strain *Stenotrophomonas* sp. Br8, which was isolated from U mill tailings pore fluids and successfully precipitated U. (VI). These Br8 cells biomass was immobilized using well-established entrapment methods such as Smidsrød and Skjåk-Braek's (1990) Na-alginate entrapment technique, as well as other modified versions of other enhanced approaches such as the production of sol–gel ceramics by gelling and drying a biomass and aqueous silica nano sols composed of tetra ethyl orthosilicate and other modified versions of other enhanced approaches Beads ranging in size from 1.5 to 2 mm were discovered after these immobilized beads were characterized.

When the highly porous alginate beads for Br8 immobilization were investigated using a multidisciplinary approach (High-Angle Annular Dark—Field Scanning Electron Microscopy (HAADF-STEM)), Environmental Scanning Electron Microscopy (ESEM), FT-IR with attenuated Total Reflectance, and so on), the results were the best. This biomaterial was utilized to complex genuine Uranium mining pore water (which contains 47 mg/L U) in the presence of glycerol—2 phosphate, resulting in reactive free orthophosphates via Br8 phosphatase activity. Over the two treatment cycles, Br8 doped alginate beads removed 1199.5 mg u/g dry biomass.

According to Uranium accumulation kinetics and HAADF-STEM/ESEM, immobilization of Uranium is a biphasic process that includes passive Uranium sorption onto the bead and sluggish active biomineralization. Thus, this lab-scale study proffers that U removal from the mining waters using alginate beads doped with the cells of *Stenotrophomonas* sp. Br8 bacterial strain is a promising strategy and further research upon scaling up this highly efficient and low-cost process in developing eco-friendly heavy metal-containing water treatment stations in future (Sánchez-Castro et al. 2021).

Microorganisms' potential in bioremediating Uranium contamination has been widely discussed. But insight into molecular resistance mechanisms is needed for this technological usage. Another study of Pinel-Cabello et al. about the above-mentioned genus *Stenotrophomonas* describes the transcriptomic and microscopic response of *Stenotrophomonas bentonitica* BII-R7 exposure at 100 and 250 μM of U.

This experiment removed around 97% of the U present in the medium after 24 h of incubation at 100 μM of U and 96% when exposed to 250 μM. Gene expression analysis revealed during the U exposure was, a higher up—regulation of genes and their inclusion in cell wall biosynthesis, transport of toxic compounds, acid and alkaline phosphatases. CreD and OmpA (cell wall proteins) help in maintaining membrane integrity, blocking the entrance of the U inside the cells during the up-regulation.

Out of 3786 genes exposure to 100 M resulted in up-regulation of 185 genes during the lag phase and 148 genes during the exponential phase, whereas exposure to 100 M resulted in down-regulation of 143 lag phases and 194 exponential phases (>1.5-fold change). During the lag phase, 250 M showed up, regulating 68 genes and downregulating 290 genes. According to microscopic observations, soluble uranyl ions bind to the functional groups of the bacterium's cell wall, where they precipitate to produce U-phosphate minerals due to phosphatase activity (PAP2 or ALP-like phosphatases).

These findings were made in a biphasic process, in which enhanced cell wall increases U biosorption to the cell surface, where it precipitates as U-phosphate minerals, which are strengthened by phosphatases. RND transporters are the transport systems that prevent accumulation of U in the cell by active efflux of the metal. These findings give us an insight into how microbes do cope up with the toxicity of U and ensure designing effective bioremediation strategies (Pinel-Cabello et al. 2021b).

The ubiquitous usage of (Se) Selenium in technologies has resulted in a broad build-up of this metalloid in the environment. Pinel-Cabello et al. examined the biotransformation of (Selenite) Se(IV) to Se(O)—nanoparticles (Se (O) NPs) in the above-mentioned strain, *Stenotrophomonas bentonitica BII*—R7. S. bentonitica was cultivated at 28 °C in LB medium with 150 rpm shaking.

After 24 h of incubation, the cells were washed twice in 0.9% NaCl before being inoculated at an initial OD 600 of 0.2 into LB medium containing 2 mM Na_2SeO_3. The cellular location and structural characteristics of Se (IV) bioreduction were shown by microscopic characterization utilizing HAADF-STEM. HAADF-STEM studies also confirmed that *S. bentonitica* produces a-Se nanospheres, which are thereafter biotransformed into crystalline Se nanostructures. Many proteins involved in Se(IV) reduction and stabilization of Se(O)NPs, such as glutathione reductase, increased in abundance in bacteria grown with Se(IV), as did the abundance of many proteins with transport functions, such as RND (resistance-nodulation-division) systems, potentially facilitating Se uptake.

In Se(IV)-treated cells, the abundance of enzymes such as glutathione synthase, glutathione-disulphide reductase, and thioredoxin-disulphide reductase appears to be increased, indicating that they play a role in the reduction of this metalloid. After 120 h of incubation, there is a 100% drop in Se(IV). However, as these authors reported the bioreduction of Se(O) to volatile methylated forms such as dimethyl diselenide (DMDSe) and dimethyl selenyl disulphide, Se(IV) bioreduction in *S. bentonitica* may not be limited to the formation of Se(O)NP (DMSeDS).

Se exposure-enhanced oxidative stress defence proteins, such as catalase/peroxidase (Hydroperoxidase I) HPI. The biotransformation of amorphous nanospheres to trigonal Se was discovered via electron microscope analysis. Overall, the findings highlight *S. bentonitica*'s ability to diminish Se bioavailability, laying the groundwork for bioremediation approaches and environmentally friendly biotechnological nanomaterial synthesis (Pinel-Cabello et al. 2021a).

Pantoea sp. TW18 is a common soil and water-borne facultative anaerobic bacteria. To the authors' knowledge, no research on the remediation of U(VI) by *Pantoea* sp. TW18 has already been published from aqueous solutions. *Pantoea* sp. TW18 was isolated from radionuclide-contaminated soils to be used in this study.

Pantoea sp. TW18 was isolated from radionuclide-contaminated soils utilized in U bioaccumulation experiments (VI). SEM was used to characterize the morphology of *Pantoea* sp. TW18 cells before and after U(VI) accumulation. Cells of *Pantoea* sp. TW18 were rod-like in shape with extensive cell secretions before being exposed to uranyl ions. The secretions of the cells appeared to be diminished after exposure to uranyl ions, and scale-like deposits stuck to the cell surface. The presence of uranium deposits on the cell surfaces was confirmed by EDX.

FTIR, XPS, and batch experimental analyses were used to explore the mechanism of uranium bioaccumulation by *Pantoea* sp. TW18. *Pantoea* sp. TW18 achieved the accumulation equilibrium (<4 h) with such a high accumulation capacity (79.87 mg/g at pH 4.1 and T = 310 K) for U in batch studies (VI). The existence of rich functional groups (carboxyl, amide, and phosphoryl groups) in the bacterium holds for accumulation efficiency, according to FTIR and XPS analyses. *Pantoea* sp. TW18 had increased U(VI) accumulation levels at extremely high temperatures (300 K to 320 K). The accumulation behaviour was simulated by the Langmuir model, with the maximal accumulating capacity of U(VI) on *Pantoea* sp. TW18 calculated from the Langmuir model being 76.98 mg/g at pH 4.1 and 300 K.

When the amount of bacterial dose was increased from 0.14 to 1.1 g/L, the effective accumulation of U(VI) by *Pantoea* sp. TW18 was increased, owing to a rise in functional groups as the bacterium's content increased. The accumulation of U(VI) on *Pantoea* sp. TW18 was unaffected by ionic strength, showing that the formation of an inner-sphere surface complexion dominated the accumulation of U(VI). These findings indicated that *Pantoea* sp. TW18 can be regarded as a promising sorbent for treating the radionuclide-contaminated water due to its availability, cheap cost and eco-friendliness (Zhang et al. 2018).

Heavy metal adsorption appears to be higher in Gram-positive cell walls than it is in Gram-negative cell walls. Because of the functional groups found on the cell wall, such as carboxyl and phosphoryl groups, Gram-positive bacteria, like *Streptomyces*, have a high heavy metal binding capacity (carboxyl groups on peptidoglycan and phosphoryl groups predominantly on teichoic acid of Gram-positive bacteria cell walls). Furthermore, some Streptomyces species are metal-tolerant and have excellent biosorption properties.

Such an instance of bioaccumulation which involves the characterization of uranium by the *Streptomyces sporoverrucosus* dwc-3 is defined in the study by Xialong Li et al. This strain was obtained and cultivated in Gause's medium from a disposal site for (ultra-) low uraniferous radioactive waste in Southwest China. This study investigated biosorption mechanisms using transmission electron microscopy (TEM), energy-dispersive X-ray (EDX) analysis, and Fourier Transform Infrared Spectroscopy (FT-IR), which aided in determining the functional groups (phosphate, carboxyl, and amide groups) and potential binding sites present in the uranium biosorption.

At a scattering angle of 165 °C, the Proton-Induced X-Ray Emission (PIXE) and Enhanced Proton Backscattering Spectrometry (EPBS) spectra were obtained. Both assisted to the uranium adsorption process. Pseudo-first-order and pseudo-second-order rate equations were used to characterize the bacterium's dynamic biosorption of U(VI). In the adsorption mechanism of U (VI) on *S. sporoverrucosus*, the pseudo-second-order model ($R2 = 0.99$) performed better.

On 100 mg *S. sporoverrucosus* dwc-3, approximately 60% of total uranium was absorbed at a 10 mg/L starting concentration. After 12 h at room temperature and pH 3.0, it had an adsorption capability of greater than 3.0 mg/g (wet weight). *S. sporoverrucosus* accumulated uranium needle-like granules on cell walls and also within the cell, according to TEM and SEM studies. These findings aided in the creation of a prospective biosorbent for the absorption of uranium from aqueous environments, as well as the knowledge of biosorption mechanisms (Li et al. 2016a).

The longer half-life (5.26 years) and emission of higher (1.17 and 1.33 meV) gamma energy have made ^{60}Co the most important radioactive element. The nuclear industry's decontamination process yields cobalt citrate, oxalate, and EDTA compounds in which the cobalt citrate and cobalt oxalate are easily removed by chemical precipitation in post-decontamination operations. While the removal of cobalt–EDTA complex, i.e. [Co(III)–EDTA], is extremely difficult because of its high stability and solubility.

[Co (III)–EDTA]$^-$ is the most transportable form of ^{60}Co, and any leakage could result in soil and groundwater contamination, posing major health risks to living things. Many physicochemical methods for removing ^{60}Co from low-level radioactive nuclear waste have been tested at the laboratory scale, but most of them have been found to be ineffective for a variety of reasons, including low decontamination factor, higher cost, higher energy consumption, and the generation of large amounts of liquid waste. The main approach for [Co(III)-EDTA]$^-$ appears to be microbial reduction.

The microbial reduction of [Co(III)-EDTA]- by *Bacillus licheniformis* SPB-2 isolated from the solar salt pan is the subject of this work by Paraneeiswaran et al. After isolation, this strain was transferred to Tris-G medium (Tris buffered medium supplemented with 0.2% glucose) and grown at 30° C. Colonies were chosen at random and transferred to Tris—G broth supplemented with 1, 3, 5 mM [Co(III)-EDTA] before being incubated at 30 °C. [Co (III)-EDTA]$^-$ resistant colonies were chosen and preserved as glycerol stocks at −80 °C. Genomic DNA was characterized and isolated using molecular techniques.

BLAST was used to check the sequences of the genes (Basic Local Alignment Search tool). Van der Voort et al. used a modified approach to produce sporulation of the *B. licheniformis* SPB-2. The spore germination assay was then performed using purified spores. The batch-mode reduction of [Co(III)-EDTA]$^-$ and adsorption assay for [Co(II)-EDTA]$^{2-}$ were carried out, respectively. The batch mode reduction showcased recurrent reduction cycles of 1 mM [Co (III)-EDTA]$^-$ in 14 days. Increase in reduction activity were subsequently seen up to 4 cycles.

An Inductively Coupled Plasma Atomic Emission Spectrophotometer was used to measure the adsorption of [Co(II)-EDTA]$^{2-}$. Using anaerobic respiration procedures, *B.licheniformis* was able to convert 1 mM [Co(III)-EDTA]$^-$ to a less harmful form [Co(II)- EDTA]$^{2-}$. The adsorption event also eliminated [Co (II)-EDTA]$^{2-}$. Surprisingly, [Co(III)-EDTA]-induced spores and [Co(III)-EDTA]-induced germinated cells both played a role in lowering [Co(III)-EDTA]$^-$. Apart from that, the bacterium had a D10 value of 250 Gy (Gy) (radiation dose required to kill 90% of cells), indicating that it is a good candidate for bioremediation of moderately active nuclear waste (Paraneeiswaran et al. 2015).

This study confirms that novel microbial isolates from naturally constrained environments are viable options for hazardous waste bioremediation.

2.2 Bioremediation Using Fungi to Remove Heavy Metals and Radionuclides

2.2.1 Bioremediation Using Fungi for Removal of Heavy Metals

The first study, conducted by Talukdar et al., discusses the application of three fungal isolates: *A. fumigatus* (Cd (II)) tolerant, *A. flavus* (Cr (VI) tolerant), and *A. fumigatus*. As we all know, heavy metal pollution in water is a major concern because these pollutants can accumulate in biological systems, causing the onset of a variety of harmful diseases. Fungus surfaces are made up of polysaccharides, proteins, and lipids such as glucans, chitins, and mannans, which contain a variety of groups, such as sulphates, amino groups, carboxyls, and phosphates. These natural components have numerous properties and are used for medicinal applications such as controlled drug release, the synthesis of advanced materials, and the elimination of unwanted entities, thereby controlling foul odours.

The samples were collected from various Indian locations, that include Haryana (Karnal, Faridabad, Panipat, Yamunanagar, Sonepat, Mullana), Punjab, Chandigarh, Jammu and Kashmir, Delhi, and Assam. Effluent and sludge were collected from various companies, sewage treatment plants, and oxidation ponds in the study by Yang et al. Bottles were kept in cold boxes until the start of the microbiological analytical processes to avoid any physicochemical changes in the samples. The concentration of dissolved Cd (II) in effluent from the electroplating industry in Assam was found to be the lowest (0.01 ± 0.00 mg/L) and the highest concentration was observed (70.35 ± 0.17 mg/L) in the battery industry in Mohali, Punjab.

The total concentration of Cd(II) was found be minimum (0.02 ± 0.01 mg/L) in effluent, for the electroplating industry and maximum (162.71 ± 1.30 mg/L) in sludge located STP (Sewage Treatment Plant), Yamunanagar. The dissolved Cr(VI) concentration was detected least (0.01 ± 0.03 mg/L) in effluent coming from battery industry situated at Assam while maximum Cr(VI) concentration (51.6 ± 0.5 mg/L) in effluent was observed at electroplating industry in Chandigarh. An output from the thermal power plant in Bhatinda was analysed wherein the total Cr(VI) concentration found was minimum (0.16 ± 0.01 mg/L) in effluent, and maximum (973.12 ± 1.54 mg/L) in sludge located in Chandigarh. The order of heavy metals concentration in the collected samples was found to follow the trend of Cr (VI) > Cd (II).

Finally, three fungal isolates were chosen for the establishment of a microbial consortium based on their heavy metal tolerance capacity: *A. fumigatus* (Cd (II) tolerant), *A. flavus* (Cr (VI) tolerant), and *A. fumigatus* (Cr (VI) tolerant). The study's findings provide fresh insights into the biological remediation of hazardous metals utilizing filamentous fungi. Because of their high adsorption potential for even minimal levels of heavy metals, these fungi are ideal for eliminating them. The results of the study will be relevant in the future evaluation of natural biosorbents, which could be found to be cost-effective in the treatment of toxic metal-laden effluents (Talukdar et al. 2020).

Mercury is a toxic element, and their disposal in the environment has been a subject of debate. It was understood that *A. flavus* strain could have a potential to be applied in contaminated soil with mercury and study regarding the same was conducted by Evi Kurniati et al., Fungi, which are abundant in the natural environment and play an important role as decomposers of organic matter and in nutrient cycling. Many metals are required for fungal growth, including Ca, Co, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn, but others, such as Ag, Al, Au, Cd, Cs, Hg, Pb, and Rb, are not.

Mercury in soil is tightly linked to organic matter or precipitated as sulphide, and it can be detected in trace amounts in soil solutions. Excavation and disposal, stabilization/solidification, electro-remediation, soil washing/leaching, and thermal desorption are examples of procedures that are not cost-effective. The accumulation of metal in the fungal biomass has been confirmed. The strain chosen belongs to *Aspergillus* sp. based on colony morphology and cell-based taxonomic analysis. Fungi have a reputation for collecting heavy metals in their mycelium and spores. By cultivating crops in polluted agricultural soil and preventing them from being consumed by humans, this technique will reduce the danger of heavy metals absorption.

A. niger as a biological leaching agent for heavy metals from polluted soil can produce a wider range of organic acids that are useful for metal solubilization. Cu, Cd, Pb, and Zn were successfully removed from the exchangeable, carbonate, and oxide fractions by these acids. On paper mill effluent, *A. niger* and *A. flavus* were used to remove heavy metals and demonstrated the ability to collect Pb, Zn, Cu, and Ni. Using potato dextrose broth medium, the growth profile of the Aspergillus flavus strain was followed for further 28 days based on mycelia dry mass.

Mercury degradation in potato dextrose during the first 8 days of incubation, the steady rise in mycelia dry mass occurred in the broth culture medium. The decrease in mercury concentration in the culture medium coincided with the increased mycelia dry mass. This shows that a utilization process took place, and a degradation mechanism was present. The overall mercury concentration began to fall on the eighth day of cultivation and continued to fall until the 28th day. This result implies that the mercury utilization process occurred, indicating that the study on fungal strains may have a mechanism for mercury contamination degradation (Kurniati et al. 2014).

Cobalt has serious impacts to human and environment, hence bioremediation or biosorption of the same is necessary. In this study by Sudhir K. Shukla et al., *Aspergillus versicolor* SPF-1, a [Co (III)-EDTA]-resistant fungus, was isolated from a solar salt pan. The fungal isolate took 5 days to remove 1 mM [Co (III)-EDTA]- from the bulk medium at pH 5.0 and 30 °C, with maximum biosorption at pH 4.0. *A.versicolor* SPF-1 extracted [Co (III) EDTA]- from the bulk media using a biosorption technique, according to SEM-EDAX analysis.

Fresh and saltwater bodies, acidic and alkaline circumstances, low and high-pressure zones, arid locations such as salt pans, and heavily contaminated areas are all places where microorganisms can be found. Even though various studies have been conducted to examine the use of microorganisms as metal biosorbents, there are just a few papers in the literature that detail microbial remediation of [Co (III)-EDTA]. This study found a [Co (III)-EDTA]-resistant fungus in a solar salt pan sediment sample from Marakkanam on India's east coast. A fungus resistant to [Co (III)-EDTA] was isolated from a solar saltern pond.

During the winter, ten grams of soil samples were collected at random from different sections of Marakkanam's solar saltern crystallizer pond and delivered to the laboratory in sterile plastic bags. *A. versicolor* SPF-1, which was isolated from a salt pan, had a higher tolerance to [Co (III)-EDTA]- and a higher MIC (Minimum Inhibitory Concentration), indicating that fungi isolated from harsh environments, such as polluted or contaminated soil, and industrialized areas, have greater tolerance potential for various heavy metals than other fungi. This strain was able to extract more [Co (III)-EDTA]- complex than other strains previously reported. Two exposure intervals (12 h and 24 h) were tested to evaluate the [Co (III)-EDTA] biosorption capacity at each pH. Separated from the water and the land in the Iraqi city of Basra, *C. tropicalis* and *C. glabrata, Escherichia coli* that has been genetically modified to have distinct efflux and sequestration mechanisms. After 10 min of treatment, the maximum metal ion concentration was 6 mg/g bacterial dry weight, indicating that nickel and cobalt absorption had increased.

On the fourth and fifth days, the greatest Co absorption (200.14 and 183.09 mg/l) from a 0.5 g/l cobalt solution was found. The maximal adsorption capabilities of calcium alginate + EPS, calcium alginate + *C.luteola* TEM05, and calcium alginate + EPS + *C. luteola* TEM05, respectively, were 55.25, 49.26, 51.81 mg/g for Co, and 64.10, 62.5, 61.73 mg/g for Cd. Maximum Co-loading (1036.5 M/g, 60 min) was achieved using *Mortierella* SPS 403 biomass, which removed over half of the 4.0 mM cobalt from the aqueous solution. SG1 has reached the maximal level of resistance to metal ions (cobalt 5). Engineered Escherichia coli expressing co-eliminated 12 g/g after 1 h of exposure. In 14 days, SPB-2 B. licheniformis was converted from poisonous [Co (III)–EDTA] to less hazardous $[\text{Co-EDTA}]^{2-}$ (Shukla et al. 2020b).

So, it could be concluded that when we have better absorption capacity when *Aspergillus* and *Candida are* clubbed together.

Using Mushrooms for the removal of arsenic is the main motive for the study mentioned below which was done by Dabrowska et al. For many years, scientists have been fascinated by the microbial metabolism of arsenic. Arsenic microbial transformations have been included into a number of systems and technologies for the bioremediation of As-contaminated waterways, soils, and wastewaters. As leaching agents for soil cleansing and the immobilization and removal of arsenic from waterways and wastewaters, dissimilatory reducing bacteria have been proposed. For both types of arsenic (As(III) and As(V)), many bacteria and fungal species have been characterized as effective sorption materials.

With the aid of mixed cultures plus, the consortia of microorganisms which contained sulphate-reducing bacteria (SRB) were shown to completely remove arsenic from acid mine drainage by the formation of amorphous orpiment ($\text{As}^{\text{III}}{}_2\text{S}_3$) and realgar ($\text{As}^{\text{II}}\text{S}$). The goal of the project is for SMC to discover potential processes and investigate causes that cause contaminated water (Spent mushroom compost). The CEC (Cationic Exchange Capacity) value of a mature, fully humified compost should be at least 600 mM/kg. The addition of gypsum, a regularly used substance to aid the composting process, is linked to a higher concentration of sulphates and calcium. Because compost stability varies in individual compost samples and determines its qualities, SMC is a set of extremely diverse components that must be examined before to use. This research proved that SMC (Spent Mushroom Compost) is a model waste organic material for studying microbial interactions with arsenic and explains why SMC is commonly used as a remediation agent in the passive treatment of arsenic-contaminated environments. The study's most important finding was that the SMC microbial community could both oxidize As (III) and reduce As(V).

The sorption experiment showed that SMC could capture both arsenite and arsenate, but desorption studies showed that arsenic could be remobilized. The extraction of SMC organic compounds by As (III) and As (V) was determined using GC–MS (Gas Chromatography-Mass Spectrometry), implying that these metabolites have binding properties. The biofilm formation test and the binding abilities of the extracted proteins directly confirmed the sequestration of arsenite and arsenate by SMC. It proved that SMC, a common waste material in passive wastewater treatment systems, could be used as an organic carbon and microorganism source in arsenic-contaminated wastewater treatment systems (Dabrowska et al. 2021).

In this part of the subtopic, a study by Damodaran et al. checked for the bioaccumulation potential of *G. vittiformis* versus the *P. clypeata* and *P. ostreatus* fungal species. Soil pollution has been accelerated over the world due to the invasion of industrialization, the use of pesticides in agriculture, and poor waste disposal procedures. Heavy metals such as Cadmium, Chromium, Cobalt, Lead, Manganese, Mercury, Nickel, and Zinc are among the top pollutants that require rapid mitigation, according to statistics from India's Central Pollution Control Board. Metals are removed from wastes by chemical precipitation, coagulation with alum or iron salts, membrane filtering, reverse osmosis, ion exchange, and adsorption. These procedures are technologically feasible, but they are not economically viable for large-scale soil remediation. In the Dakshina Kannada District of Karnataka, India, ten fungal species were gathered from two separate municipal trash disposal regions. Basidiomycetes were confirmed to be present in all ten isolates. Soil pollution has been accelerated over the world as a result of the invasion of industrialization, the use of pesticides in agriculture, and poor waste disposal procedures.

Removal of Cu (II), Cd (II), Cr (VI), Pb (II), and Zn (II) in single metal systems, metals from polluted soil were examined using newly isolated macrofungi, *G. vittiformis.* The newly identified macrofungi strains were first screened for heavy metal resistance before being tested for bioaccumulation potential. The fungus, *G. vittiformis* (M6), had a higher accumulation potential in both mycelial and fruiting body stages of its life cycle (M5) when compared to *P. clypeata* (M5) and *P. ostreatus* (M5). The soil pH and incubation period were discovered to have a significant impact on metal bioaccumulation from the soil slurry during the mycelial stage of the fungus *G. vittiformis.*

Wild non-edible mushroom species like *G. vittiformis* were found to be more efficient in gathering heavy metals from soil than food species like *Pleurotus* sp. and *Agaricus* sp., highlighting the importance of establishing mycoremediation on a large scale. The BAF (Bioaccumulation Factor) values of *G. vittiformis* for Cu (II), Cd (II), Pb (II), and Zn (II) were found to be above one at an initial metal concentration of 50 mg/kg, indicating its suitability as a bioremediating agent. Even though the mushroom failed to produce fruiting bodies for other metals at the same concentration, the BAF values of *G. vittiformis* fruiting bodies for Cd (II) and Pb (II) metals at 100 mg/kg concentrations were much higher. Heavy metals, Cu, Cr, and Zn poisoning on *G. vittiformis* were severe enough to suppress and skip the production of fruiting bodies as a result (Damodaran et al. 2014).

In comparison with food species like *Pleurotus* sp. and *Agaricus* sp., wild non-edible mushroom species like *G. vittiformis* showed high efficiency in gathering heavy metals from the soil, highlighting the importance of establishing mycoremediation on a large scale.

In another research by Cui et al., for heavy metal bioremediation, three types of microorganisms were chosen from mine tailings in Anshan. The strain's species was then determined using internal transcribed spacer (ITS) analysis. The microbial agent was generated with the following superior ratio, which was decided by our lab: The proportions of *Mortierella* sp., *Actinomucor* sp., and *Mucor circinelloides* were 1: 1.5: 3. Mn and Zn were remedied using *Actinomucor* sp. and *Mortierella* sp., which were isolated and identified from the soil. Heavy metal pollution in mine tailings constituted a severe hazard to the environment as mineral exploitation progressed.

Anemia, reproductive failure, impatience, renal failure, and neurodegenerative damage are all symptoms of lead accumulation in the body. Other heavy metals, such as manganese, zinc, and copper, can lead to hypophosphatemia, heart disease, liver damage, and sensory disturbances, among other things. The microbe entered a logarithmic growth phase after a period of adaptation, with the fastest growth rate occurring between 4 and 10 h.

The microbe's physiological and morphological characteristics were more consistent at this point, the generation interval was shorter, and its resistance to the environment was stronger. End of logarithmic growth phase beginning of stable period was the strain's optimal period. At this stage, the microbe had significant biological characteristics and high metabolic activity, making it suitable for the research. This rule was used to determine the best ratio for a composite microbial agent. Sand, 19.2% medium sand, 43.5% fine sand, and 3.68% clay make up the soil particles, which have a low water holding ability. The pH of the soil was 8.35, making it a saline alkali soil.

Because of the low water content (1.93%) and lack of organic matter in the tailings, there were few soil organisms (1.15%). The assessment and research were based on the background value of Liaoning Province, China, and the Grade II standards of the Chinese environmental quality standard for soil. For each soil sample, none of the heavy metals surpassed the Grade II threshold. The values of Pb, Mn, and Zn in this study were seen to be higher than the background values, indicating notable enrichment of these metal contaminants in the tailings soil. It is essential to research and develop an effective and clean bioremediation technology.

Because of their great concentration, Mn and Zn were chosen as primary elements for further study. Based on the features of tailings soil, a special formula of a composite microbial agent was investigated. The agent formula was based on the differences in growth curves between *Mucor circinelloides, Actinomucor* sp, and

Mortierella sp. The main mechanism of bioremediation, according to thermodynamics and kinetics of adsorption, is a chemical reaction of ion exchange and complexation based on a monomolecular layer. The microbial community is used to monitor the bioremediation effect on soil contamination. The modifier formulation and microbiological fraction of the composite microbial agent must be adjusted based on the concentration and composition of heavy metals, as well as the physical and chemical qualities of the soil (Cui et al. 2017).

Amorim et al. investigated two types of yeast species isolated from Brazilian mining water and identified as *Meyerozyma guilliermondii* and *Meyerozyma caribbica* using biochemical and phylogenetic analysis. In small-scale batch experiments conducted over a 1-week period, both isolates survived, and their colonies expanded in up to 32 mM of Mn^{2+}, and they were able to remove 100% of Mn^{2+} from the culture media. Manganese (Mn), the metal we'll be focused on in this section of the chapter, is a key element for many species, as it's required for vital processes including growth, development, and homeostasis. Mn is a constituent of mitochondrial manganese superoxide dismutase water-splitting enzyme in photosystem II, and is a cofactor for a variety of enzymes, including various decarboxylases of the tricarboxylic acid cycle, RNA polymerases, and numerous glycosyltransferases. Mn is essential for photosynthesis, but excessive exposure can cause toxicity.

Mn poisoning in humans is largely shown in the CNS (Central Nervous System) and has symptoms that are comparable to Parkinson's disease. Mn is a heavy metal that can be present in groundwater and wastewater as a result of mineral breakdown and/or leaching from rocks and soils. Elevated Mn levels produce a variety of morphological, physiological, and metabolic dysfunctions, which can have a variety of negative consequences.

Plating of mine water sample was done on Sabouraud, Potato, and Mineral media. Three yeast isolates were then selected on YPD plates containing 2.5 mM Mn^{2+} ion, the same quantity of Mn as observed in the mine water collected. Then there was Mn^{2+} bioremediation, followed by biochemical and phylogenetic analysis to find and protect potential yeast strains for bioremediation. We identified the isolates after separating the three yeast strains from the mine water. *Candida guilliermondii* was identified in all the yeast isolates. *M. caribbica* and *M. guilliermondii* were found in a mine's water in south-eastern Brazil, and *M. caribbica* was able to remove 0.91 MM of Mn^{2+} in only 4 days of cultivation on YPD medium, whereas *M. guilliermondii* took 6 days under the same conditions.

M. caribbica and *M. guilliermondii*, whether using their live or dead biomass for biosorption, could be effective biosorbents in the removal of Mn^{2+} ions. *M. caribbica* has a faster manganese removal rate than *M. guilliermondii*, with 1088 mg $Mn.day^{-1}$ and 0.324 mg $Mn.day^{-1}$, respectively (Fig. 3). The researched yeasts were shown to be effective at biosorption of the Mn^{2+} ion as well as quantifying the effectiveness of using their biomass for biosorption in wastewater bioremediation procedures. The biomass of these two yeasts could be used as biosorbents to cure Mn-contaminated waterways, according to the findings (Amorim et al. 2018).

B. bassiana strain grows naturally in soil, so it would be ideal enough to be utilized for the bioremediation process, such a study of removal of multiple heavy metals was

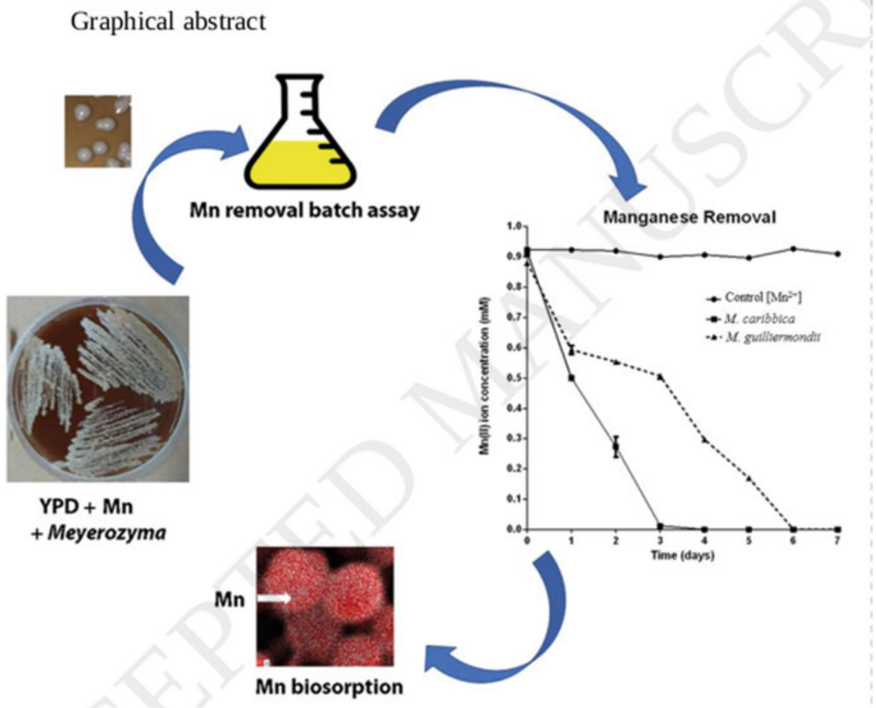

Fig. 3 Graphical abstract. Reprinted with permission from Amorim, S. S., Ruas, F. A. D., Barboza, N. R., de Oliveira Neves, V. G., Leão, V. A., Guerra-Sá, R., 2018. Manganese (Mn^{2+}) tolerance and biosorption by Meyerozyma guilliermondii and Meyerozyma caribbica strains. Journal of Environmental Chemical Engineering 6, 4538–4545. https://doi.org/10.1016/j.jece.2018.06.061

done by Gola et al. The aim of this study was to see how well an ecologically friendly fungal strain could deal with single and multiple metal stresses, as well as to figure out how metal exposure affected the fungal strain's morphological traits and surface qualities. This study's purpose was to look at *B. bassiana*'s bioaccumulation of single and multi-metal ions [Cd (II), Cu (II), Cr (VI), Ni (II), Zn (II)] abreast. Overall results showed that *B. bassiana* strain will survive under the moderately polluted conditions, heavy metal remediation from wastewater contaminated with numerous heavy metals found in the environment can be successfully used by increasing metal concentration to provide good performance of the strain in variable metal stress. At three different temperatures, biomass growth was detected, with 30 °C being the most appropriate temperature for biomass production, with 84.5% removal.

At pH 4, *B. bassiana* showed a considerable decrease in metal removal capability (52.6%) from the multi-metal mixture as compared to the neutral pH (84.5%). A positive charge on the fungus cell wall is created by the connection of hydronium ions with the cell surface at low pH, providing a repulsive force against heavy metal cations in the solution. The effects of heavy metals were illustrated using SEM, AFM, TEM, FTIR, and specific functional groups contributing to metal uptake were identified. The multi-metal mixture had the maximum metal removal (84.5%) and did not vary much in pH from 6 to 8. As a result of its bio-pesticidal and metal-remediation abilities, *B. bassiana* is a viable option for multi-metal remediation from irrigational waters (Gola et al. 2016).

The hyphae in the control condition had a long ribbon-like structure, were uniformly shaped, and were loosely packed. Individual heavy metals disrupted the ribbon-like shape of hyphae, making them short and tightly packed. Denser, closely packed malformed hyphae with shorter length were found in the multi-metal spiked media, suggesting synergistic toxicity of heavy metal combinations. When exposed to heavy metals, the fungus's toxicity reaction results in morphological alterations in the hyphae. The morphological mechanism for overcoming heavy metal toxicity could be fungal hyphae aggregation in the presence of heavy metals. Aggregation reduces the overall surface area of the fungal hyphae exposed to the hazardous environment.

S. cerevisiae is one of the well-studied species of yeast and showed a varied level of application is the industry as well, in this part of the chapter we would be studying their effectiveness to bioremediate. Sathvika et al. here focuses on chromium bioremediation by yeast.

Hexavalent chromium is classified as a mobile oxyanion carcinogen that can contaminate groundwater. Biosorption is considered a viable option for removing toxic metals because it is inexpensive and can treat large amounts of effluent with abundant available biomass. The interaction between polar functional groups and microwave energy enhances yeast immobilization on the biopolymer matrix, and the cross-linking of cellulose and glutaraldehyde enhances the cell surface of the microorganism. Detoxification of carcinogenic metal ions such as chromium Cr (VI) using yeast immobilized on a biodegradable polymer matrix such as cellulose has not been reported.

A simple and novel microwave-aided green strategy was employed to encapsulate yeast in glutaraldehyde cross-linked cellulose and then use it to extract Cr (VI) from an aqueous solution. The NH_2, COOH, and OH functional groups on the yeast cell surface could be important in interacting with the Cr (VI) oxyanion. Although a residential microwave oven has several limitations, it has lately been found to be useful in a variety of applications, including the creation of nano-sized metal oxides, protein hydrolysis, and glycogen to glucose conversion. These applications are notable for their efficiency and reproducibility.

Cellulose and the other chemicals used in biosorbent manufacture are non-toxic and safe to handle, and the low and intermittent microwave exposure is safe enough that hazardous vapours do not build up inside the oven. The pH conditions in the aqueous phase, as well as the presence of Cr (VI) ions in various forms ($HCrO^{-4}$; $Cr2O_7{}^{-2}$; $CrO_4{}^{-2}$), influence the interaction between Cr (VI) and the microbe immobilized biopolymer and zero-point charge or isoelectric point of the biosorbent surface. All these qualities are classified as extensive or additive, and the first step, which involves biomass immobilization and subsequent adsorption on the surface, influences the overall free energy, enthalpy, and entropy changes related with adsorption.

Cr (VI) oxyanion transport from the solution phase to the biosorbent surface is influenced by the concentration gradient across the biosorbent-solution interphase. The negative ΔG values obtained at various temperatures used to assess chromium absorption reflect this, and because the concentration of Cr (VI) on the solid surface is larger than in solution, the spontaneity of the adsorption process is indicated by the free energy difference. Because real effluent, such as tannery wastewater, contains significantly more Cr (III) than Cr (VI), it is critical to monitor chromium uptake in the +3-oxidation state. The Cr (VI) from the eluate was diluted and used in the tests that followed.

From the study, it can be concluded that microwave irradiation is an excellent method for immobilizing yeast with a biodegradable cellulose polymeric substrate and for bioremediation as a long-term solution for chromium detoxification. The immobilization of yeast takes 3 h in the traditional technique of preparation. It takes only 200 s to immobilize yeast in a microwave oven and has a strong adsorption capacity of 23.61 mg/g for Cr (VI). The chromium adsorption capacity of cellulose was found to be only 49.6% when yeast was immobilized inside the biopolymer matrix, but when yeast is immobilized within the biopolymer matrix, the cellulose adsorption capacity is increased two-fold, greatly enhancing its metal uptake. Based on the findings, this technology has the potential to improve the potential of microwave chemistry in biosorbent preparation for the detoxification of a variety of different contaminants (Sathvika et al. 2015).

The coming part of the subtopic focuses on the study by Mahmoud et al. where they checked for an eco-friendly approach of using calcium alginate with yeast to tackle chromium bioremediation. Toxic metal pollution is a global environmental issue. In view of the fact that their persistence, biomagnifications, and accumulation in the food chain, metals released without effective treatment constitute a major hazard to public health. Chromium concentrations in electroplating and leather tanning facility effluents range from tenths to hundreds of micrograms per litre. According to the US-EPA, Cr^{6+} discharge to surface water is controlled limit of 0.05 mg/L, whereas total chromium discharge is regulated to the limit of 2 mg/L.

The absorption percentage and specific uptake of metal ions are used to determine their biosorption capacity (Q). Under a variety of experimental circumstances that affect the biosorption study, batch experiments are used to explore the affinity and capacity of the BPMB (Biomass/Polymer Matrices Beads) for chromium sorption from synthetic solution and tannery effluent wastewater. These variables include pH, temperature, stirring rates, BPMB dosages, and chromium ions concentrations. At pH 3.5, Cr^{6+} uptake capacity reached 77% and 154 mg/g, respectively. These findings indicated that metal ion biosorption on the BPMB might occur via two different processes. Because chromium is used in a variety of industrial applications, bioremediation of this metal is of particular interest. To make BPMB, Baker's yeast cells were immobilized in alginate extract.

These beads have excellent potential for chromium removal from contaminated locations with concentrations ranging from 200 to 1000 mg/L, according to our observations. The biosorption process is influenced by variables such as the pH of the solution, initial concentration, biosorbent dosage, and contact durations. Understanding the mechanism of chromium removal by biomass/polymer matrices beads is critical for tailoring the chromium bioremediation process and lowering treatment costs (Mahmoud and Mohamed 2017).

2.2.2 Bioremediation Using Fungi for Removal of Radionuclides

The study by Song et al. helps us assess the use of *A. niger* for radioactive waste treatment. Due to the advancement of nuclear research and technology, pollution caused by radionuclides has gotten a lot of attention. For the treatment of radionuclide-contaminated wastewaters, coagulation-flocculation, precipitation, adsorption, ion exchange, and membrane filtration have all been used. These technologies are frequently utilized, but they have several drawbacks, including complicated measurement and operating processes, high costs, and secondary pollutants. The effects of experimental conditions such as pH, ionic strength, mycelia concentration, and temperature on the accumulation of Co (II) and Eu (III) on *A. niger* were investigated. Surface complexation and precipitation are thus to blame for *A. niger*'s substantial accumulation of Co (II) and Eu (III) at pH > 8.0. Increased ionic strength may lead mycelia to agglomerate, lowering the number of radionuclide sites accessible on the surface of *A. niger*.

According to the above theory, radionuclide accumulation on *A. niger* is regulated by outer-sphere surface complexation at low pH and inner-sphere surface complexation at high pH. Concurrently accumulation of radionuclides resulted in increased in the total accumulation of 2 radionuclides and a moderate decrease in the total accumulation of two radionuclides, compared to solitary accumulation of each radioactive. Distinct species in solution may have a strong competitive relationship. Cd (II) and Co (II) exhibited similar simultaneous accumulation behaviour. These findings suggest that *A. niger* could be an effective biomaterial for the treatment of radioactive wastewater. At roughly 402.0 eV, a new weak N 1 s band develops, indicating the synthesis of protonated amines ($-NH^{2+}-$), which is linked to the formation of $R–NH^2–Co$ (II)/Eu (III) complexes. The high adsorbability of *A. niger* is indicative of the presence of nitrogen- and oxygen-containing functional groups on the mycelial surface, which could form complexes involving radionuclides, according to XPS spectrum investigation.

A. niger, a radionuclides-resistant bacteria isolated from radionuclide-contaminated soil, was utilized to remove Co (II) and Eu (III) from a radioactive solution at the same time. *A. niger* surfaces had plentiful nitrogen- and oxygen-containing functional groups, according to the results of FTIR, potentiometric acid–base titration method, and XPS. On *A. niger*, overall concentration of Co (II) and Eu (III) was higher than single radionuclide accumulation capacities. From the Langmuir isotherm analysis, the accumulation data can be represented, and presence of

A. niger was showing higher adsorbability for radionuclides. The outcome of the same makes concrete use case of *A. niger* in treatment of radionuclides pollution soon (Song et al. 2016).

Another example here mentions the joint functioning of *A. niger* in conjunction with *S. Podophyllum* (Plant), and this would be for bioremediation of Uranium, this approach was studied by Chao et al. The necessity for uranium resources is growing in tandem with the rapid expansion of nuclear power, which encourages the growth of uranium mining and metallurgy. Uranium mining and metallurgy produce a lot of waste rock, tailings, and uranium-containing effluent. When exposed to long-term rainfall, uranium in uranium-containing waste rock and uranium tailings gradually leaches out and enters water in the air. Because there are so many coexisting ions in wastewater and the proportion of uranium is so low, typical physico-chemical techniques are ineffective, leading to high treatment costs and secondary contamination.

Because of its low cost, safety, environmental protection, and ability to treat several contaminants at once, bioremediation technology has risen in popularity in recent years, making it suitable for treating huge amounts of wastewater with low-concentration pollutants. Purification ability improved ($P < 0.05$) with the addition of *A. niger* to *S. podophyllum*, and removal efficiency for the top group grew by 7%. As a result, *S. podophyllum* is a good candidate for bioremediation of water with low levels of uranium.

A. niger by modifying the chemical form of uranium entering the root cells of *S. podophyllum*, increasing cell wall immobilization and subcellular compartmentalization, and transferring it from the roots to the stems and leaves, the phytotoxicity of uranium entering the root cells of S. podophyllum was reduced. *S. podophyllum*'s calcium efflux was reduced by *A. niger*. Uranium stress was reduced in *S. podophyllum*, which aided their growth by increasing uranium removal efficiency and enrichment. According to the findings, microbial-assisted phytoremediation can be used to treat low-concentration uranium-containing wastewater (Chao et al. 2019).

S. commune is a common white-rot fungus that can break down complex plant biomass, including lignin, and has a global distribution. Incubation with *S. commune* considerably decreased high uranium and rare earth element concentrations in mine seepage water. It has also been demonstrated that *S. commune* can absorb and accumulate cadmium from the surroundings. This study by Traxler et al. found the application of *S. commune* in the Chernobyl Exclusion Zone (CEZ).

Bacteria and fungi in soil play an important role in element cycling and soil formation. It is impossible to overstate their influence on agriculture, human nutrition, health, and renewable energy generation, and land-use changes will have an impact on fungal and bacterial diversity. Fungi that can break down the lignin found in plant matter are required for decomposition of plant matter. For soil fertility, their contribution to mineralization processes in the soil is critical. Human impact is low in this area, which has become a valuable home for rare animal and plant species.

We were able to demonstrate that *S. commune* can live and even flourish in the soil, but unlike the other soil-dwelling fungus we studied, it spreads and grows rapidly. Due to the impossibility of extracting RNA in the field laboratory, based qPCR was employed to determine the residual load of *S. commune* DNA in the field soil. Decreased bioavailability, in turn, might explain survival even in soils with high amounts of metals or radionuclides. One year after inoculation, the white-rot basidiomycetes *S. commune* was found. White-rot fungi such as *Phanerochaete chrysosporium, Trametes versicolor, Pleurotus ostreatus*, and others have previously been documented to require an additional carbon source to thrive in soil. *S. commune* appeared to outperform these fungi, since it lasted several months in this experiment without any extra carbon source. Experiments in the lab might indicate that living without supplementary nourishment is possible.

We were able to demonstrate that at the CEZ test field site, even a saprotrophic fungus that isn't often found in soil may live. With one instance, where either wood must have been examined or inoculation, a mistakenly lost section of mycelium may have been implanted unintentionally. *S. commune* was not observed in spaces not inoculated. We were able to demonstrate the spreading of the fungus within native soil using this set-up. Plants were planted in part of the region to see if they were affected positively or negatively (Traxler et al. 2021).

2.2.3 Bioremediation Using Fungi for Removal of Both Heavy Metal and Radionuclides

With regard to fertilizers as well, there is growing environmental concern, not only because of their pertinence in the environment but also due to their ability to mobilize. In this study, Abd El Hameed et al. find out information with regard to fungi from phosphatic fertilizers. Heavy metals are chemical elements which include 69 elements, of which 16 are synthetic. Metal cations are mobilized and immobilized by soil microbes, affecting their availability to plants. *Penicillium, Aspergillus, Pseudomonas, Saprophyticus, Bacillus* and *Phanerochaete* have all been reported to be effective at removing heavy metals like chromium and nickel.

The goal of this research is to see how well-isolated fungal species from phosphatic fertilizers can extract uranium and other heavy metals. Only 5 of the 26 fungal cultures isolated on medium from various phosphate fertilizer and phosphatic ore samples were the best for growth on medium supplemented with metal ions at concentrations (150 ppm). These isolates were chosen for further research. The preliminary categorization of fungal isolates into the genus *Aspergillus* was based on their morphological features.

Various *Aspergillus* species have been described as effective heavy metal reducers in this regard. Identification of many heavy metal tolerant isolates was performed by laboratory culture as *Aspergillus niger* (Pb_2, Cr_{10}, Ni_{19}, Ni_{27}, Ni_{33}), and *Aspergillus flavus* (Pb_7, Pb_8, Ni_{35}, Ni_{36}), respectively, in a study. Several fungal cultures were obtained from phosphate fertilizer and rock phosphate, according to various research. Five fungal isolates were seen to be highly efficient for growth on a liquid medium supplemented with high concentrations of various heavy metals. Based on visual characteristics, these isolates were recognized as *Aspergillus* sp. (Abd El Hameed et al. 2015).

2.3 *Bioremediation Using Algae to Remove Heavy Metals and Radionuclides*

2.3.1 Bioremediation Using Algae for Removal of Heavy Metals

Microalgae bioremediate through two mechanisms: bioassimilation and biosorption which possess the potential to develop as ‘algal blooms’ in contaminated water and assimilate different contaminants. After harvesting and lipid/protein extraction, algal biomass might be employed as an effective biosorbent. Also, green macroalgae, seaweed and their derivatives of alginate emerge with higher amount of affinity for various metal ions. The following depicts certain research studies on algae and their bioremediation techniques.

Microalgae sorbents are those microalgae that binds heavy metals passively to their cellular structures via the biosorption process. *Chlorella vulgaris* and *Phormidium tenue* are the two species of microalgae which are collected from the Wadi Hanifah Stream in Riyadh (Kingdom of Saudi Arabia). These species help in the elimination of hazardous heavy metal cobalt from aqueous solution and further in brief was investigated in this work. The findings showed the eliminatory Co^{+2} ions percentage rose steadily, peaking at 85% for *C. vulgaris* at pH of around 5.5 and 90% for *Phormidium tenue* at pH 6, before decreasing. Before and after the process of Co^{+2} biosorption, the FTIR spectrum curves of the biomass of *P. tenue* and *C. vulgaris* were in the range of 400–4000/cm. This data showed that the active groups of the biosorbent that might be responsible for binding of the Co^{+2} in the process of biosorption.

For the dry biosorbent before the process of Co^{+2} biosorption, FTIR peaks were obtained at 3400, 2929, 2524, 1650, 1442, 1080, 881, 700, and 620 cm^{-1} and then shifted to 3424, 2927, 2525, 1653, 1429, 1145, 1049, 878, 685, and 630/cm after the uptake of Co^{+2} ions by the biosorbent. Characteristic of the –NH and –OH group were shown by the peaks at 3415 cm^{-1} and 3450.40/cm whereas peaks at 2854/cm and 2896.58/cm were from the C–H expansion. The presence of C=O expansion (COOH) were confirmed by the peaks at 1640 cm^{-1} and 1636 cm^{-1} wherein the peaks at 1541/cm and 1539.56/cm gave confirmation of an amide group. Again, peak formation at 1456/cm and 1470.48 cm^{-1} were a form of C=O expansion wherein the peaks at 1395 cm^{-1} and 1383.54 cm^{-1} indicated the CH_2 binding vibration and carboxylate expansion. The P=O and C–N expansion manner was revealed at 1235 cm^{-1} and 1230.58 cm^{-1} peaks. *P. tenue* control cells were displayed under SEM and TEM examinations in Fig. 4 (SEM 1A and TEM 1B).

The *P. tenue* cells’ ultrastructure was enclosed by a cell wall (CW) and had distinct components of cells like (Gg) glycogen granules and vacuoles. SEM (2A) and TEM (2B) images in Fig. 4 indicate the influence of Co^{+2} on the *P. tenue’s* ultrastructure following the biosorption process, which showed that some algal filaments undergone fragmentation and loss of the round apical cells. Furthermore, bioaccumulation of Co^{2+} gave rise to thickening of cell wall and higher inclusions of crystalline inside the cell, which could be ascribed to certain defensive strategy against Co^{+2} ions. The

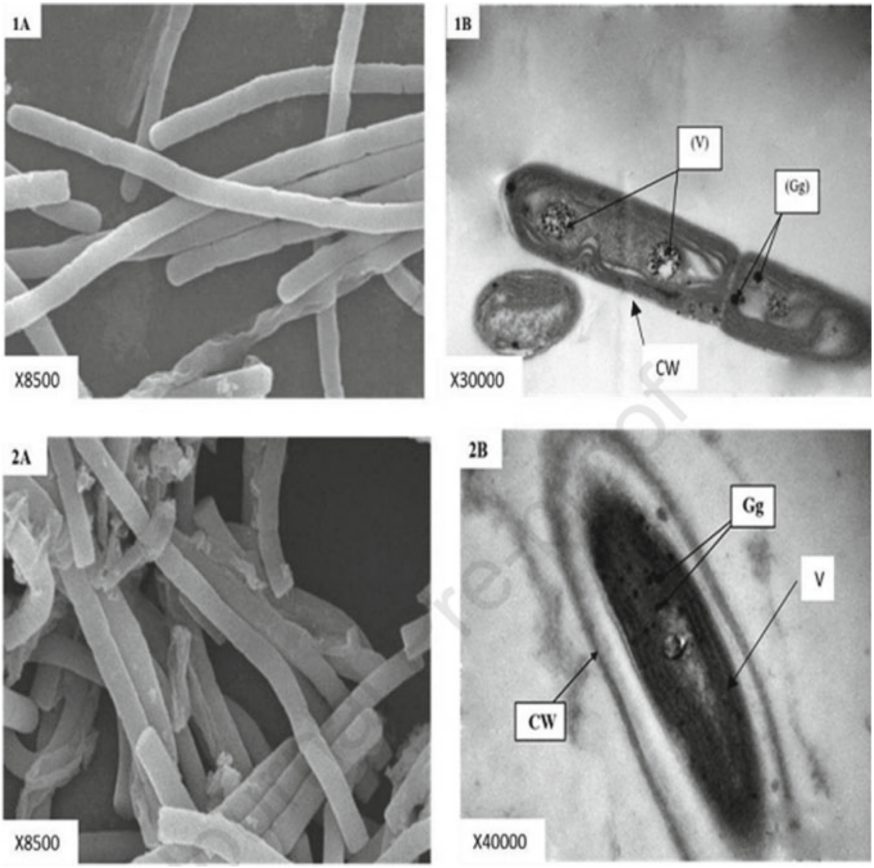

Fig. 4 SEM (1A) and TEM (1B) micrographs of P. tenue before the absorption process (control) and SEM (2A) and TEM (2B) micrographs of P. tenue after the absorption process. Gg = Glycogen granules, CW = Cell wall, V = Vacuole. Reprinted with permission from (Abdel-Raouf et al. 2022)—Abdel-Raouf, N., Sholkamy, E.N., Bukhari, N., Al-Enazi, N.M., Alsamhary, K.I., Al-Khiat, S.H.A., Ibraheem, I.B.M., 2022. Bioremoval capacity of Co^{+2} using Phormidium tenue and Chlorella vulgaris as biosorbents. Environmental Research 204, 111630. https://doi.org/10.1016/j.envres.2021.111630

C. vulgaris cells increased in shape, size, and thickness of the cell wall after the process of biosorption. Moreover, the cell's internal structure seemed disorganized.

Langmuir and Freundlich isotherm models were obeyed by biosorption of Co^{+2} ions in which the results displayed higher R^2 values (>0.9) in many cases. In this study, removal of *Phormidium tenue* of about 94% of Co^{+2} (ideal conditions of pH 6) which consisted of contact duration of about 30 min, the initial concentrations (50 mg/L) and the dose of biosorbent (1 g/L), whereas *Chlorella vulgaris* removed 87% of Co^{+2} under the same conditions except pH 5.5 and contact duration of around 60 min. Finally, it was clarified that *Phormidium tenue* and *Chlorella vulgaris* consist of the potential of being microalgae for efficient and effective biosorption of cobalt under aquatic conditions (Abdel-Raouf et al. 2022).

Seaweed has been a key species for monitoring environmental conditions in coastal environments. In Asia, several red, brown, and green seaweeds are classified as Cd, Zn, Cu, and Pb biomonitors. These are effective biomonitors for concentrations of heavy metals in coastal saltwater.

Hence, the present study targets the potential bioremediating effects of the seaweed *Gracilaria lemaneiformis* on the heavy metals from a zone of mariculture, South China. The samples were collected from five zones (*Gracilaria* cultivation zone, G; Fish culture zone, F; Shellfish culture zone, S; Transition zone, T; Control zone, C) from December 2014 to July 2015. The concentrations in the different culture zones showed variations such as Cd (0.04 ~ 1.02) μg/g; Cu (1.19 ~ 37.70) μg/g; Pb (8.45 ~ 74.45) μg/g; Zn (36.80 ~ 201.24) μg/g. The sediment samples were weighed, and acid digested by the US-EPA Method 200.2 protocols. The metals like Zn, Cd, Pb, and Cu concentrations were analysed by ICP/AES instrument. The Zn concentrations decreased in the order—C > F > T > S > G, while the Cd, Cu, Pb were observed in this order—F > C > S > T > G.

The heavy metal enrichment of *Gracilaria* caused the lower concentrations metals like Cu, Zn, Pb, Cd and Zn in the cultivation zone of *Gracilaria* in comparison with the other four zones. *Gracilaria* cultivation helps in absorbing the heavy metals from seawater and sediments present on the surface. These are released through the process of disturbance and remineralization. This consecutively increases the concentration of these toxic metals in seawater. However, increasing volumes of *Gracilaria* will give rise to higher quantity of toxic metals, decreasing the metal concentration in seawater. Thus, this results in a decrease in the combination of organic matter in surface sediments and the heavy metals. In this way, the study revolves around seaweed *Gracilaria* and its significance in bioremediation of toxic metal contaminants in mariculture ecosystems, also improving the aquatic environment and quality (Luo et al. 2020).

Many lakes in economically developed regions are afflicted by algal blooms, particularly cyanobacteria, which are heavily contaminated with toxic metals. The quantities of Zn and Cd ions in eutrophic freshwater habitats are frequently over the World Health Organization (WHO) guidelines. To focus on remediation of such heavy metals, the following research served its purpose.

The concept of physiological response and the accumulation ability of *Microcystic aeruginosa* to Cd and Zn was studied by Deng et al. *Microcystic aeruginosa* being a commonly blooming species in (eutrophic) freshwater lakes and its capability to form higher biomass in a short period of time, adds its advantage in removing toxic ions (metallic) present in the lakes. This alga was cultured in BG11 medium at 25 ± 1 °C, later treatments were conducted using modified medium of BG11 for around 144 h, in which Zn and Cd levels were also measured. Using an inductively coupled plasma-mass spectrophotometer, Zn and Cd accumulation levels in *M. aeruginosa* were obtained.

Concentrations such as <0.1 mg/l of Cd and Zn were less influential on the growth of algae. Higher concentrations (>0.1 mg/L) showed higher activity of esterase that is from 42.5% to 621.9%), activity of superoxide dismutase ranging from 12.8% to 45.4% and malondialdehyde content ranging between 18.2% and 103.9% of *M. aeruginosa*. Dramatic cell division inhibition was observed (from 12.6% till 70.0%) and photosynthetic performance (from 7.1% to 53.1%). More Cd and Zn ions were acquired by *M. aeruginosa* when the initial amounts of both the ions increased. Thus, the findings conclude that this alga has the potential for remediation of domestic sewages, industrial wastewater, eutrophic freshwater lakes during the blooming phase of cyanobacteria, where metallic ions are >0.1 mg/L (Deng et al. 2020).

Microorganisms as heavy metal biosorbents offers potentially effective alternative to standard procedures for the bioremoval of metals from wastewater, like pounding waters present in mine tailing's sites. Microalgae has the potential for its usage in the bioremediating toxic metal-polluted locations. This in turn is the owing to their propensity in absorbing and accumulating heavy metals from these surroundings with the help of adsorption (extracellular) and absorption (intracellular) mechanisms.

Therefore, evaluation of the two microalgae species, *Scenedesmus spinosus* and *Chlorella vulgaris* for removal of bioremoval of the heavy metals, is the aim of C. Urrutia et al.'s research. These two microalgae were investigated for tolerance to metal (MTW) Mine Tailings Water in northern Chile. They were also studied for synthetic treatments of Mo and Cu (0.1 and 0.5 mg/L). After 72 h, the impact of the tested treatments on *C. vulgaris* microalgae cells was observed using (SEM–EDX) Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy and Confocal Laser Microscopy (CLSM). Morphological changes in the *C. vulgaris* can be seen when crystallized salts attach to its surface. EDX examination revealed that 19% of calcium was present after the treatment of MTW, which explains the observation of crystalline salts. CLSM showed that fluorescence levels were higher in the Cu (0.1 mg/L), MTW and Mo (0.5 mg/L) treatments.

To analyse the heavy metal treatment effect on cell viability, a BD FACS Canto II flow cytometer with two lasers (blue 488 nm and red 633 nm) was employed for this assessment. The effects of Cu, MTW and Mo on the growth rate of *S. spinosus* and *C. vulgaris* after incubation period of about 72 h were then compared with controls. The Tukey test ($p < 0.05$) was conducted for the above experiment which is depicted in Fig. 5.

In comparison with the Control, Cu (0.5 mg/L) and MTW treatments depicted considerable decrease in the growth rates (p 0.05) of *S. spinosus*. The major findings revealed that *C. vulgaris* cultivated with MTW treatment demonstrated better tolerance. In fact, this microalga has a remarkable efficiency of removal for Cu and Mo in MTW: 64.7% and 99.9%, respectively. In addition, after duration of 72 h, Cu (55%) and Mo (80.3%) eliminations were detected at 0.5 mg/L (synthetic concentration treatments). Furthermore, as compared to the control treatment, a greater protein content and a minimum variation in the lipid content could be studied through characterization of the microalgae biomass subjected to MTW. It also might be employed in biorefinery processes. Thus, this study demonstrates the capacity of *C. vulgaris* in removing heavy metals from (MTW) mining tailings water and the effects that occur in the cells of microalgae (Urrutia et al. 2019).

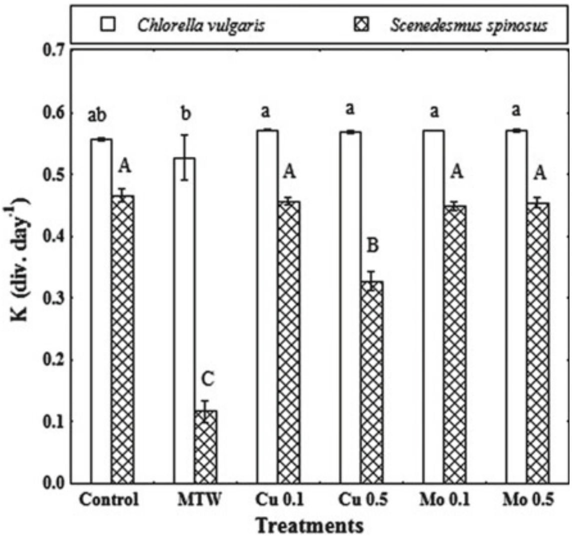

Fig. 5 Growth rate of *C. vulgaris* and *S. spinosus* exposed to MTW, Cu, and Mo solutions, after 72 h. The different letters refer to significant differences in mean values among different treatments of each microalgae species ($n = 3$), determined using the Tukey test ($p < 0.05$). Reprinted with the permission from Urrutia, C., Yañez-Mansilla, E., Jeison, D., 2019. Bioremoval of heavy metals from metal mine tailings water using microalgae biomass. Algal Research 43, 101659. https://doi.org/10.1016/j.algal.2019.101659

Various techniques such as chemical precipitation, solvent extraction, ion exchange, electrochemical separation, membrane separation, etc. have been proposed for separation and pre-concentration of (Rare Earth Elements) REEs. The two most common methodologies for pre-concentration and separation of REEs from various matrices are solvent extraction and ion exchange. The electrochemical treatment and chemical precipitation processes are ineffective when metal ion concentration in the aqueous solution is in the range of units of about 100 mg/L. It did produce large amounts of sludge which was essential to be treated before its discharge. As these procedures are not economically feasible, a necessity to develop a cost-effective and eco-friendly way to recover REEs from aquatic environments was required. The present study focuses on (two types of cyanobacteria) genus *Arthrospira* were tested by David Sadovsky et al., for cerium (III) ion's biosorption from the aqueous environments.

The aim of this research was exploring the cerium biosorption potential consisting of two types of *Spirulina, f*irstly, an endemic type (ES) present in the northern Negev Desert, Israel and secondly, a commercial dry powder (CS).

At the initial pH values of 5.0 ± 0.1, 6.1 ± 0.6 and 9.2 ± 0.9 mg/g for ES and CS, respectively, the highest biosorption capacity was fulfilled. From the aqueous solution, the biosorption of Ce^{+3} was performed by two types of (dried) *Spirulina* biomass which was demonstrated further in batch experiments.

(1) Effective biosorbents were said to be both ES and CS types, as they helped in removing (Cerium) Ce^{+3} from the aqueous solutions.
(2) The Ce^{+3} biosorption rate was observed at maximum percentages (for about 90% of total Ce^{+3}) wherein it was removed within 70 min. This was revealed in kinetic studies.

(3) The isotherms revealed that both equilibrium models (Freundlich and Langmuir) showed to describe the sorption process which suggested an intermediate behaviour (between a mono and a multilayer adsorption mechanism).

Langmuir model clearly stated that the higher sorption capacities found were 38.17 and 18.05 mg/g for CS and ES, respectively. It indicated that CS acted as a better biosorbent in comparison with ES. NaCl concentrations (up to 5 g/L) showed only a minimal influence on the biosorption process of cerium. After three cycles of sorption and desorption, the efficiency of desorption was obtained better than 97% with 0.1 mol/L HNO_3, with no notable decline in biosorption capacity. Thus, these findings demonstrated the viability of recovering cerium (REEs) from the industrial wastes with the usage of *Spirulina* biomass (Sadovsky et al. 2016).

The successful toxic metal-containing wastewater treatment has become a daunting technique because of scarcity for cost-effective treatment methods. Ion exchange, chemical precipitation, electrolytic extraction, membrane processing, and adsorption are the major common methodologies for removing metal ions from wastewater. However, most of these treatments have drawbacks such as high expenditures, a significant chemical input, and partial elimination. Adsorption is one such effective technique that is frequently being used for eliminating metal ions from aqueous solutions. One such example of sorption technique is studied by Meng et al., on Zn (II) and Linear alkylbenzene sulfonates (LAS).

Synthetic surfactants such as LAS are primarily utilized in the manufacturing of detergents, other cleaning products and toxic metal such as zinc is widely employed in a varied industrial sector primarily in galvanizing and producing brass and some other alloys. Doses ranging from 100 to 500 mg d^{-1} of Zn (II) are hazardous to the mankind.

Thus, the current research of Meng et al. is focused on using batch techniques, as in the potential remediation of surface water polluted with LAS and zinc (Zn (II)) by sorption on *Spirulina platensis*. The results revealed that biodegradation of LAS can be carried out by *Spirulina platensis*, with biodegradable rates of 87%, 80%, and 70.5% after 5 days when the initial concentrations were 0.5, 1, and 2 mg/L, respectively. *Spirulina platensis* has a maximal Zn (II) absorption capacity of 30.96 mg/g. The presence of LAS might boost the maximal Zn (II) absorption capability of the alga, which can be linked to raised bioavailability. The LAS rates of biodegradation by *Spirulina platensis* raised with Zn (II) concentration and achieved a maximum limit when the concentrations reached 4 mg/L. The synergistic impact of Zn (II) and LAS was demonstrated in the joint toxicity test. More research is needed to corroborate these findings (Meng et al. 2012).

Inorganic arsenic (iAs) in its dissolved dominant form in the surroundings is well established to significant hazards to both environmental and health of humans. Freshwater-containing arsenic has been thoroughly researched the behaviour and bioaccumulation of dissolved iAs in the presence of Extracellular Polymeric Substances (EPS) which remains as a critical knowledge gap. This has been the first thorough research of iAs-EPS interactions in *Chlorella pyrenoidosa* exposed to arsenite (As^{III}) and arsenate (As^{V}). The study aimed at

(1) investigation of accumulation capacity of arsenic and kinetics of uptake in *C. pyrenoidosa* for its hyperaccumulator potency;
(2) characterising self-produced algal cell EPS and determination of its effects on iAs intracellular uptake, surface adsorption and overall bioaccumulation in this freshwater green alga;
(3) investigating iAs-EPS interactions with the usage of multiple data which includes morphology of cells and their spectroscopic analysis at atomic and molecular levels.

The arsenic absorption capacity raised from 0 to 300 mmol/L and then the uptake rate constants (Ku) observed in the bio-dynamic model were higher for As^{V} than As^{III} (0.63–11.57 L g^{-1} h^{-1} vs. 0.44–5.43 L g^{-1} h^{-1}).

Characterization of EPS being excreted by *C. pyrenoidosa* and understanding the role of EPS in iAs bioaccumulation via intracellular uptake and surface adsorption which was investigated with the usage of various atomic, morphological and molecular evidence, including SEM, TEM, EDS, FTIR, 3D-EEM, and XPS. At the cell-water interface, interaction of EPS with iAs via $–NH_2$, C–O–C, and C–O–H functional groups in polysaccharide and tyrosine-like substances was observed. EPS and its presence may effectively boost arsenic surface adsorption while limiting intracellular absorption, hence improving algal tolerance to iAs and iAs bioaccumulation (EPS-C). EPS-covered cells showed a greater capacity of arsenic adsorption than (EPS-F) EPS-free algal cells due to surface adsorption which is EPS-enhanced and decreased intracellular absorption. The total decrease in maximal uptake capacity in intact algal cells (35% for As^{III} and 23.3% for As^{V}, respectively) promotes cell resistance to the noxious effects of iAs.

Thus, the above findings of the study describe that the *Chlorella pyrenoidosa,* common freshwater green alga, has a subsequently higher arsenic uptake capacity (BAF = 390 L/Kg), rapid adsorption and (2 h) kinetics of intracellular uptake, capability of detoxification in transformation of iAs species into lesser toxic forms, and tolerance to higher levels of As^{III} and As^{V} (EC20: 163 and 300 μmol/L). Since EPS from algae and other microorganisms is widely dispersed in soil, sludge, aquatic, and engineered systems, the findings of this study will help us better understanding of arsenic biogeochemistry and investigating algae-based bioremediation techniques for eradicating iAs and other toxicants of the environment (Zhang et al. 2020).

Phytoremediation is the most promising approach for decontaminating aquatic environments by using plants, algae, and fungus. The utilization of macroalgae has gained popularity due to its ability to absorb harmful components from their surroundings. As the key components of their cell walls, marine algae biosynthesize glycoproteins and polysaccharides. In this research of Dalia M. S. A. Salem et al., one such example of macroalgae using biosorption method was performed wherein two (dried) marine macroalgae species, *Colpomenia sinuosa and Ulva fasciata,* were employed for remediating of cobalt contaminated solutions. The optimal biosorption occurs at pH 6.0 for *U. fasciata* and pH 7.0 for *C. sinuosa*, with contact duration of 60 min for both species, starting Co (II) concentrations of 50 mg/L for *U. fasciata*

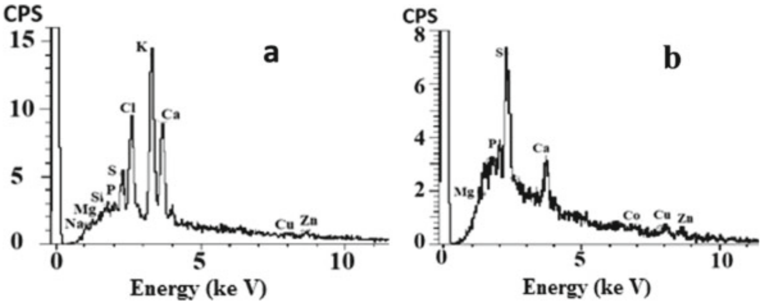

Fig. 6 Energy-dispersive X-ray spectra of (a): *C. sinuosa* before Co(II) biosorption and (b): *C. sinuosa* after Co(II) biosorption. Reprinted with permission from Salem, D. M. S. A., Moawad, M. N., El-Sayed, A. A. M., 2021. Comparative study for bioremediation of cobalt contaminated aqueous solutions by two types of marine macroalgae. Egyptian Journal of Aquatic Research 47, 13–19. https://doi.org/10.1016/j.ejar.2021.02.002

and 80 mg/L 0 *C. sinuosa*, respectively, and dose of 0.05 g/50 mL biomass for both the species.

Characterizing the adsorbents was revealed in this above Fig. 6 which represents the EDX spectra of biomass (*C. sinuosa*) before and after biosorption of Co (II). The maximal biosorption capacity q_{max} of *C. sinuosa* (62.5 mg/g) was significantly higher than *U. fasciata* (40.0 mg/g). It was discovered that the process of biosorption involving *U. fasciata* and *C. sinuosa* adhered to the second-order kinetic model. The higher R^2 values revealed that the Langmuir model was more suitable than the Freundlich model for the adsorption equilibrium of Co(II).

The binding energies of cobalt are seen by the peaks ranging from 6.77 to 7.11 keV. The proportion of Co (II) ions contained in the biomass was found to be 2.8%. As a result, the EDX spectra established *C. sinuosa*'s bio sorption of Co (II) ions. Thus, the ability of two marine macroalgal species to remove Co (II) ions from the aqueous solutions via biosorption has been examined. According to the results, *C. sinuosa* outperformed *U. fasciata* in the eradication of Co (II) ions from the solutions at high and low metal concentration of ions. This would be owing to the high concentration of functional groups in *C. sinuosa*'s cell wall, which contains alginate.

The wastewater from Edku Lake was used in studying the adsorption behaviour of *U. fasciata* and *C. sinuosa.* Lower biosorption capacity in wastewater was obtained from *U. fasciata* and *C. sinuosa* that is around (1.33 and 0.24 mg/g, respectively) in comparison with deionized water (1.79 and 0.89 mg/g, respectively). In wastewater, *C. sinuosa* and *U. fasciata* had a low biosorption capability compared to deionized water which may be due to interference of ions in the drainage water. Thus, based on the above research study, *C. sinuosa* is found to be safe, promising and effective biosorbent for removal of Co (II) ions and remediating drainage water (Salem et al. 2021).

The highly hazardous and carcinogenic metal hexavalent chromium may be found in industrial effluents at quantities hundreds of times higher than the permissible levels for natural aquatic environments. Various efforts have been taken to develop viable technology to remove it from aqueous solutions. Among these, phycoremediation has recently received a lot of interest.

In this research by D. Ociński, J. Augustynowicz, K. Wołowski et al. described a unique aquatic reservoir located near a company of chemicals that generated compounds of Cr (VI) for over a century. Cr (VI)-contaminated ground water is a resultant of rainfall infiltration of the previous chromium-waste dump in this area. On this landfill, production debris was accumulated for almost 40 years, until the 1960s. Since 1990s, polluted groundwater has been gathered in the reservoir for the additional Cr (VI) chemical detoxification. From a biological standpoint and the perspective, the aquifer fostered the creation of a stable aquatic ecosystem which is more than 20 years old. Due to this, the community of algae discovered in the form of a distinctive biological mat has developed mechanisms that allows the algae in colonization and flourishing at exceptionally higher levels of Cr (VI). (Nearly 6150 times the upper limit for surface water.)

The average proportions of the genera of the biological mat include as follows: *Tribonema* sp. 31% > *Klebsormidium* sp. 20% > *Stigeoclonium* sp. 14% > *Mougeotia* sp. = *Ulothrix* sp. = *Melosira* sp. (diatom) = *Dolichospermum* sp. 7% > *Spirogyra* sp. 5% > *Oedogonium* sp. 2%. Further taxonomic studies founded a composition consisting mixture of filamentous algae, upon identification it denoted three species of *Tribonema,* namely *T. vulgare, T. microchloron, and T. viride.* The *T. vulgare and T. microchloron* being highest representation in the biological mat. Reduction being the major process of Cr (VI) detoxification followed by Cr (III) biosorption, which might be accomplished by complexation and ion exchange mechanisms.

Desorption experiments were performed on algae to know the binding patterns of chromium. FTIR and XPS studies demonstrate the functional group's interaction with Cr (III) and Cr (VI) wherein the most significant differences were seen in bands at 1221, 1452, and 1740 cm^{-1}. These bands can be attributable for different carboxyl stretches. After desorption in HNO_3, the newly created bands at 1740 cm^{-1} (free COOH) and 1452 cm^{-1} (COO), and the rise in the band at 1221 cm^{-1} (C–O stretching in COOH), indicated the importance of carboxylic functional group in an ion exchange mechanism for cations of Cr (III).

The characterization of algal biomass collected from the natural habitat (absorbed chromium with raw biomass, and biomass after desorption of chromium) by 0.1 M NaOH and 0.1 M HNO_3 was performed. Despite the presence of Cr (VI) in the examined system of aquatic environment, the chromium bounded to the algae (>90%) was in the (trivalent chromium) Cr (III) form, with only low Cr (VI) yields of recovery. This consistency with recent research on the eradication of Cr (VI) from aqueous solution using algal biomass demonstrated that reduction is a key aspect of the remediation process. Reducing Cr (VI) is said to be a pH dependent process, only under very acidic conditions, (pH 1–pH 4), the Cr (VI) process of biomass oxidation be efficient. The exopolysaccharides with a higher number of -OH groups may play an essential part in the reducing Cr (VI) to Cr (III) by algae.

The investigations depicted that the reservoir inhabited by the algae can be employed as the cost-effective 'bioreactor' capable in reducing chromium content to it's acceptable limit with some modifications. Furthermore, the investigations undertaken are vital for obtaining in-depth understanding and should be useful in the community's relevance for the future application as a possible Cr(VI) biosorbent on a worldwide level (Ociński et al. 2021).

2.3.2 Bioremediation Using Algae for Removal of Radionuclides

Nuclear energy accounts for around 9.7% of total world energy supply and is recognized as one of the cleanest, most promising, and carbon-free sources of energy. Radioactive energy has a drawback that it generates almost 2000 metric tonnes of nuclear waste per year. Furthermore, considerable amounts of radioactive nuclides have been discharged in the environment which causes nuclear power plant accidents. Among those radioactive nuclides released, there are major concerns regarding 137—Caesium (^{137}Cs), a nuclear fission product, because of its long half-life of about 30 years, high water solubility, powerful gamma-ray emissions and higher bioavailability.

In the research study of Kim et al., utilization of, *Desmodesmus armatus* SCK, a photoheterotrophic microalga to remove Caesium (Cs^+), following a recovery protocol with the usage of magnetic nanoparticles was examined in his work. The comparison of three microalgae (*D. armatus* SCK, *C. reinhardtii* CC-124, and *C. vulgaris* UTEX265) findings revealed that *D. armatus* SCK eliminated most of the Cs at 10 °C and 25 °C. *D. armatus* SCK removed the high amount of Cs^+ (52.2 ± 0.4 mmol/L), following *C. reinhardtii* CC-124 (6.1 ± 0.0 mmol/L and *C. vulgaris* UTEX 265 (8.2 ± 0.9 mmol/L). Cultivation of these microalgae was performed using Tris–acetate phosphate (TAP) medium which consists of Hunter's trace metal solution, Beijerink's solution and Tris–acetate solution with an exposure to fluorescent light (120 mmol m^{-2} s^{-1}).

To assess the further removal of Cs, supernatants were allowed to pass through 0.22 mm membrane filters after undergoing centrifugation (3000 rpm for 5 min). The non-radioactive Cs^+ in the filtrates (soluble quantities) were quantified with the help of inductively coupled plasma-mass spectrometry and radioactively soluble ^{137}Cs in the filtrates were measured with the usage of the multichannel g-spectrometry analyser. The ion-chromatography analysed the concentration of K^+ filtered supernatant samples. The use of K-depleted SCK cells increased Cs removal efficiency by more than 20% when compared to normal cells. Heterotrophic mode with the inclusion of volatile fatty acids (VFAs), particularly acetic acids (HAc), also improved Cs elimination by K-depleted *D. armatus* SCK. The specific activity of ^{137}Cs was determined from its 661.7 keV peak.

Magnetic nanoparticles, polydiallyldimethylammonium (PDDA)-FeO3, were used to efficiently capture the Cs^+ uptaken by the microalga. This strain finally removed <99% of radioactive ^{137}Cs from solutions containing 10, 100, and 1000 Bq m/L^{-1}. As a result, combining *D. armatus* SCK, a K-starved microalga, with VFAs might be a potential way to extract Cs from liquid wastes (Kim et al. 2020).

Another example of radioactive Caesium's biosorption from polluted water by microalgae *Haematococcus pluvialis* and *Chlorella vulgaris* by Lee et al. Its Caesium uptake was in comparison with other microalgae, *Anabaena* sp. and *Chlorella vulgaris*, under different cell conditions. By bioaccumulation mechanism, radioactive Caesium was rapidly removed by photo-induced *H. pluvialis* red cyst. The *H. pluvialis* red cyst removed about 95% of the soluble ^{137}Cs in 48 h, both *H. pluvialis* intermediate cells along with *C. vulgaris* demonstrated 90% ^{137}Cs uptake efficiency with a slower uptake rate. The build-up of Caesium via the potassium transport channel is associated with the acceleration of Caesium absorption by astaxanthin-inducing red cyst. After an year for monitoring of biosorption, it was observed that 40% of the ^{137}Cs remained in *collapsed H. pluvialis* cell fragments.

This nature of cellular collapse following Caesium uptake was caused primarily due to the introduction of dense cells of microalgae into Caesium-contaminated solution. This severe culture condition was unable to be conducive to survival of the cell and its growth. This data implies that for long-term bioremediation, stability and bioaccumulatory properties of microalgal ^{137}Cs absorption should be considered (Lee et al. 2019).

The degree of removal of toxic radionuclides is determined based on types of radionuclides and the removal procedure (biosorption or bioaccumulation). Thus, investigation regarding biosorption and bioaccumulation of ^{241}Am, ^{90}Sr, ^{137}Cs, ^{233}U, ^{239}Pu, and ^{237}Np by *Arthrospira platensis* CNMN-CB-02 biomass for removal of radionuclides was performed by I. Zinicovscaia, et al. EDTA-free Zarrouk medium of cultivation medium and this medium with the inclusion of sodium polyphosphate was being used for bioaccumulation experimentations. Uptake of ^{233}U was seen to be raised in the existence of polyphosphate ions. Also, *A. platensis* CNMN-CB-02 biomass showed increased uptake capacity for ^{90}Sr, ^{239}Pu and ^{241}Am.

The concentration levels of the concerned radionuclides were determined using liquid scintillation methodology by a Tri-Carb-3180 TR/SL radiometer. For ^{239}Pu and ^{241}Am, rate of accumulation was 98.7% and 99. 4%, respectively. The efficiency of elimination of *A. Platensis* CNMN-CB-02 biomass for ^{90}Sr was reported to be around 90% which also can be considered very high. This accumulation occurred through Ca $^{2+}$ transport system.

In the biosorption, higher biosorption capacity for ^{90}Sr, ^{239}Pu, ^{241}Am, and lower in case of ^{233}U. Kinetic models were applied wherein pseudo-first-order model served as best fitting model for ^{90}Sr, ^{239}Pu and ^{237}Np and, with the coefficient determination (0.98–0.99). By pseudo-second-order model, the data obtained for ^{137}Cs, ^{241}Am and ^{233}U depicted that radionuclides biosorption is due to chemisorption. Also, the studies of desorption showed that *A. platensis* CNMN-CB-02 biomass might be reused for biosorption process and the higher strength of radionuclide binding to phosphorylated biomass was observed. Thus, this cyanobacterial biomass can be taken into consideration as a potential species. As *A. platensis* CNMN-CB-02 is an extremophile, it is hugely tolerant to gamma-rays making it the main candidate for eradicating radionuclides from alkaline solutions, salt and in situ bioremediation methodologies (Zinicovscaia et al. 2020).

2.4 Bioremediation Using Plants to Remove Heavy Metals and Radionuclides

2.4.1 Bioremediation Using Plants to Remove Heavy Metals

In the following part of the chapter, we are going to study the role of plants in the bioremediation process. Plants do act as strong bioindicators as any change in their natural habitat may be a visible in their morphology.

With rapid population growth, tremendous technological advancements, and substantial changes in land usage, human effect on the maritime environment has intensified. Many countries' policies have changed to reflect the belief that the ocean's capacity to sink our waste is finite. The sponge *Chondrilla nucula* was studied by Ferrante et al. to see if it could be used as a bioaccumulator and bioindicator of contaminated ocean environment for those with high levels of toxic metals, in order to aid the development of new environmentally friendly technologies for environmental recovery. The goal of this research was to see if *C. nucula*, a typical sponge found in the Mediterranean Sea, might be used as a bioremediator for inorganic pollution in marine environments.

This study investigated the ability of *C.nucula*, a common marine sponges found in the Mediterranean rocky environment, to acquire Cd, Cu, and Pb in contaminated sites in vivo, as well as its potential for bioremediation via transplanted individuals. According to available literature, variability in trace metal bioaccumulation and organism response to the environmental stress can be obtained at a variety of scales. The preliminary purpose was to determine background concentrations and the species accumulation strategy by detecting Cd, Cu, and Pb in specimens and sand samples collected from the control site. We discovered background levels that were low enough to allow the organisms to be used in following in vivo investigations, and low values of bioaccumulation factor (BAF) ranked as BAF Pb < BAF Cd < BAF Cu with values of 0.037 ± 0.006, and 1.380 ± 0.405 and 2.490 ± 0.560, respectively.

The bioaccumulation factor appropriately captures each metal's varied accumulation behaviour since accumulation is a function of metal availability in the environment. Biomonitoring and bioremediation are two of the most ideal difficulties in the field of maritime environmental protection. It showed extraordinary resistance to high Cu water-borne concentrations up to 0.8 mg/L, with consistent and equal absorption rates across all exposure levels and no symptoms of stress.

Cd tolerance is moderately reduced as Cd concentrations rise. The absorption rates are stable throughout the exposure levels, while metallothioneins are detected at the second (0.2 mg/L) and third (0.4 mg/L) levels, respectively. Increased Pb concentrations resulted in a significant reduction in sponge tolerance. A substantial drop in absorption rates was found with exposure to the third Pb level (0.8 mg/L). The expression of metallothioneins was seen during the second (0.4 mg/L) and third (0.8 mg/L) levels of exposure, as well as cell necrosis from the second level of exposure (Ferrante et al. 2018).

In another interesting study, Saqib et al. used blue pine shavings (*Pinus wallichiana*), walnut shells (*Juglans regia*), and testa chickpeas (*Cicer arietinum*, Indian variation) for removal of arsenic from drinking water. The adsorbate concentration of the arsenic test solution was maintained at 200 g/L whereas the concentration for adsorbent biomass, i.e. blue pine biomass and walnut, was maintained at 20 g/L and 40 g/L of, respectively, at pH 8. Interestingly, blue pine 90% sorption efficiency with enhanced % removal of arsenic, whereas walnut indicated an optimum quantity with more than 80% sorption efficiency.

For blue pine and walnut, the effect of pH in the range of 2–13 (20 °C) on arsenic removal was investigated using biosorbent dosages of 20 and 40 g/L, respectively, equilibrated for 20 and 40 min, and an arsenic concentration of 200 g/L. At lower concentrations up to 100 g/L, arsenic removal efficiency was high, with removal efficiencies of 94% and 88% (maximum) for blue pine and walnut, respectively. Further increases in arsenic content resulted in decreased sorption of 60–85% up to 500 g/L. The influence of temperature on arsenic removal efficiency was investigated at temperatures ranging from 10 to 50 degrees Celsius. Further increase or decrease in temperature resulted in lower sorption efficiency for arsenic removal by biomass of both blue pine and walnut. *P. wallichiana* shows good arsenic biosorption (arsenic removal efficiency of >90%), which demonstrates its potential for treating the drinking water.

The abatement procedure was also influenced by arsenic concentration, which was found to be effective for a wider range of values (i.e. >200 g/L). *P.wallichiana* biomass followed both the Langmuir ($r^2 = 0.99$) and Freundlich ($r^2 = 0.97$) isotherms. The bioaccumulation factor appropriately captures each metal's varied accumulation behaviour since accumulation is a function of metal availability in the environment (Saqib et al. 2013).

Phytoremediation is a low-cost remediation technique. Many plants, including Brachiaria decumbens, *Brassica juncea, Elsholtzia splendens*, and *Silene vulgaris, Atriplex halimus, Pennisetum purpureum*, and *Phragmites australis*, as well as bioenergy plants like *Arachis hypogaea, Cannabis sativa, Helianthus annuus, Linum usitatissimum,* and *Ricinus communis,* are currently grown. Andreazza et al. investigated the high bioenergy in the castor bean plant (*Ricinus communis L.*) for phytoremediation of copper-contaminated soils in this study. The height of each plant was measured in relation to the main stem of the castor bean plant, which ran from the base to the tip. After 57 days of development, the castor bean plants in the Inceptisol reached an average height of 20 cm. The plant effective number for the branches and the plant effective number for the overall plant were used to measure a hyperaccumulator plant's potential to repair polluted soil. The TI (tolerance index) of castor bean plants was lowest when the plants were planted in Cu mine waste, with values of 60%, 39%, 66%, 85%, and 91% for plant height, fresh mass, and dry mass of the roots and shoots, respectively.

Castor bean plants grown in both Inceptisol and Mollisol had low macronutrient concentrations in the roots for K, Ca, Mg, and S in general. The roots of castor bean plants cultivated in Cu mine waste have large macronutrient concentrations, with the greatest quantities of K, Ca, Mg, and S. When castor bean plants were grown in native soil, Inceptisol, or Mollisol, the nitrogen content in the roots was much higher, with values of 1.74, 1.69, and 1.45 g/Kg, respectively.

Castor bean plants had a high tolerance index when grown in both Cu-contaminated vineyard soils and Cu mining waste, but a modest reduction when grown in Cu mining waste. The results demonstrated high Tolerance Index (TI) values for castor bean plants when the plants were cultivated in the Mollisol and Inceptisol soils to produce roots and shoots phytomass relative to several potential energy crops, such as *Arachis hypogaea, Brassica rapa, Cannabis sativa, Carthamus tinctorius, Glycine max, Helianthus annus, Linum usitatissimum, and Ricinus communis*, they were grown for 28 days in soil that was contaminated by Cd, with values between 15 and 91%. Specifically, castor bean plants cultivated in the Cu mining waste exhibited TI values ranged in between 85 and 91% for the dry mass of the roots and shoots, respectively.

Despite the fact that the heavy metals were different, our research revealed that the castor bean plant has a high potential tolerance for development in places polluted with various heavy metals, particularly Cu. Cu concentrations beyond a certain threshold can impair macro and vitamin absorption, as well as induce certain ocular poisoning symptoms in oats plants. Castor bean plants growing in vineyard soils polluted with Cu and Cu mining waste had a higher MER index than that other hyper-accumulator plant Solanum nigrum contaminated with other heavy metals such as As and Cd, according to the findings. So, when biomass of shoots plus total plants is included, the plant effective number is defined as the number of plants required to take up 1.0 g of metal.

The plant effective number for Pb shoots for *Piptatherum miliaceum*, a Smilo grass hyperaccumulator 659 plant, was about 1000 shoots, and its value for Cd was around 4300 full plants.

These findings suggest that castor bean plants have a high potential for Cu phytoaccumulation throughout the plant, particularly in the roots. When all the variables are considered, as well as the number of plants per hectare that may be employed, our PEN (plant effective number) values might be dramatically reduced, allowing for increased efficiency in the use of castor bean plants in phytoremediation (Andreazza et al. 2013).

Heavy metal pollution has become a critical environmental issue due to rapid industrial development. Bioremediation has gotten a lot of press as a good way to detoxify effluents and soils that are also cost-effective and environmentally benign. Microalgae seems to be one of the most promising candidates for the removal of heavy metals since it has high efficiency and selectivity, as well as a low cost. Immobilized metabolically active microalgae have also been studied for heavy metal bioremediation as they have ability to accumulate more metals can be reused with reduced clogging property in aqueous solution. Shen et al. used a combination of water-hyacinth leaf biochar pellet (WLBp) immobilized with Chlorella sp. to improve Cd (II) removal efficiency and examine the Cd (II) absorption mechanism. The capacity of Chlorella sp. to build biofilms was tested on four different carriers. The following processes were used to make the water-hyacinth leaf pellet (WLp), water-hyacinth root pellet (WRp), water-hyacinth leaf biochar pellet (WLBp), and water-hyacinth root biochar pellet (WRBp). On the four carriers (WLp, WLBp, WRp, WRBp) with distinct surface hydrophilic qualities (WLp, WLBp, WRp, WRBp) with different surface hydrophilic properties were tested (see Fig. 7). The two biochar-based carriers had better surface hydrophilicity (91.73 ± 2.63% for WLBp and 77.92 ± 2.26% for WRBp) than the two raw biomass-based carriers (74.57 ± 1.41% for WLp and 69.82 ± 3.81% for WRp). The WLBp and WRBp surfaces had extremely few hydrophobic functional groups compared to the WLp and WRp raw material, as evidenced in the spectra from 2800 to 3000/cm.

Hydrophobic functional groups, like –C–H stretching of alkyl groups, were substantially related with this range. Additional hydrophilic functional groups found in WLBp included C–O–C symmetric stretching at 1027.9/cm, –OH deformation of –COOH, and C–O stretching of aryl esters at 1236.2/cm, all of which led to increased surface hydrophilicity.

Immobilized *Chlorella* sp. was used to test the effects of cadmium treatment on water-hyacinth-derived pellets. Cd (II) elimination efficiency was shown to be positively related to both immobilization efficiency and bioaccumulation capacity. With a complex composed of the best possible WLBp carrier and immobilized algae in stationary growth phase, illuminated with an intensity of light of 119molm-2s-1, a maximum Cd (II) removal rate of 92.5% was achieved, resulting in an immobilization

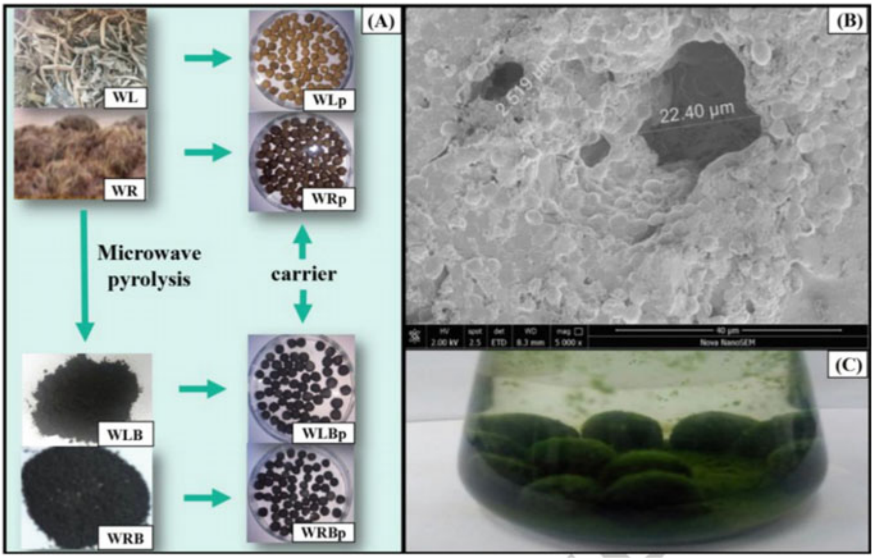

Fig. 7 The complex of immobilized Chlorella sp. on water-hyacinth-derived pellets: (A) Pellet preparation, (B) Field emission scanning electron microscopy (FESEM) micrograph, (C) Microalgae coverage on the WLBp surface. Reprinted with the permission from (Shen et al. 2018)—Shen, Y., Zhu, W., Li, H., Ho, S. H., Chen, J., Xie, Y., Shi, X., 2018. Enhancing cadmium bioremediation by a complex of water-hyacinth-derived pellets immobilized with *Chlorella* sp. Bioresource Technology 257, 157–163. https://doi.org/10.1016/j.biortech.2018.02.060

efficiency of 89.3% and a bioaccumulation capacity of 13.81 mg/g. According to regeneration testing, both *Chlorella* sp. WLBp and Chlorella sp. WLBp may be economically recovered and reused. According to regeneration tests, both Chlorella sp. and WLBp may be recovered and reused at a low cost (Shen et al. 2018).

Many inorganic pollutants pollute numerous ecosystems, including air, water, and soil, causing major difficulties. Toxic metal compounds are released into the environment due to human activities that contaminate not just surface water bodies like ponds, lakes, and reservoirs, but also groundwater the following seepage into the water table. Most heavy metals come from anthropogenic sources, such as mineral ores, metallurgical industries, paints and ceramics, wood preservatives, dyes, and pesticide manufacture.

Smelting processes, the battery industry, thermal power plants, and other sources of nickel (Ni), emit the metal. Zinc smelting, e-waste, paint sludge, incinerations, waste batteries, and fuel combustion are all sources of Cadmium (Cd) in water bodies. Lead (Pb) is a waste-product of lead acid batteries, electronic trash, smelting processes, paints, coal-fired power stations, ceramics, and the bangle industry. Heavy metal-polluted lakes were identified in and around Bangalore in this study by George et al., based on reports from the KSPCB, 2002, and numerous other scientific studies on Bellandur Lake (Ramesh and Krishnaiah 2014), Varthur Lake (Vardanyan et al. 2008), and Hebbal Lake. The approach was followed to manufacture Citric Acid altered Moringa oleifera leaf and bark (CAMOL and CAMOB) using sundried leaf and bark samples as well as the leaves and bark. Batch biosorption investigations in conical flasks were carried out to optimize pH, metal ion concentration, contact duration, and temperature.

For metal ion removal from polluted water bodies, *Moringa oleifera* bark can be employed as an alternate sorbent. When compared to seeds, most studies show that 60 to 90% of metals are removed. Following the removal of heavy metals from aqueous solutions by biomass, the recovery of the metal and the biosorbent's reusability are crucial considerations. This might be accomplished by using a metal desorption method to weaken the heavy metal biomass connection. Additional research should be focused on the creation of innovative, ecologically friendly technologies (George et al. 2016).

Heavy metal poisoning of terrestrial and aquatic ecosystems has emerged as a global environmental issue. The primary source of heavy metals in the environment is mining and the improper disposal of industrial solid/liquid wastes. Heavy metal contamination of freshwater resources in metropolitan areas is at an alarming level, owing to the dumping of untreated or inadequately treated sewage and industrial wastewaters. Adsorption, chemical precipitation, electrochemical techniques, coagulation-flocculation, flotation, ion exchange, and membrane filtration are among the traditional metal-remediation procedures.

These technologies have several benefits, including design and operation versatility, large treatment capacity, high removal efficiency, and fast kinetics, but they also have disadvantages, such as toxic waste or other by-product generation, excessive cost of maintenance, and high energy demands. The study by Verma et al. wanted to determine how efficient duckweed (*Lemna gibba*) is at removing heavy metals (Pb and Cd) from water at different pH levels and metal loading. The goal of this study was to see how pH and metal concentrations in aquatic media affected the removal effectiveness of a duckweed system with *L. gibba* as the test species and to check how metal load and aqueous media pH affected the rate of metal removal and also biomass productivity in a duckweed-based phytoremediation system.

Pb and Cd elimination in setups with varying metal ion doses and pH. During the experimentation, there was a substantial variation in removal rate across different metal strengths. Pb elimination (percentage) ranged from 60.1 to 98.1% at various pH levels in all duckweed testing setups. The greatest *L.gibba* elimination efficiency was observed at pH 7 in the 2 mg/L set-up, whereas the maximum Pb removal was obtained at pH 5 and 7 in the 5 mg/L and 10 mg/L load sets, respectively. In terms of removal effectiveness, any experimental setups with varied pH ranges may be constructed.

The purpose of this study was to determine how the rate of metal removal and biomass productivity in a duckweed-based phytoremediation system were impacted by metal load and aqueous media pH. The findings revealed that metal removal was related to the quantity of metal in the medium, with pH 5 and 7 proving to be the ideal range for optimal system performance. The metal uptake yield and BCFs (bioconcentration factor) recommended pH 7 and medium and high Pb and Cd metal loading for the intended metal removal results. After modifying the metal load and medium pH for enhanced system performance, our findings justify the use of *L. gibba* as a biological agent in the design of a metal bioremediation system. In the case of a decentralized treatment option, this could be a cost-effective alternative treatment strategy (Verma and Suthar 2015).

Numerous physicochemical methods for bioremediation of heavy metals from the aquatic and terrestrial environment are not feasible because they are energy-consuming and expensive. A biological therapy system that could replace these standard treatment modalities is urgently needed. The use of aquatic macrophytes for the phytoremediation of industrial waste has emerged as a simple, cost-effective, and self-sustaining alternative to standard treatment methods.

Wetland plants including cattail (*Typha latifolia*), common reed (*Phragmites australis*), and motha have been reported to contain heavy metals (*Cyperus malaccensis*). The focus of Chandra et al. research will be on *P. cummunis* bioaccumulation. *P. cummunis* accumulated most metals (Cr, Fe, Mn, Ni, Pb, and Zn) in its shoots, except for Cd and Cu. Total metal accumulation was in the sequence Fe (2813) > Mn (814.40) > Zn (265.80) > Pb (92.80) > Cr (75.75) > Cu (61.77) > Ni (45.69) > Cd (4.69). The majority of the metals were collected in the roots of *T. angustifolia* and *C. esculentus.* Mn translocation was greatest in the leaves of *T. angustifolia* and *C. esculentus,* followed by roots and shoots. The following metals accumulation pattern has been observed in *T. angustifolia* and *C. esculentus.*

Three wetland plants accumulated heavy metals in an aqueous metal solution, revealing their phytoremediation capability. Metals collected greater in *P. cummunis* shoots than in roots or leaves. *P .cummunis* had TF (Translocation factors) more than 1 for Cr, Fe, Mn, Ni, Pb, and Zn, but TF for Cd and Cu. The shoots/roots ratio was less than one for Cd, Cr, Cu, Fe, Mn, Ni, and Pb, *T. angustifolia* and *C. esculentus,* and larger than one for Zn. The shoots/roots ratio was less than one for Cd, Cr, Cu, Fe, Mn, Ni, and Pb, *T. angustifolia* and *C. esculentus*, and larger than one for Zn.

T. Angustifolia shoots accumulated the most Zn, as seen by this. Based on heavy metal accumulation and biochemical markers, the order of phytoremediation capacity was *P. cummunis* > *T. angustifolia* > *C. esculentus*. *P. cummunis* and *T. angustifolia* had higher relative biomass than *C. esculentus*, indicating that *P. cummunis* and *T. angustifolia* have more potential for heavy metal cleanup from industrial wastewater (Chandra and Yadav 2011).

One of the most dangerous elements present in water is arsenic. Arsenic can be discovered in the water due to the breakdown of minerals from volcanic or sedimentary rocks, as well as the dilution of geothermal fluids. This element is used in lasers, semiconductors, the glass industry, pharmaceuticals, and pigments, to name a few. Agriculture and industrial effluents discharge might be regarded the primary source of arsenic pollution in water. Alvarado et al. published the following findings in their investigation.

There were no significant differences between treatments on a wet basis. This might be due to the control treatment plants having a high humidity percentage. Plant density increased on a dry basis between the first and 14th days in the case study of the water hyacinth and decreased between the 15th and 21st days. This might be because the plants reproduce during the first cycle, while the vessels' available space and nutrients decrease during the second. Between the two species, there were no significant differences in bioaccumulation capacity or mg of As/kg of plant. *L. minor* exhibited a lower removal rate (140 mg As/ha d) and a 5% removal recovery rate.

The water hyacinth exhibited a removal rate of 600 mg As/ha d and an 18% removal recovery under test circumstances. The eradication efficacy of water hyacinth has improved as a result of the biomass generation. The climatic circumstances in the trial zone were more conducive to the water hyacinth's capacity to remove itself, and this had an influence on the species' ability to do so. The species' development was unaffected by arsenic pollution levels of 0.15 mg/L. Due to the climatic conditions of a somewhat dry tropical forest in Tarabana, Edo, Venezuela, the Water Hyacinth is a reliable option for arsenic bioremediation in rivers (Alvarado et al. 2008).

Because of the emission of gaseous pollutants, solid wastes, and heavy metals, the proliferation of industries and the surge in the expanded production of many categories of consumer products have generated major environmental difficulties. Almost all living and non-living creatures are at risk from these toxins. When human skin is exposed to wastewater containing Cr (VI) for an extended period of time, dermatitis and eczema develop.

The utilization of Biochar (BC) derived from Neem (*Azadirachta indica*) leaves as a bio resource, activated by pyrolysis at 450 °C for removing Cr (VI) from synthetic wastewater was described in this work by Thangagiri et al. After mixing the BC with Cr (VI)-containing synthetic wastewater, the char successfully removed Cr (VI) by adsorption [Cr (VI)NBC]. The elemental composition of NBC is C (70.52%), O (26.27%), N (3.19%), and S (0.02%), whereas Cr (VI) loaded NBC is C (63.77%), O (31.30%), N (2.82%), and Cr (VI) loaded NBC is C (63.77%), O (31.30%), N (2.82%), and Cr (VI) loaded NBC is C (63.77%), O (31 (2.12%). For the NBC and Cr (VI) NBC, the N/C ratios are calculated to be 0.045 and 0.044, respectively.

The BC maintained after the NBC procedure X-ray diffraction investigation revealed the existence of graphitized and amorphous carbon, as well as trace amounts of metal oxides. The existence of a flake-like morphology of carbonaceous char and elements such as C, N, O, and S was verified by FESEM (Field Emission Scanning Electron Microscope) and EDX (Energy-dispersive X-ray spectroscopy) investigations. FTIR was used to show the many vibrational modes present on the BC. After mixing the BC with Cr (VI)-containing synthetic wastewater, the char successfully removed Cr (VI) by adsorption [Cr (VI)NBC]. The presence of Cr (VI) on the char was assumed, as indicated by the EDX study.

The zeta potential examination of the surface charges produced on the BC and Cr (VI)NBC revealed that the adsorbed Cr (VI) occurs in the form of chromate, yielding negative zeta potentials. BET analysis was used to assess the surface area, pore volume, and related parameters of NBC; the results demonstrated that a large surface area (38.593 m^2/g) leads in a greater amount of adsorbed Cr (VI). WCA (water contact angles) investigations were carried out with the use of a WCA goniometer and the biochar's hydrophilic nature.

The NBC's adsorption behaviour towards Cr (VI) was studied at different pH levels, and the results revealed that the adsorption process was pH dependent. Cr (VI) adsorption on NBC was best (58.54 mg/g) when the pH was low. The adsorption experiment, which was carried out at various temperatures, revealed that the adsorption followed the Langmuir-type isotherm. A fixed-bed column investigation was used to further test NBC's adsorption capabilities for the elimination of Cr (VI).

The BTC (break through point), PAZ (principal adsorption zone) length, fractional capacity of NBC, and other parameters related with this study were calculated to disclose the adsorption characteristics of NBC. The char was utilized in the adsorption experiment after it was alkali treated to reactivate the Cr (VI)-adsorbed char. After six cycles, the adsorption effectiveness dropped from 96 to 57%. Finally, a cost analysis was undertaken based on the cost of biochar per tonne in relation to the removal of Cr (VI), and the results demonstrated the material's excellent cost efficiency for wastewater treatment (Thangagiri et al. 2022).

Heavy metal contamination of our environment is caused by a variety of economic activities such as dyeing mining, plating, vehicle production, and metal processing. This contamination has resulted in a slew of environmental issues. These compounds wind up in aquatic ecosystems, which frequently include high amounts of contaminants that can be hazardous to creatures. Heavy metals are among the pollutants that are carried and stored in the environment. Contaminants and heavy metals are frequently absorbed naturally by aquatic vegetation.

The main goals of the study by Syukorwere et al. were to determine the heavy metal removal efficiency of an integrated Phytogreen system made up of two different aquatic plants, *Typha angustifolia* sp. and *Limnocharis flava* sp., in the traditional oxidation pond process, as well as the relationship between retention time and heavy metal removal. The metal uptake of the plants boosted the heavy metal removal when they were cultivated separately and jointly. While working on contaminated soil phytoremediation, this approach was able to remove more Cd and Zn from S4 wastewater (49.3% and 59.6%, respectively) than Ahmadi et al. (2014), who used maghemite nanoparticles to treat aqueous solutions (20% and 52%). Feizi and Jalali (2015) investigated removal efficiency of heavy metal from aqueous solutions using sunflower, potato, canola, and walnut shell residues and found that nickel removal efficiency was around 55%, whereas the current study achieved a higher nickel removal efficiency of 62.2% when two plants were used.

Another study looked at removal efficiency of heavy metal from aqueous solutions using sunflower, potato, canola, and walnut shell residues and found that the nickel removal efficiency was around 55%, whereas the current study achieved a higher nickel removal efficiency of 62.2% when two plants (*Typha angustifolia* sp. and *Limnocharis flava* sp.) were used together. In a study on the assimilation of heavy metals by maize plants, Mohammad et al. (2015) achieved 21.2% Zn and 29% Cu removal, whereas this work achieved 38.4% and 32.8% higher Zn and Cu removal.

In a traditional oxidation pond procedure, the integrated phytogreen systems consisting of two different aquatic species, namely *Typha angustifolia* sp. and *Limnocharis flava* sp., proved to be a reliable and environmentally appealing solution. It is said that the integrated phytogreen system is made up of two different aquatic plants, namely *Typha angustifolia* sp. and *Typha angustifolia* sp. In a traditional oxidation pond procedure is a reliable and environmentally friendly alternative.

Typha angustifolia sp. was found to remove the most heavy metals (Cd, Cr, Cu, Fe, Mg, Ni, Pb, and Zn). When a combination culture of the two plants was used instead of a monoculture, metal removal efficiency increased, and *Limnocharis flava* sp. The above-mentioned heavy metals were eliminated by natural precipitation *Limnocharis flava* sp., which was equivalent to the net sorption in plants. Apart from Zn and Pb, for all heavy metals tested. Two separate aquatic plants, *Limnocharis flava* sp. and *Typha angustifolia* sp., make up the scientific contributions of this integrated phytogreen system. The use of this green technology in industry could make the removal of heavy metal processes more cost-effective (Syukor et al. 2016).

2.4.2 Bioremediation Using Plants for Removal of Radionuclides

Biosorbents have recently received a lot of interest as a wastewater treatment option. Freundlich and pseudo-second-order kinetic models were used to model the biosorption process. According to thermodynamics, biosorption was endothermic and spontaneous. Waste banana peels were used to evaluate the elimination of Sr (II) from aqueous solution in this study. At 120 rpm, pH 7, 323 K temperature, 439 μm sized biosorbent, and a contact time of 10 min, the maximum biosorption capacity of Banana Peels Powder (BPP) had been found to be 41.5 mg/g. Up to 5 cycles of BPP regeneration and reusability were successfully tested.

Biosorption data was analysed using a variety of models, including pseudo second-order kinetics. In comparison with the Langmuir model, studies of biosorption isotherms show that the Freundlich model is better at describing the biosorption process. The feasibility, favourability, spontaneity, and endothermic character of the process were all expressed by thermodynamic studies. Using 0.1 N (Nitric acid) HNO_3 at 303 K, 66.7% of the strontium ions from BPP were desorption. Five repetitions of biosorption and desorption were completed successfully with BPP. The morphological variations in BPP during biosorption and desorption were validated using SEM images. The amino, hydroxyl, carbonyl, and carboxyl groups all play a role in biosorption, according to FTIR analysis of BPP. The results showed that BPP was effective at bioremediating strontium from aqueous solutions (Mahindrakar and Rathod 2018).

Uranium is primarily used as a nuclear reactor fuel. Because of the global scope of this application, uranium-contaminated waste is generated at every stage of the nuclear fuel cycle, from mining and mineral processing to uranium enrichment, fuel rod manufacture and reprocessing, nuclear power generation, decommissioning, and plant operation. Apart from radiation, uranium causes both carcinogenic and non-carcinogenic risks to human health, with nephritis being the most common symptom.

U (VI) has been recognized as an inveterate human carcinogen by the US Environmental Protection Agency. U has a maximum permitted limit set by the EPA (VI). The optimal concentration of Uranium in drinking water should be less than 20 μ/L as stated by WHO guidelines of 1998 and 2003. According to WHO studies, the daily tolerated intake of U (VI) is 0.6 g/ kg of body weight per day. Uranium pollution has a variety of negative consequences for humans and other organisms. The development of affordable and environmentally friendly biosorbents as prospective heavy metal-remediation methods has been a key focus area in recent decades. GK (gum kondagogu,) is a biopolymer that was previously evaluated for its potency as an adsorbent for toxic heavy metals such as mercury. This research by Sashidhar et al. gives data on GK (gum kondagogu), which is a biopolymer that was previously evaluated for its potency as an adsorbent for toxic heavy metals such as mercury.

In line with previous research, the efficacy of GK was assessed in this study for U (VI) adsorption in aqueous and simulated effluents, with U (VI) ions adsorbing up to 50% of their own mass. Adsorption of hazardous metals from industrial effluents has been reported using chitosan, rice straw, activated sludge, filamentous fungus, sawdust, neem (*Azadirachta indica*) bark, and gum kondagogu at pH 4 and 5.

Gum kondagogu was discovered to be one of the most cost-effective and efficient biosorbents for hazardous metals in aqueous and simulated effluents. For various GK concentrations, the Langmuir model describes the sorption data with r^2 values of 0.996. Earlier studies have firmly established the ability of a natural carbohydrate biopolymer gum kondagogu, to remove toxic metal ions such as Cd^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} and total Cr present in industrial effluents.

GK was investigated for its morphological, adsorption and metal interaction behaviour with various toxic heavy metals (Cd^{2+}, Cr^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+}). The efficacy of this biopolymer in bioremediation of uranium from aqueous solutions and synthetic nuclear power wastewaters is highlighted in this work. The investigation suggests that GK could be used as an environmentally friendly biosorbent product based on green chemistry principles (Sashidhar et al. 2015).

3 Conclusion

Radionuclides and heavy metals are highly emerging pollutants in the present environment, both of which are very harmful to living species, including humans. Although natural sources contribute to this pollution, manmade activities have significantly increased exposure to heavy metals and radionuclides. This chapter cited technologies used in bioremediation, adapted by various researchers which effectively helped in removing toxic metals and radionuclides. The employment of bioremediation procedures over previously employed traditional ones will undoubtedly be the way ahead in developing a green, sustainable approach for removing toxic metals and radionuclides from the environmental atmospheres and safeguarding living creatures from the harm. Further studies on diverse bacterial, fungal, algal and plant species, their metabolism and pathways will lead to greater insight on better eradication of these heavy metals and radionuclides, while employing bioinformatics tools in studying the molecular mechanisms will aid in storing and utilizing this information aptly along with efficient removal of toxic contaminants.

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Water Reuse Planning, Policy, Monitoring Requirements and Standards/Criteria

Shipra Jha and Garima Tyagi

1 Introduction

Water reuse is the main target for sustainable development across the world and minimises the water scarcity, improvement in the water quality by controlling the various parameters.

- To determine the impact of water bodies mainly responsible for water scarcity, to analyse the effect of human activity on water bodies includes irrigation, for environmental purpose, rural and urban use. These will help in planning and analysing the depth of existing water scarcity problem.
- To identify the origin of water for proper distribution to address the water needs.
- It is important to evaluate the volume of wastewater for better planning to treat and distribute for public use.
- To determine the safe water treatment method for protecting the environment.
- To identify the other requirements includes energy, cost, etc., which is linked with the treatment of different types of waste water.
- Conduct comparative analysis for various water treatment methods with other substitute methods.
- Identify the funding agencies and programme to plan, operate and develop the water reuse strategies.
- For dividing roles, responsibilities and to assure that agreements needs to signed among managers and users for smooth implementation.
- To enact the safe monitoring and control of waste water treatment for environment and public health.

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In many areas of the world, witnessing that water supply is available either through groundwater or canal in absence of other water resources including Perennial River. In case of ground water, water quality is also becoming health concern due to the presence of dissolved solids, high concentration of metals or minerals and necessary to treat before use (Angelakis et al. 2001; Asano and Levine 1996; Arceivala 1999; Armon and Kott 1996; Asano 1998, 2004).

There is need to focus on planning and improving the guidelines for reuse wastewater, especially treatment of urban and industrial waste water includes planning to explore the possibility for waste water management Asano et al. (2007). The waste water planning includes spatial plans, relational database planning management and River Basin management plan. According to integrated plan, there are nine key steps for water reuse. The water reuse has economic benefits in wide range of sectors which includes Industrial, municipal and agricultural sector. Extension of water reuse provides job opportunity in the area of water treatment, maintenance and providing solution for water scarcity (AWPRC Study Group on Health Related Water Microbiology 1991; Ayers and Westcott 1985; Carley 1985; Ayres et al. 1992).

2 Urban Water Management

For maintaining water management, water conservation is important step to preserve water sources includes groundwater, river, lake catchments area, water bodies' treatment and conserving flood water. For floodplains restoration, restricting the area for construction and announcing the green belt areas (Ayuso-Gabella et al. 2011; Gilmer and Hughel 2008; Bastos et al. 2008; Bevilacqua et al. 2014). The practice to be adapted for the construction of building, without disturbing the morphology of natural water sources. For domestic use, large volume of water is used in flushing, washing and gardening. The domestic wastage can be controlled by using water fixtures which control the excessive flow of water. And flush water can be replaced using treated water (BIO 2015; CAC 2003; Bralts and Kesner 1983).

2.1 Efficiency of Water

To overcome water scarcity issue, treated water use can aim for sustainable development to supply water for irrigation and other commercial purposes**.** According to European Union policy published in the year 2007 about water conservation, the saving water must be priority and water quality must be improved in all possible ways. In addition development for water supply, infrastructure can be explored including alternatives, water price policy and development of clean water supply policy (Centre for Science and Environment 2009; Clark et al. 1977; Clarke 1993; CPCB 2000).

Globally, there is major research on water efficacy and to improve water management system. According to President's Water Resources Policy Commission in the year 1950 published methodology for American citizen, which highlight that people can no longer waste water and water resources cannot be handled discriminately. Similarly, there is need to store water for future use. The report of New Mexico office of State Engineers in 1997 emphasised that any activity reduces water from public supply, reduces waste of water, prevents pollution and increases water recycling method (Crook 1985; Dishman et al. 1989; Cullet 2006; Dean and Lund 1981; Dellapenna and Gupta 2009).

Water audit is a tool to address the water conservation by minimising wastage through pipe leakage and improving water quality. It also includes pipe fitting and properties, water management at domestic level, maintenance, sources of water supply and increase profit for municipality (Drewes et al. 2013; EC 2009).

2.2 Rain Water Harvesting System

Rainwater collecting has been a part of ancient societies all over the world for thousands of years, from Israel's Negev desert to Morocco's Anti Atlas, and from Central America's Mayan Civilisations to the remote Pacific island of Fiji (EC 2014; El-Ghamam 1997; Eslamian et al. 2010). The ancient practise of rain water harvesting is gaining significant public policy attention in regions around the world, such as the Gold Coast of Australia, Germany and sub-Saharan Africa, as the intensity of water droughts and shortages projected worldwide increases, as does the escalation of floodwater caused by intense storm events. To assist reduce the negative consequences of increased floods; an appropriate storm water management system must be developed (ENSIC 1995; EPPC 1989; Eslamian and Tarkesh-Isfahani 2010b; Fatta-Kassinos et al. 2016).

Rainwater harvesting has been found to solve flooding-related storm water issues while also providing a resource for water-stressed areas. Rainwater is being used by more than 100 million people throughout the world. RWH, in its widest definition, is a method of collecting and storing roof runoff for non-potable uses in the home, commercial, institutional and industrial sectors, rather than drinking water (Florida Department of Environmental Protection 2002; Food and Agriculture Organization (http://www.fao.org/corp/statistics/en/); Gallup 1973). Harvesting has five controllable components: (1) catchment surface, (2) conveyance system, (3) filter, (4) storage tank(s) and (5) water pump(s). The catchment surface determines how much rain can be collected and, as a result, the size of the storage tank required. Rainwater must be conveyed from the roof to the storage tanks, which necessitates the use of conveyance systems. Each inch of rain produces approximately 623 gallons of water per 1000 square feet of roof surface (Gibney et al. 2014; Gleick 1993; Grabow 2001; Jofre et al. 2016; Kretschmer et al. 2000).

The filter's primary goal is to prevent pollutants (such as airborne particles, tree leaves and animal dung) from being transported straight into the cistern during the initial flush of a rain event. Small above-ground 65-gallon tanks to enormous subterranean tanks capable of storing thousands of gallons of rainwater are all examples of storage tanks. Particles settle in a well-designed storage tank, which inhibits algae and bacterial growth. Pumps are used to convey rainwater to its final destinations, either directly or indirectly, depending on the type of collecting system utilised (Laws et al. 2011; Lazarova 2000, 2001).

Gravity fed, directly pumped, and indirectly pumped rainwater harvesting systems are all capable of transporting rainwater to structures for non-potable usage. To supply the quantity of pressure head needed for toilet flushing, gravity-fed rain water collecting systems require cisterns to be positioned on top of a building's roof. In an indirectly pumped RWH system, storm water is pumped to a second holding tank, usually on the roof, and then gravity transports the water to toilets. Rainwater that is immediately pumped bypasses the holding tank and is delivered to the desired location (Leverenz et al. 2002, 2007; Lindsey et al. 1996; Loos et al. 2013).

The following are some of the benefits of employing a RWH system that is pumped indirectly:

(1) Because it relies on gravity to transfer water to toilets, it provides water for non-potable needs in the event of a pump failure.
(2) Saves energy by allowing the water pump to function at full capacity rather than only when the supply is needed.
(3) Can be linked to a backup water main line in the event that the water level in the header tank drops below a certain level and has to be replenished with potable water (Fig. 1).

Rainwater harvesting lessens the pressure on storm water pipe systems, reducing peak runoff and minimising flooding of existing infrastructure. Municipalities may build smaller and less expensive storm water management systems by expanding the number of localised rainwater collection devices (Maeda et al. 1996; Mantovani et al. 2001; Metcalf and Eddy 2003; Mara et al. 2007).

Decreased storm water discharges to a central municipal system also extends the life cycle of the storm water management system by lowering replacement costs and prolonging the service life of infrastructure assets. Rainwater collection enhances storm water infrastructure by separating roof runoff from traditional centralised storm water conveyances and lowering the cost of storm water infrastructure correction and/or restoration (Méndez et al. 2002; Metcalf and Eddy 1974). Rainwater collecting not only alleviates the difficulties created by storm water runoff, but it also lessens the demand for public water sources, lowering the need for additional reservoirs and wells. Consumers utilise municipally provided potable water for both potable and non-potable applications. Rainwater may be used to augment both potable and non-potable water needs, but it must be adequately filtered and treated before being used because it does not satisfy ordinary drinking water requirements untreated. Rainwater collecting reduces the amount and pace of storm water entering recipient rivers, acting as a storm water quality management buffer (Metcalf and Eddy 2007; Ministry of Housing and Urban Poverty Alleviation-Government of India and United Nations Development Programme [UNDP] 2009; Ministry of Urban Development-GoI 2010).

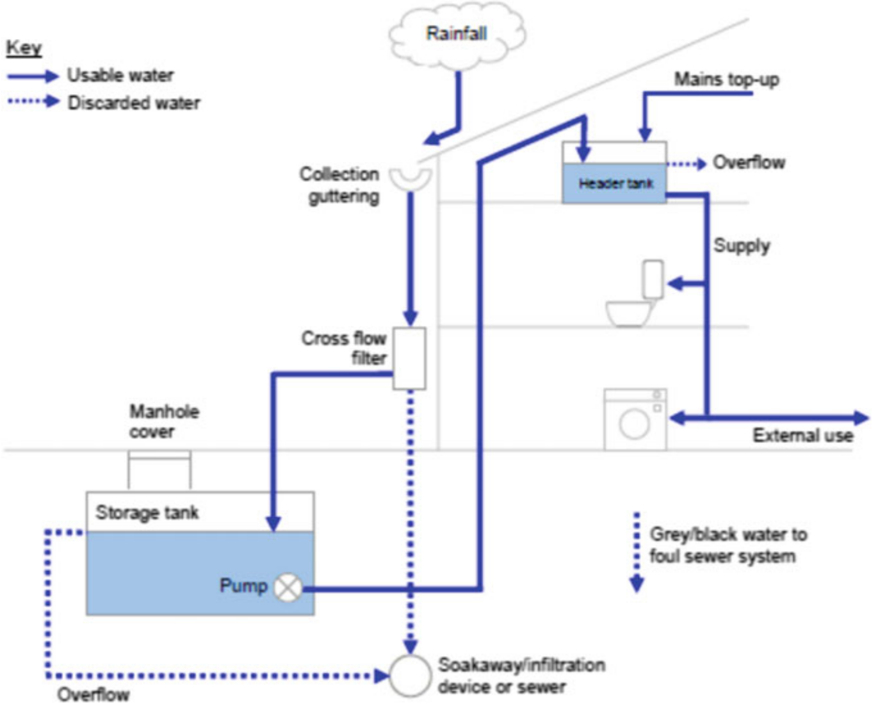


Fig. 1 Indirectly pumped rain water harvesting system (Roebuck 2007). *Source* Roebuck, R. (2007) A Whole Life Costing Approach for Rainwater Harvesting Systems. Doctoral Thesis, University of Bradford, Bradford, UK

3 Risk Assessment in Water Treatment Plant

Globally, many parts of country treated wastewater are used for irrigation purpose and research data confirmed that 20 million ha are irrigated using partially diluted waste water, treated water or raw water (Bastos and Mara 1995; Bralts and Kesner 1983; Bucks et al. 1979). It is well documented that if waste water directly discharged into nature may severely affect the marine life and other ecosystem. Waste water generates in large quantity and to overcome water scarcity, it is becoming trend to use treated water for irradiating agricultural crops. The pressure is increasing on existing waste water treatment method due to increasing risk of infectious disease, including increase in rota virus infection, enterococcus strain enters to top layer of soil, fruits affected during development phase with *Escherichia coli*, natural pathogens gets destroy from soil or alterations in soil properties due to treated water used in irrigation (Cirelli et al. 2007; Pettygrove and Asano 1985). In wastewater treatment industry, due to chemical exposure, chlorine gas leakage may increase the chances of health hazards to workers. Therefore, it becomes mandatory to analyse, develop protocol with advanced personal protective tools to control, training programme and emergency protocol and link the wastewater treatment process with laboratory-based method to implement sustainable environment (Thornton et al. 2013; USEPA 2012).

4 Role of Government

Water reuse may differ from country to country depending upon agriculture, industrial and domestic requirements and can be important alternative to water resources available in the region. Instead of releasing contaminated water in ocean, treating waste water for reuse needs proper water quality assessment along with purification techniques, planning and guidelines (Ministry of Water Resources-GoI 2008, 2009). Guidelines are mainly focussed on various legal aspects developed by Members of WHO and EPA. Legal standards targeted on human health along with responsible for critically analysing the ethics which might use in particular country or geographic region enfold various kinds of use of reused water and the standards made for the reuse purpose do not harm the existing water resources (Table 1). In addition, reuse water may not only the alternate but also scale down the deduction of groundwater and land (Mori 1993; National Research Council 1994; Navarro and Jiménez 2011).

4.1 Unnoticed Water Conservation Initiatives

- It is important to make National water Frame work law for water management instead of dividing under different ministries with different objectives.
- Public participation is completely dead in water management.
- It is essential to make action plan for official department who fails to provide timely water supply.
- The importance of groundwater supply is undervalued as main source of water in many areas.
- Promoting the use and storage of rain water harvesting, wastewater reuse needs to be implemented in action plan.

5 Research and Development

Initiation of water reuse technologies projects may bring progressive development in this field. Various National & international organization have potential to plan and structure the water reuse sector (Negreanu et al. 2012; NRC 1996). The planning of waste water management may open up the doors for contributors for implementing the projects along with the support of financial donors or agencies. Research and

Table 1 Water management technological policies for transforming water efficiency and conservation

Country	Policy	Proposed year	Objectives
Ministry of Environment, Spain	New water policy Framework	2006	Environmental, Legislative, Economics, Technical and socio-political
South Africa	National Water law & Policy Framework	2000	Equal access to water, equality in water distribution, Protecting ecosystem, improvement in management strategy
California, USA	Policy principles on water conservation and water—use efficiency	2005	By introducing specific measures (pricing strategies, commercial retrofit) reduce state-wide per capita water
Orillia, Canada	Water conversation and Efficiency Plan	2014	To reduce total water produced at water filtration
Singapore	Four Tap Programmes	2001	To meet water demand, increase recycling of water and desalinated water supply
Texas, USA	Water conservation pricing	2004	Environmental protection, water shortage problem, water system improvement
Denmark	Sustainable Groundwater Management	2014	Develop innovative method and tools for ground water mapping on large scale
Israel	Reduction of non-revenue water	1999	Stipulates all water resources are public property. Waste water recycling and for reusing
USA	Water Sense	2006	Promote the value of water efficiency and label products for information resources
New York, USA	Toilet Replacement Program	1994	To reduce waste water streaming into city sewars, connect water metre device for water usage

(continued)

Table 1 (continued)

Country	Policy	Proposed year	Objectives
Mumbai, India	Reuse of treated waste water at Naval Civilian colony	2005	Treating wastewater for local reuse in non- portal water requirements (irrigating garden, park and for plantation

Development facility can be used for preparing protocols to be followed for the execution projects (Page et al. 2010).

After the government approval for successful water reuse techniques and before implementing legally in public, it is important to make people aware through Electronic media, Mass media, workshops and conferences about need for water reuse, importance of water quality and issues related with water (Petterson et al. 2001; Prosser and Sibley 2015).

6 Role of Industries in Water Reuse Planning

Water reuse is the practice of reclaiming water from various sources and treating and reusing it for beneficial applications such as farming, potable water supply, underground refilling, industrial activities and ecological rehabilitation. Water reuse can supplement existing water sources and improve water stability, conservation and resiliency (Regnery et al. 2013; Research and Markets 2008; Roebuck 2007). Water reuse can be classified as either intentional or uncontrolled. Unintentional water reuse refers to instances in which a type of water is largely made up of previously utilised water. Unexpected water reuse is prevalent where municipalities obtain their water supply from rivers that obtain treated effluents from settlements upstream (Vigneswaran et al. 1996).

In most process industries, water is used for a variety of purposes. Environmental rules governing the discharge of effluents are becoming stricter for industrial processes and systems that use water. Fresh water is becoming increasingly scarce, making it a valuable commodity in more and more countries and a critical commodity in some areas of the world (Sadik and Shawki 1994; Salgot et al. 2006). The rate at which things are changing has heightened the demand for better water management and wastewater reduction. Water conservation measures can successfully reduce total fresh water demand in water-using processes, reducing the quantity of wastewater created as a result. This can lower the cost of obtaining fresh water as well as the expense of treating effluent streams. This paper gives a quick review of modern approaches and methodologies, which are divided into many categories: Water footprints and life-cycle assessment (LCA); water/wastewater minimisation—incorporating Water Pinch, Mathematical Programming methods and integrated water-energy minimisation (Shah et al. 2009; Shuval et al. 1997; Thanner et al. 2016).

Water systems developed with the objective of usefully recycling a recycled water supply are referred to as planned water reuse (Chart 1). Usually, organisations may aim to optimise their overall water consumption by recycling water as much as possible inside the group before reintroducing it to the ecosystem. Almost every industrial plant has the opportunity to reuse/minimise wastewater (Toze et al. 2010; United Nations 2006). The results of reusing the secondary treated wastewater as an additive in the cooling tower show that a high conductivity water source can provide excellent corrosion protection. It is necessary to take into account the variability of some components of wastewater, especially phosphates, calcium. The reuse of recycled wastewater from a demineralise can take many forms—from wastewater reuse regenerated in subsequent regeneration to combine cation and anion regenerated waste to provide neutral wastewater as a component of the cooling tower (Pettygrove and Asano 1985; Thanner et al. 2016; Thornton et al. 2013).

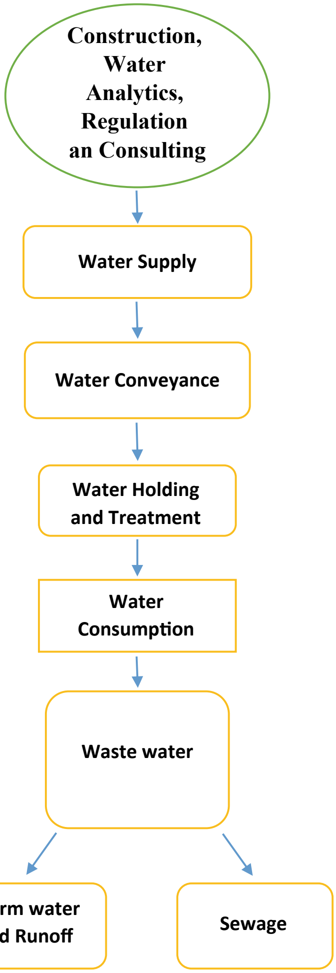
Municipal wastewater, industrial purposes and cooling water, precipitation, agricultural runoff and return flows, and generated water from organic extractive operations are all examples of groundwater with ability for reuse. Such water sources have been sufficiently processed to fulfil "suitable requirements" for a certain next use (USEPA 1992; Ginneken and Oron 2000).

Reusing water in industry has the potential to significantly reduce water supply and wastewater treatment costs for businesses while also reducing demand on water resources. Wastewater may be utilised inside a company or across many companies through smart manufacturing. The wastewater may be reused directly or processed before reuse, depending on the kind and quality of the wastewater (i.e. recycled).Industrial water usage accounts for 22% of total world water use. In 2009, industrial water usage in Europe and North America accounted for half of overall water use, but industrial water use in developing countries varied from 4 to 12% of total water consumption. As emerging countries become more industrialised, industrial water demand may possibly grow by a factor of five, putting a significant strain on water supplies (Toze et al. 2010; United Nations 2006).

The reuse of wastewater is one way to reduce water usage in industry. Reducing industrial water use can reduce water withdrawals from local water sources, improving water availability and community relations, increasing productivity per water input, lowering waste water discharges and their pollutant load, and potentially lowering thermal energy consumption and processing costs. In the industrial sector, it is possible to repurpose wastewater, both within the business as well as between businesses. This can lead both to decreasing water bills as well as reducing wastewater treatment costs. Water can either be recycled (reused) directly or treated and reused (recycled) depending on the contaminants it contains and its future use.

When used properly, wastewater can be reused directly by businesses. Process water, which comes from industries such as cooling and heating, contains few

Chart 1 Industrial water cycle



contaminants after use. For example: irrigation, washing, pH addition and fire protection can both be achieved by reusing rainwater and process water. Water reuse can also be done directly between businesses. This process is known as "industrial symbiosis" and involves exchanging waste products to benefit both parties. During industrial symbiosis, all members benefit by either reducing the costs of wastewater treatment or decreasing the inputs required in their production process (USEPA 2003, 2004; Valentina and Akica 2005).

The three common ways of industrial symbiosis are by-product exchange, utility sharing and ancillary services sharing.

- In an industrial symbiosis, wastewater is directly reused in the following ways: Water exchange between businesses and subsequent reutilisation (irrigation, washing pH adjustment, firefighting, etc.).

- Anaerobic digestion can be used to generate electricity from large volumes of organic waste or wastewater.
- Reuse wastewater to grow plants or animals.
- Short rotation plantations.

6.1 Recycle

If wastewater isn't acceptable for direct reuse, decentralised wastewater treatment systems can be used to decrease pollutants to a safe level for reuse. This can be done within a company for internal usage or between companies. Wastewater treatment and reuse between businesses can reduce treatment costs for all firms, making reuse more cost-effective (USEPA 1981, 2004).

- The following are some options for decentralised wastewater treatment systems: Non-planted filters
- Waste stabilisation ponds.
- Aerated ponds.
- Constructed wetlands (see free-surface, horizontal, vertical and hybrid constructed wetlands).
- High-tech solutions include activated sludge, membrane bioreactors, sophisticated oxidation processes, ozonation, activated carbon, rotating biological contactors and anammox.

The precise treatment option chosen is determined on the final quality desired. If space is sufficient and water loss through humidity is not a concern, a natural treatment such as a free-surface built wetland where some nutrients remain in the water could be appropriate.

Some sophisticated solutions, such as membrane filtration and activated carbon, may potentially provide treated wastewater of higher quality than freshwater, allowing the treated wastewater to be reused as process water in applications requiring high water quality.

6.2 Optimisation

Water quality should be examined for chemical composition, including pH, nutrient concentrations, pathogens and other factors, to guarantee that the (pre-treated) wastewater or grey water is suitable for reuse (USEPA 2003, 2004).

6.3 Cost

- By reusing process water, businesses may save money on water bills and wastewater treatment.
- By lowering input costs or wastewater treatment expenses, industrial symbiosis benefits all enterprises engaged.
- Implementing reuse or treat-and-reuse strategies necessitates a significant upfront expenditure, which can be expensive (depending on modifications required and technology).

6.4 Applicability

Almost any firm may implement wastewater reuse strategies. While direct reuse techniques are simple to execute, the expense of installing wastewater treatment systems may prevent businesses from reusing wastewater. The possibility for wastewater reuse between enterprises is determined by factors such as the distance between them (transport costs) and the volume and quality of wastewater produced. If wastewater treatment is required, the involvement of numerous enterprises might greatly lower treatment costs, allowing wastewater to be reused (Virginia Cooperative Extension Materials [http://pubs.ext.vt.edu/452/452-014/452-014.html]).

Economic factors generally influence industrial water reuse production activity and manufacturing levels. Treatment instability is frequently reflected in effluent quality uncertainty. Water reuse management, such as safety issues during periods of noncompliance with water quality regulations, is significantly influenced by quality uncertainty. Incorporating stochastic programming into the water reuse model is required to address uncertainty concerns. GIS might also be utilised to improve a model's capabilities in data processing and other water resource management applications, in addition to visualisation (Sadik and Shawki 1994).

Manufacturing units started reusing significantly from 1990s and for the similar reason reuse of water adopted in the city area, which includes drought area, highly populated area and dry spell region for environment compliance and water conservation. Many countries have installed water distribution lines to meet the demand including for water processing. The industries like metal working facilities; petroleum refineries are benefiting from recycled water (USEPA 1992; Dean and Lund 1981).

As a result, significant industrial operations in California, Arizona, Texas, Florida and Nevada use recovered water for cooling water and process/boiler-feed requirements. Because of their huge water requirements for cooling, ash sluicing, rad-waste dilution and flue gas scrubber requirements, utility power plants are suitable sites for reuse. Petroleum refineries, chemical plants and metal working facilities are just a few of the industrial facilities that use recovered water not just for cooling but also for process demands.

7 Environmental Protection Agency

In 2012, the EPA issued the Guidelines for Water Reuse as a general reference for water reuse techniques, and in 2017, the Potable Reuse Compendium was produced to explain current practises in the fast emerging area of direct potable reuse. The EPA promotes water reuse as part of integrated water management and encourages states to design their own water reuse policies based on the Clean Water Act and the Safe Drinking Water Act. To that end, the EPA will continue to participate in active engagement and research with a variety of partners and stakeholders to ensure that water reuse is safe for people. Many towns have started or are starting to build centralised water reuse programmes, involving storm water runoff and wastewater recycling. They're also becoming more interesting in decentralised systems that collect and treat onsite-available waters for non-potable purposes, which including grey water and rainfall.

Civil societies (especially those in arid regions) require federal guidelines on different forms of water reuse and fit-for-purpose treatment procedures as the demand for alternate water sources grows. The EPA's research, which includes collaborations with outside partners, is providing recommendations on new and current water reuse techniques, with the ultimate objective of increasing the quantity of high-quality water while minimising other major ecological consequences (Wellington et al. 2013; WHO 2001).

Included in the research is:

- Considering alternate groundwater for utilisation.
- Increasing knowledge of the possible health concerns, economic efficiencies and life-cycle consequences of different water reuse techniques and treating combinations.
- For onsite non-potable water systems, developing treatment objectives and monitoring surrogates.

There is just so much groundwater that can be consumed. To keep up with rapid urbanisation, increased resource rivalry, and climate concerns, drinking water suppliers must implement best industry practises for water usage as well as innovative methods that account for variations in water supply. It is critical that we lower the amount of water we use and better manage our water consumption, from the source to the amount of wastewater we must treat. Water efficiency best practises can be pursued by drinking water system owners and operators (Westrell et al. 2004).

Water providers can also take measures to guarantee enough source capacity and system preparedness for changeable water quality. Navigate through the tabs to uncover resources on storm water management, rainfall patterns and water maps data.

Table 2 Design to implement efficiency and water conversation

Provisional/temporary (6 months)	Medium duration goal	Permanent (12–24 months)	Projects in India
Technical committee	Set up Bureau of water efficiency	Set criteria for water conservation	Restoration plan for Lake Ngaroto, Waipa district, New Zealand
Define objectives	Protocols and testing facilities	Set metre scale to measure efficiency and water storage	Reuse of treated waste, Mumbai, India
Set up goal	Set up standards and rating	Set up monitoring and evaluation programme	Urban lake management for Jakkur lake, Bangalore
Conduct training	Water conservation Act	Adopt method to reduce water need of the city	Rain water harvesting system, Goa university, India
Implement existing rule	Conducting workshops and training	Keep updating database after each water audit	Decentralised waste water treatment, Delhi, India
Promote awareness	Awareness campaign	Develop urban drainage system	Reuse of treated waste water at Aravind Eye hospital, Puducherry, India

8 Legal and Institutional Provision

For waste water management, there are many guidelines, protocol for the improvement for water quality through enhancing water supply and water auditing. Various government organisation, authorities and ministries in urban region are responsible to involve in water conservation (Table 2). Different organisations includes Central public department, Central water commission and Central ground water Broad provide water monitoring and technical support (Westrell et al. 2004; World Bank 1995; World Health Organization [WHO] 1989).

9 Water Reuse Action Plan

WRAP was created in conjunction with water industry stakeholders. The policy's actions are designed to accelerate reuse and address local and national hurdles on a variety of themes, including technical, institutional and financial constraints. Over 100 action leaders and partners are collaborating to promote reuse across the country (Fig. 2).

Many regions are finding it difficult to satisfy their long-term water demands as the climate changes. Reusing wastewaters and rainwater for agronomic, non-potable or even potable purposes can provide a more stable source of water than

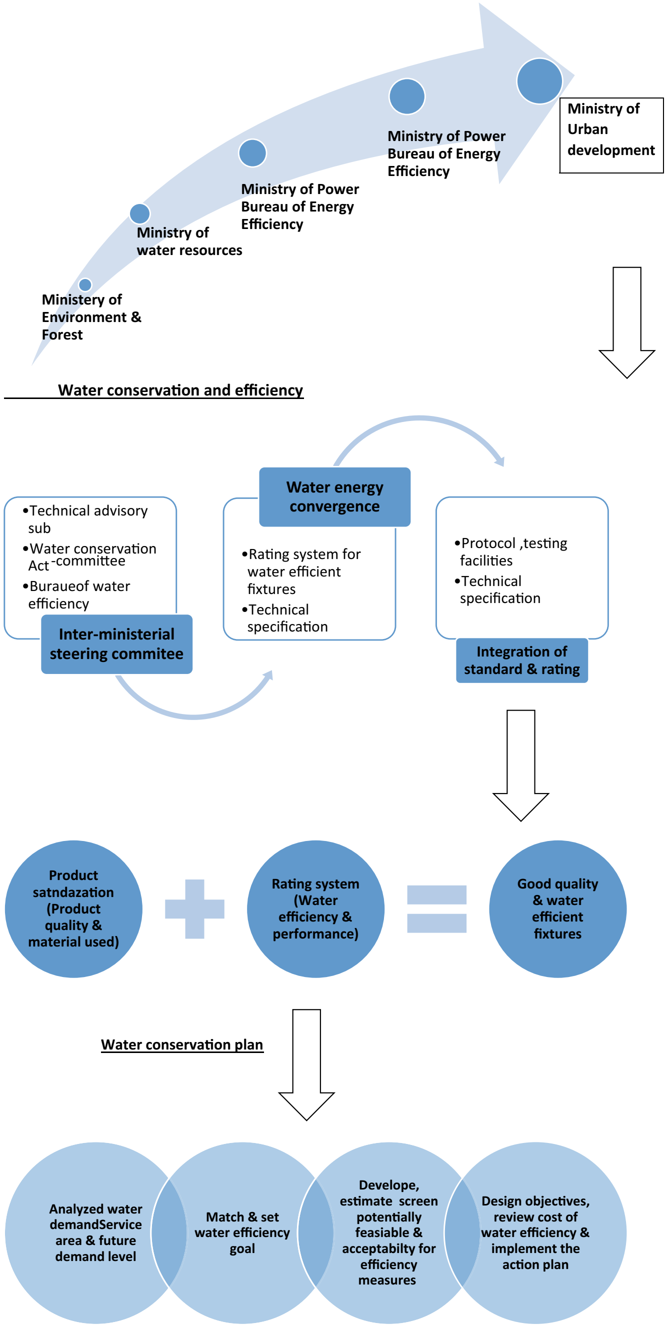


Fig. 2 Policy roadmap for urban water efficiency and conservation

conventional raw water sources. The ability to include water reuse into a majority's freshwater portfolios can help to mitigate the effects of climate change. Finally, the WRAP collaboration aims to make water reuse more accessible, simple to execute and considerate of climate and environmental justice concerns. The combined efforts of the organisations and entities involved in WRAP initiatives can help to expand the corpus of reusable knowledge and expertise for the benefit of everybody (Prosser and Sibley 2015; [92]; Xie et al. 1993).

On February 27, 2020, the United States Environmental Protection Agency (EPA) and its government partners issued the National Water Reuse Action Plan: Collaborative Implementation. Through integrated and collaborative water resource planning, the plan draws on the knowledge of both the corporate and governmental sectors to design ambitious initiatives that accelerate the adoption of water recycling. To give input on the strategy, the Water Reuse Association led a coalition of partner water groups and our members. Investing in water reuse creates contemporary, healthy and economically viable civilisations. From coast to coast, treated wastewater is used in:

In Idaho, where recycled water is used on crops, 2000 tonnes of nitrogen and 500 tonnes of phosphorus are kept out of rivers and streams.

The New England Patriots' stadium was built in Foxborough, Massachusetts, thanks to an onsite, decentralised water recycling technology.

In Orange County, California, recycled water is purified to supply the drinking water demands of one-third of the population.

In Nevada, the Tahoe-Reno Industrial Centre is attracting 100 enterprises that will bring nearly 20,000 new employments to the high desert thanks to the availability of reclaimed water.

10 Conclusion

For reducing misuse of water, to overcome negative effect on human health, develop equality in water distribution, enhancing water quality, it is essential to have water policy to address water reuse demands for national and global development for decentralised way. The water reuse policy should be made which can be suitable under various climatic and environmental conditions.

According to the area specific, different countries need modified water policy and to fulfil the requirement, broad guidelines need to be made which can be adjustable in various locations and situations including industrial sites, agricultural field and near high population density. The risk assessment can be analysed by using the Quantitative microbial risk assessment (QMRA) method suggested by WHO, using post-treatment health protection control process including peeling, product washing and using disinfectants. Reuse and recycling of water resources is important and fall under the resources management tool maintains sustainable agricultural development.

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Wastewater Treatment and Reuse in Future Cities

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1 Introduction

Rapidly depleting as well as elevating the rate of freshwater demand, however, wastewater reuse seems to be one of the most essential necessities of the present scenario. Agriculture consumes 92% of all water consumed in the world. Around 75% of freshwater is utilized for irrigation that comes from river systems and groundwater sources (Clemmens et al. 2008; Hoekstra and Mekonnen 2012). The statistics are very concerning for the countries that are experiencing a water shortages. According to investigators, 45% of the world population lives in water-stressed reservoirs, indicating a water shortages for irrigation. As a result, wastewater reuse in agriculture activity is an excellent source for replacing freshwater usage in agricultural activity (Contreras et al. 2017). Treated wastewater is commonly used for non-drinking purposes such as agriculture, firefighting, land irrigation, golf course irrigation, groundwater recharge, toilet flushing, vehicle washing, cooling purpose in thermal power plants, and building construction. Treated wastewater irrigated agriculture benefits agricultural yield as well as the livelihood opportunities of millions of small-scale farmer's worldwide. Globally, the reuse of this water for agriculture activities varies greatly, varying from 1.5 to 6.6%. More than 10% of the worldwide people consumes agricultural products grown with wastewater irrigation (Ungureanu et al. 2020).

Treated wastewater reuse has grown drastically, with volumes increasing by 10–29% annually in the United States, Europe, China, as well as nearly 42% in Australia

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(Aziz and Farissi 2014). China is the major country in Southeast Asia for treated wastewater reuse, with an approximate 1.3 million hectares, followed by India, Vietnam, and Pakistan. At the moment, it is approximated that only 38.3% of India's urban wastewater is treated (Singh et al. 2019). Israel is the biggest consumer of treated wastewater for agricultural land irrigation, using 90% treated wastewater. Untreated wastewater is used as an irrigation source in many low-income countries in Africa, Latin America, and Asia. But at the other side, middle-income nations, like Tunisia, Saudi Arabia, and Jordan utilize treated wastewater for the purpose of agriculture irrigation (Balkhair and Ashraf 2016; Mohsin et al. 2021). Household water as well as treated wastewater possess a wide variety of nutrients like nitrogen (N_2), phosphorus (P), sulfur (S), and potassium (K); however, the majority of the N_2 and P available in wastewater could be easily acquired by plants, hence, it is extensively utilized for irrigation (Poustie et al. 2020; Sengupta et al. 2015). The high nutrient availability in treated wastewater decrease the usage of fertilizers, enhances crop yields, improves soil fertility, and it may lower crop production costs (Jeong et al. 2016).

Numerous health concerns were raised when wastewater was reused for agricultural irrigation. Irrigation by industrial effluents, either directly or even in combination with domestic water, posed a greater risk. Heavy metal as well as pathogen pollutants raises health risks because of heavy metals were also non-biodegradable and also have a long biological half-life. It comprises a range of toxic elements, including copper (Cu), chromium (Cr), manganese (Mn), lead (Pb), nickel (Ni), iron (Fe), and zinc (Zn) (Mahfooz et al. 2020). These toxic metals start accumulating in topsoil and are absorbed by plant roots; they enter the animal and human bodies via ingestion of leafy green vegetables and acute exposure of contaminated soils. As a result, evaluating the health dangers of these wastewater irrigation seems to be critical, particularly in adults (Mehmood et al. 2019). Various wastewater treatment methods should be used before releasing wastewater into rivers, agricultural lands, and soils. As a result, this chapter also proposed several wastewater treatment methods, which were partially tested at the laboratory scale by various researchers.

For more than a decade, wastewater reuse has been one of the most pressing global health issues, with consequences for both environment and public health. In 1973, the WHO issued guidance to maintain good health by making it easier to use wastewater as well as excreta in agriculture and aquaculture (Organization 1973). In the lack of epidemiological studies with such a small risk strategy, the initial guidance was prepared later in 2005 (Carr 2005). Nonetheless, researchers evaluated the epidemiological proof and health problems associated with wastewater reuse for agricultural irrigation. Harmful health risks are associated with effluent or gray water reuse due to microbial hazards (i.e., contagious pathogens) and chemical or pharmaceutical exposure. According to studies, wastewater exposure can lead infectious diseases that are associated to anemia as well as intoxicated cognitive and physical development (Amoah et al. 2018). The consumption of wastewater is estimated to rise in the next decades due to growing population and a rising disparity in the consumption and supply of water. In developed countries, the usage of treated wastewater is governed by strict regulations. Moreover, in developing countries, the direct utilization of direct wastewater is obvious in the absence of reasonable regulatory policies, resulting in severe public health and environmental concerns. Because of such issues, we briefly describe the WWT methods, reuse possibilities in various cities, and future challenges associated with it in this chapter.

2 Mineralization of Emerging Contaminants (ECs) in Wastewater

2.1 Classification and Major Sources of ECs

Table 1 represents the basic classification of ECs as well as their key sources. Approximately, 70% of the ECs identified in environment samples are personal care products (PCPs), and pharmaceutically active compounds (PhACs), with the other 30% being agricultural and industrial chemicals (Das et al. 2017). For example, more than 200 PhACs have been reported worldwide in rivers streams with the maximum reported concentrations of 0.006 g/L for antibiotic ciprofloxacin. Tamoxifen, an anti-cancer medicine, was also found in the river water with concentrations ranging from 25 to 38 ng/L (Das et al. 2017; Shah Maulin 2020). According to investigators, the concentrations of hormones, antibiotics, antidepressants, analgesic chemicals, lipid regulators, and chemotherapeutic medications in the environment range from 0.00004 to 0.063 g/L. Besides this, high-usage compounds such as preservatives and sunscreen agents are commonly detected at concentrations more than 0.1 mg/L. For example, the sun screen ingredient, 4-benzophenone was identified in environment samples at concentrations ranging from 3.597 to 5.790 mg/L. The pollutants are classified as dangerous to aquatic species, if their EC50 values are 0.01–0.1 g/L, toxic if they are 0.0.001–0.01 g/L, and extremely toxic if they are 0.001 g/L (Cleuvers, 2003).

ECs can reach the water and groundwater environments from a variety of sources (Table 1) that can be divided into 5 subgroups: industrial, household wastewater, animal farming, hospital, and agricultural activities. Among other things, household effluent is among the biggest sources of PCPs and PhACs into the environment. Similarly, PCPs such as toothpaste, sunscreen lotions, shampoos, and so on are released into the environment by human washing operations (Yang et al. 2017). The discharges of PCP, pharmaceutical, biocides, and other chemical production facilities are important producers of ECs to the environments. Hospital discharges also incorporate antibiotic resistant bacteria/genes, drug conjugates, radioactive elements, pharmaceutical metabolites, and other ECs. Other significant sources of ECs are discharge from dairy farming and agricultural operations, notably in the forms of steroid hormones and insecticides used to boost yield. The amount of ECs emitted by these sources is determined by the pesticides/biocides employed, the properties of the surface water bodies, and the meteorological circumstances. Other sources

Table 1 General classification as well as major sources of ECs

Class	Important sub-class	Representative ECs	Major sources
PhACs	Antibiotics, Nonsteroidal anti-inflammatory drugs (NSAID), Antidepressant, Hormones, Lipid regulators, Beta-blockers and Anticonvulsants	Diazepam, Ciprofloxacin, Diclofenac, Testosterone, Clofibric acid, Metoprolol and Carbamazepine	Pharmaceutical industry pollutants, Domestic effluent, Hospital wastewater, aqua culture and wastewater from livestock farms
Perfluorinated alkylated substances (PFASs)	Perfluorocarboxylic acids and Perfluorosulfonic acids	Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonate (PFOS)	Surface water, Wastewater, Ground water, and Sediments
Active pesticides	Organochlorines, Organophosphate, Pyrethroids, Triazine derivative and Phenylpyrazole	Parathion, Imazalil, Paraquat, Imidacloprid, Thiacloprid, and carbofuran	Agricultural waste runoff, Surface water and Aquaculture effluent
PCPs	Fragrances, Insect repellants and Sunscreen agents	Galaxolide, N′-diethyltoluamide (DEET) and 4-benzophenone, N,	Surface water, Landfill leachate and WWTP waste
Artificial Sweeteners	Saccharin, Aspartame, and Sucralose	–	Surface water, Landfill leachate and WWTP effluent
Industrial chemicals	Plasticizers and Fire retardants	Dimethyl adipate (DMAD) and Tris (1-chloro-2-propyl) phosphate (TCPP)	Domestic effluent and Industrial wastewater
Endocrine disrupting chemicals (EDCs)	Bisphenol, Xenohormone, and Phthalates	Bisphenol A (BPA), Xenoestrogen, and Dioctyl phthalate (DOP)	Ground water, Portable water, Secondary sludge, Sediments, and Soil
Surfactants	Ionic surfactants and nonionic surfactants	Tweens (Polysorbates) and Sodium lauryl sulfate (SLS)	Domestic wastewater and Industrial effluents
Biocides	Herbicide, Fungicide, and Molluscicide	Butachlor, Epoxiconazole, and Metaldehyde	Aquaculture wastewater, Agricultural effluents, and Surface water
Dyes	Acidic dyes, azoic dyes, basic dyes, nitro dyes, vat dyes, and sulfur dyes	Methylene blue, Methylene orange, Orange red, Rhodamine B, Direct orange 26, and Basic brown	Domestic wastewater and Industrial effluents
Regulated compounds (RCs)	Pesticides and Poly aromatic hydrocarbons (PAHs)	Chlorpyrifos and Phenanthrene	Surface water, Agricultural effluents, Sediments, and Soils

of ECs in the surroundings include irrigations with reclaimed water, waste disposal facility leaks, landfills leaching, marine discharge, and so on (Yang et al. 2017).

2.2 Primary WWT Technologies

The sedimentation, screening, and floatation are used in the primary treatment. However, certain suspended, colloidal and fine materials cannot be adequately removed using the techniques described above. Mechanical flocculation and chemical coagulation are used in some circumstances (Atul et al. 2012; Shah Maulin 2021a, 2021b). In chemical coagulation, the adding of various chemicals coagulants such as $FeSO_4$, alum, polyelectrolyte, lime [$Ca(OH)_2$], and ferric chloride ($FeCl_3$) are employed for flash mixing. This approach was carried out in a clariflocculator or a settling, and a flocculation tank. Coagulants like aluminum iron as well as salts are extensively employed in wastewater treatment. To minimize color, suspended solids, BOD, and COD, chemical treatments are utilized. The chemical coagulation technique effectively decolorizes insoluble colors but is ineffective in reducing soluble dyes (Thamaraiselvan and Noel 2015).

2.3 Secondary WWT Technologies

The dissolved, color, and colloidal organic matter occurring in dyes wastewater are stabilized during secondary WWT (Xue et al. 2019). The bacteria, fungi, yeast, algae, and other microorganism are used in this method. This process might be contributed to aerobic or anaerobic in nature. The algae, bacteria, fungi, and various type of microbes use different compounds especially organic compounds for food and undergo the following changes: (1) Coagulation and flocculation of colloidal compounds, (2) Oxidation dissolved organic compounds (DOC) to CO_2, and (3) mineralization of nitrogenous organic materials to NH_3 that are transformed to nitrite (NO_2^-) and then nitrate (NO_3^-). Anaerobic treatment is usually utilized for garbage decomposition. The functionality of this process varies with pH, temperature, waste loading, absence of oxygen, and concentration of hazardous materials. Aerobic treatment of various type of dyes has been shown to be ineffective in the majority of instances, although it is currently utilized as a standard treatment strategy (Thamaraiselvan and Noel 2015; Ghaly et al. 2014).

2.4 Tertiary WWT Technologies

The dyes industrial effluent contains a variety of hazardous dyes. To eliminate specific contaminants, they require sophisticated treatment methods or tertiary treatment.

Tertiary treatment is often employed to decompose the organic color components through adsorption and dissolved solids by membrane filtering processes. Many pollutants can be eliminated by treating wastewater with ozone (O_3) or the other oxidizing agent (Atul et al. 2012).

3 Membrane Technologies for WWT

Membrane filtration is now a cutting-edge WWT method. Water is permitted to move by a porous type of membrane in this procedure. If the solutes are higher than that of the porous structure of the membrane, it would be confined and the remaining solution will be flowed from the membrane. When the process of filtration takes place, solutes retained on the surface of filter are removed continually. The membrane filtration procedure are divided due to the diameter of the pores. Pressure-driven membranes treatments are categories into four: (1) reverse osmosis (RO), (2) ultrafiltration (UF), (3) microfiltration (MF), and (4) nanofiltration (NF) (Valero et al. 2015). NF membranes have pore size ranging from 0.1 to 10 mm during minimum applied pressure. UF membranes have pore size ranging from 2 to 100 nm at the maximum applied pressure, as well as a poor water permeability. MF and UF are using the same sieve process that is an innovative and long-lasting mechanism. The kind of membrane filter employed for the purpose of separations are resolute by various parameters like the properties of dyes, the various dyeing method, and the chemicals nature of toxic pollutants (Chekli et al. 2012).

3.1 Ultrafiltration

UF uses less pressure as compared to the NF and RO, making it extremely effective. Dye removal from the effluents is achieved through utilizing a polyether sulfone (PES) membranes. PES has porous membranes with diameters of 1 and 10 kDa that are used to remove dyes. The 1 kDa PES membrane removes dyes with an efficiency of 80–100%, but the 10 kDa PES membranes are inefficient. The UF technology is better suited for usage as a pre-treatment step (Tomizawa et al. 2016).

3.2 Nanofiltration

The permeability of NF is greater, and the trans-membrane pressure is lower. NF uses lower amount of energy as compared to the MF. That's why NF is a promising technology for treating dye industrial wastewater. Many studies have concentrated on the mineralization of synthetic dyes from dye industry effluent throughout the last decade (Deghles and Kurt 2016).

3.3 Microfiltration

This method is utilized as a pre-treatment for NF or RO. To remove dye through textile wastewater, MF with 0.1–1 *u*m porous membrane has been used (Valero et al. 2015).

3.4 Reverse Osmosis

The RO method is utilized to remove dyes as well as decolorize various colors from dye effluent. Decolorization and chemical complex removal from dye house effluent may be accomplished in a single reverse osmosis phase. Most ionic chemicals are retained by the RO membrane at the rate of 90%. Chemical substances, hydrolyzed dyes, and minimum salts can all be removed via reverse osmosis (Valero et al. 2015).

3.5 Membrane Modules and Selection

The large membrane areas have been necessary for big scale filtration systems, such as industrial applications of membranes. Such areas are financially loaded at what are regarded as modules. Membranes modules are classified into four types: plate and frame modules, spiral wrapped modules, tubular modules, and hollow fiber modules (Merkel et al. 2012). These are briefly discussed below.

3.5.1 Plate-and-Frame Module

It is one of the very first modules generated. It is made up of feed spacers, membrane, and product spacers that are imbedded together through a metallic frame. These spacers keep the membrane from adhering together to provide pathways for stream and material flows. It should be noted, however, that this module is only used for specific purposes, such as the treatment of wastewaters with significant concentrations of suspended particles, such as landfill leachate (Gu et al. 2011).

3.5.2 Spiral Wound Module

This membrane modules are really the most often used in RO and NF applications. The arrangement provides a high compressibility, resulting in a large membrane surface. A multitude of permeate spacers, membranes, and feed spacers are looped across a perforated central collecting tube in this configuration. These are then transferred to a tubular pressure vessel. Water enters the spiral wound module perpendicular to the membrane. Permeate runs perpendicular to the membrane surface, via the permeates spacer, and eventually into the central collecting tube. This component has the benefit of being easily replaceable and scalable for large industries (Lee et al. 2011).

3.5.3 Tubular Module

This module is made up of an outer shell, which is attributed to as the housing. This shell has a tubular shape. A fiberglass pipe is incorporated inside this tubular shell, along with a semi-permeable membrane. The liquid to be considered is pumped inside the tube with pressure. The filtrate from the membranes enters the shell via the pipe and is collected by the influent outflow. Tubular membrane are well suited to the treatment of fluid with higher filler contents (Obotey Ezugbe and Rathilal 2020).

3.5.4 Hollow Fiber Module

In a pressure vessel, this module form stores a bundles of hollow tubes, regardless closed or open end. Hollow tubes are made up of a permeable non-specific substrate surface with a thickness of around 200 m as well as an active layer with a thickness of more than 40 nm. This layer is the actual membrane, although it requires support to sustain hydrostatic pressure. Depending on their application, hollow tubes modules are either outside feed or inside feed. The outside feed category is selected for highly pressurized uses (up to 70 bar), whereas the inside feed category is selected for low and medium pressure uses (Obotey Ezugbe and Rathilal 2020).

4 Sustainable Treatment Types

After the specifications for a sustainable WWT system has been outlined, there are numerous possibilities from which to select the most suitable technology for a certain region. This chapter will also go through sustainable WWT techniques such as (i) lagoons/wetlands, (ii) USAB (anaerobic digesters), and (iii) SAT technologies (Jhansi and Mishra 2013).

4.1 Lagoons/Wetlands

The natural factors work together to clean wastewater in wetlands treatment, resulting in WWT. A number of small ponds serve as stabilizing lagoons, with water duckweed acting as heavy metal accumulators. A variety of plankton, bacteria, and algae work together to further filter the water. This technology in underdeveloped nations has a competitive advantage over traditional, automated treatment processes because it provides a higher degree of ecological balance and economic sustainability. The system enables complete resource recovery. If substantial, non-arable territory is available, lagoons systems could be regarded a beneficial technique. However, in most large cities, the criterion for land available is not reached. Flat property is in great demand due to rising urban growth and agricultural reasons. The utilization of wetlands must take into account the climate. The system has drawbacks that may render it unsustainable in some areas. Clogged up with sprinkler and drip irrigation facilities, especially using oxidation ponds effluent, may be one of the mechanical issues. Plugging is caused by biological overgrowth in the emitter orifice, sprinkler head, as well as high quantities of microalgae and suspended particles (Jhansi and Mishra 2013).

4.2 Anaerobic Digestion

When there is limited availability of land, alternative potential treatment is anaerobic digestion. In the oxygen free environment, anaerobic bacteria breakdown organic molecules, producing methane and CO_2. Methane may be recycled and utilized as an alternate source of energy. Other advantages include a 50–80% decrease in total bio-solids quantity and a biologically stable overall waste sludge that can function as rich manure for agriculture. Thus far, anaerobic digestion treatment was used in Colombia, India, and Brazil, either to replace more expensive ASM or to reduce the necessary pond area. Several Brazilian cities have expressed attention in using anaerobic digestion treatment like a decentralized treatment process for underdeveloped, sub-urban communities. The benefit of anaerobic digestion treatment is that it could be used on both a local and big scale. As a result, it is a viable alternative for a developing community (Jhansi and Mishra 2013).

4.3 Soil Aquifer Treatment (SAT)

SAT is a geo-purification method in which moderately cleansed sewage wastewater is preciously recharged and then collected for future reuse. The wastewater is purified further before being blended with fresh groundwater via recharging into unsaturated surface soil. In water-stressed locations, treated wastewater can be a significant resource for enhanced groundwater supplies. In water-scarce places like the Mid-East and portions of sub-Saharan Africa, effluent has become a significant source that, with proper treatment, may be used for groundwater refilling, agricultural, and urban uses. SAT methods are economical, effective at pathogen eradication, and simple to use. The majority of the expense of a SAT is for draining water through the recovery reservoirs that typically costs \$30–60 U.S. dollars per m^3. The most significant benefit of SAT is that it eliminates the pipe-to-pipe link of directly recycling purified wastewater through the treatment plant. While the water reclaimed from the SAT systems is far superior to the influent, it may still be of poorer quality than that of the natural groundwater. As a result, the system must be constructed and operated in such a way that it does not contaminate the natural groundwater and uses just a fraction of the aquifers. To ensure for proper soil-aquifer processing, the distance among infiltration ponds and wells must be as wide as feasible, generally 45–106 m. These processed wastewaters include both necessary nutrients for plant growth (phosphorus, nitrogen, and potassium) and micro-nutrients (Jhansi and Mishra 2013).

5 Advanced Oxidation Processes for WWT

Pollutants like PhACs, dyes, consumer products, pesticides, and industrial wastes were identified inside the water reservoirs in recent years (Huerta-Fontela et al. 2010). Aside from agricultural and urban run-offs, WWT plants effluent is regarded as the most substantial pollutant emitters. Because traditional physical and biological WWT could only slightly remove pollutants, they remain in WWT plant effluents discharged into rivers and streams. Because AOPs oxidize a huge variety of pollutants, they provide a feasible and efficient attenuation alternative. AOPs are focused on the in situ production of strong oxidants toward the oxidation of organic matter. This involves the procedure depends on the production of OH radicals ($^{\bullet}OH$), which account for the vast of accessible AOPs, as well as procedures depending on other oxidizing species that favor chlorine or sulfate radicals. Several various process methods that were studied for usage as AOPs (Klavarioti et al. 2009).

Several AOPs, particularly those including UV-irradiation and ozonation, are very well developed and fully operational in WWT and water reuse infrastructure. However, latest research on a variety of emerging AOPs for WWT (e.g., plasma, electrochemical AOPs, electron beam, microwave, or ultrasound-based AOPs) has been continuously being published through numerous researchers (Stefan 2017). The enormous amount of various studies as well as a growing amount of suggested technologies and procedure combinations pose a significant challenge for a critical analysis of AOPs in terms of process costs (i.e., chemical input, energy depletion), general feasibility (e.g., oxidation byproduct formation and physical footprint), and sustainability (i.e., carbon footprint, resource usage) to facilitate comparison of their effectiveness with the other AOPs as well as appropriate treatment options (Stefan 2017).

5.1 Ozone-Based AOPs

Ozone (O_3) has been utilized in WWT as a disinfectant and oxidant. Ozone is an exceedingly selective oxidant that attacks mainly electron-rich functional groups like amines, double bonds, and activated aromatic rings. Because its reactions throughout real aqueous systems mostly comprise the development of $^\bullet$OH, ozonation itself is also regarded an AOP. The reaction's initiation, on the other hand, is relatively slow, with a 2nd order rate constant of 70 $M^{-1}s^{-1}$. Furthermore, radicals are produced as a byproduct of ozone reaction with organic compounds (primarily amine and phenol functional groups) (Buffle and von Gunten 2006). Such reactions are significant contributors to radical production, particularly during ozonation of secondary industrial effluent. Ozonation at high pH, as well as the combinations O_3/H_2O_2, O_3/UV, and O_3/catalysts, is procedures for actively initiating radical formation (Buffle and von Gunten 2006).

5.2 Electrochemical AOPs

Chaplin recently reviewed electrochemical AOPs (eAOPs) for WWT applications. Doped SnO_2, PbO_2, RuO_2, boron-doped diamond (BDD), and sub-stoichiometric and doped-TiO_2 electrodes are the most commonly utilized electrode varieties in this procedure. Hydrodynamic parameters must be viewed for eAOPs procedures, as energy utilized to pump the water may account for the majority of energy usage in this procedure. This is particularly true if low current densities have been utilized to increase $^\bullet$OH generation performance, thereby extending cumulative treatment time as well as pumping energy demands (Miklos et al. 2018).

5.3 Photocatalytic AOPs

In the last few years, there has been a lot of research in the usages of photo-active catalysts in oxidations procedures in WWT. However, there are various photocatalysts (e.g., TiO_2, AgO, ZnO, or WO_3), research has primarily focused on two types of oxidation reactions based on the solubility of the catalyst: However, there are various photocatalysts (e.g., TiO_2, AgO, ZnO, or WO_3), research has primarily focused on two types of oxidation reactions depending on the absorption of the catalyst (Miklos et al. 2018):

Homogeneous Photo-Fenton processes:

$$\mathrm{Fe(OH)^{2+} + h\upsilon \rightarrow Fe^{2+} + {}^\bullet OH} \tag{1}$$

Semiconductor (SC) (TiO_2)-based heterogeneous photocatalysis (Simonsen et al. 2010):

$$TiO_2 + h\upsilon \rightarrow (e^- + h^+) \tag{2}$$

$$h^+ + ^- OH_{ad} \rightarrow {}^{\bullet}OH_{ad} \tag{3}$$

The photoreduction of Fe(III) by UV and visible light accelerates the Fenton process; therefore, the quantum yield of such reaction is comparatively lower. Because of the high UV absorption of Fe(III)-polycarboxylates, photo-Fenton procedures with an organic ligand (e.g., ferrioxalate) having a greater quantum yield as well as its effectiveness. Furthermore, the ferrioxalate complex could absorb radiation with a wavelength of up to 550 nm, allowing it perfect for solar-powered AOPs (Hislop and Bolton 1999).

A SC material is typically studied as suspended nanoparticles or immobilized on various substrates. If enough photons strike the surface of photocatalyst, an electrons are injected to the conduction band (CB), leaving a hole (h^+) in the valence band (VB) (Eq. 2). These species have the ability to cause reductive or oxidative transitions of water components, either directly on the SC interface or through radical's reactions (Eq. 3). Photocatalysis is unique in that it combines oxidation and reduction mechanisms, whereas other AOPs rely solely on $^{\bullet}OH$ radicals reactions (Vohra and Davis 2000; Oliveira et al. 2015). The catalyst is also non-toxic as well as photochemically stable. The drawback of full-scale heterogeneous photocatalysis application is primarily due to two factors: (1) isolation of colloidal photocatalyst from water after treatment and (2) mass transfer restrictions to the interface of the immobilized photocatalyst on various substrate (Vickers 2017). Despite significant research work in the sector of photocatalysis, the procedure is infrequently used in municipal or industrial WWT facilities owing to the reduced quantum yield for the production of $^{\bullet}OH$ radicals (Miklos et al. 2018).

5.4 UV-Based AOPs

These procedures are basically relies on UV-irradiation (primarily UV-C) and the interaction of UV-light with various radical promoters. UV-fluences used for AOPs are typically greater than 200 mJ/cm^2 and thus surpass UV-dose demands for inactivation of many of these pathogens, such as UV-resistant microbes. The mostly used UV-based AOPs is the mixture of H_2O_2, or with other radicals initiators being studied include persulfate and chlorine. Research examined the use of NO_3^- in conjunction with medium pressure (MP) lamps like an appropriate UV-based AOP to formed oxidants (Miklos et al. 2018).

5.5 *Physical AOPs*

5.5.1 Electrohydraulic Discharge (Plasma)

Electrohydraulic discharge reactors were evaluated as AOPs in WWT. Strong electric fields used within water or between gas and water phase start both physical and chemical processes. Aside from the direct oxidation reaction of pollutant in the water, the discharge produces a variety of UV radiation, oxidizing radicals, and shock waves that can enhance oxidation reaction (Jiang et al. 2014).

5.5.2 Ultrasound

Ultrasound (US) sonication of water at 20–500 kHz causes the generation and collapse of microbubbles due to acoustical pulse stimulated deformation and rarefaction. Upon attaining an essential resonance size, such bubbles completely collapse violently, generating transiently extreme temperatures, extremely high pressure waves, and reactive radicals. Thermal degradation and several radical reactions are used to destroy water contaminants. In laboratory experiments, sonochemical procedures have been shown to oxidize a variety of aquatic pollutants (Mahamuni and Adewuyi 2010). However, the use of ultrasound requires a significant amount of energy, resulting in a quite low electrical performance of this AOPs in contrast to certain other technologies. As a result, the coupling of UV-irradiation with ultrasound (sonophotolysis), photocatalysts, oxidants (H_2O_2, O_3), or both (sonophotocatalysis) is receiving more attention. These hybrid procedures may provide additional benefits. However, significant improvements in energy performance are frequently achieved as a result of the higher effectiveness of the coupled procedures (Goel et al. 2004).

5.5.3 Microwave

The use of extremely energetic radiation mostly in microwave frequency range (300 MHze300 GHz) for the purpose of oxidation reaction of water pollutants has been investigated. Microwaves, in conjunction with catalysts or oxidants (H_2O_2), are used to aid in the degradation of the organic contaminants. Though the internal compound vibration, microwaves could accelerate rate of a reaction and stimulate selective heating of pollutants. Microwaves can also produce UV-light through an electrodeless discharge lamp in cumulative MW/UV reactors. Unfortunately, the majority of the microwave radiation used is transformed into heat. In addition to the low electrical effectiveness, cooling devices must be used to avoid treated wastewater from overheating (Miklos et al. 2018).

6 Current Treatment Technologies

To be considered effective, every treatment must take into account operational costs, environmental friendly nature, and the various demands for water quality in connection to water applications in order to optimize the reuse depending upon the fit-for-use principle of water. Wastewater should also be released in accordance with preset restrictions. The management method's reliability and stability must also be considered. Having emphasized the severity of poor management of different effluents above, this section discusses many ways that have been used for the management of these effluent, as well as their advantages and disadvantages (Ijanu et al. 2020).

6.1 Physicochemical Treatment

Due to its capability to break the complex organic compounds present in the wastewater effluents in a controlled environment as well as in a fairly short time period (e.g., a few hours), this techniques of industrial effluents have recently been chosen over biological treatment (Ijanu et al. 2020).

6.1.1 Zero-Valent Iron (ZVI) Treatment

ZVI particles are low cost, abundant, and non-toxic materials and are easily recoverable by magnetism; they have recently been utilized for the treatment of both inorganic and organic contaminants in various wastewaters (Tomizawa et al. 2016). Oxidative degradation, precipitation, reductive degradation, and adsorption are the methods they use to remove pollution. Despite of their accomplishments, spent ZVI particles are difficult to regenerate, making the procedure economically unfavorable because there is a need to replace the materials after the treatment (Tomizawa et al. 2016).

6.1.2 Electro-Oxidation

Electro-oxidation is a method in which voltages and other parameters of an electrode are tuned for in situ creation of oxidizing as well as reactive species. As organic substances degrade directly on the electrode, electrolysis creates various oxidative radicals (e.g., chlorine, hydroxyl). Many researchers have looked into the use of electro-oxidation in the management of coffee processing wastewater (Ibarra-Taquez et al. 2017). In other work, research employed a dimensionally stable Ti anode with a level of pollutant after coagulation/flocculation pre-treatment. After biological pre-treatment, investigators, investigated the elimination of COD and color, finding that steal anode was efficient in eliminating COD in wastewater effluent (Ijanu et al. 2020).

6.1.3 Membrane Filtration (MF)

It is a mechanical separation method for removal of impurities as small as microns. This method has been reported to be a successful approach in the treatment of paper and pulp industry effluent as well as a co-treatment in several ways (Ibarra-Taquez et al. 2017; Vickers 2017). Microfiltration has a typical pore size of 0.1–2 *u*m, NF has a pore size of 1–5 nm, UF has a pore size of 2–100 nm, NF has a pore size of 1–5 nm, and reverse osmosis has a pore size of less than 1 nm. Despite its widespread use, the fundamental disadvantage of MF is that it just separates but cannot alter any of the wastewater's components. Fouling and clogging of the membrane is another major problem with this approach. Recent research has focused on aspects such as treatment before MF, rate of pressure (Vickers 2017), and more frequently, electric treatment of membrane; additional factors include pH temperature adjustment. An operator friendly pressurized membrane system membrane bioreactor plant has been created by a Malaysian water treatment firm to treat and re-utilize wastewater from various waste producing industry. Despite the fact that the approach effectively removes COD and grease and free oil, there is no discussion of acidity and color removal, which is a key concern with various manufacturing effluent (Ijanu et al. 2020).

6.2 Biological Methods

Several types of filtration have been investigated in conjunction with bioreactors to enhance biological degradation, keeping in mind the environmental and health effects of dumping coffee wastewater directly into the environment. Biological treatment is commonly used; one of the main goals of this method is to remove BOD; however, this method has been proven to be unsuccessful in removing color and other acidic constituents of waste effluent, and processes take a long time to decompose organic compounds. Anaerobic/aerobic treatment, enzyme treatment, activated sludge, spray irrigation, immobilization, and the utilization of bioreactors are examples of biological treatment (Wang and Zhuang 2017; Novita et al. 2012).

6.2.1 Expanded Granular Sludge Bed (EGSB) Bioreactor

Several anaerobic procedures for treating CPWW have been devised and examined, including flow pattern investigations, Kinetics, toxicity inhibition, optimization, start-up, and process conditions. The hydraulic retention time (HRT) is among the significant independent factors for controlling this type of anaerobic bioreactor, as it has a direct impact on anaerobic digestion processes and transfer of hydrogen, as well as the possibility of cell washing (Cruz-Salomón et al. 2018). This sort of bioreactor has been verified to be an effective system for the treatment of high organic load effluent, but it has one main disadvantage that it does not eliminate nutrients (P and N). Another issue with anaerobic biological degradation is the high quantity of fermentable organic compounds in the effluent, which causes rapid acidification of the wastewater and substantial VFA generation. As a result, for the anaerobic bioreactor to work well, the content of VFA must be monitored. Because these parameters affect performance of EGSB bioreactor, it is vital to monitor VFA content and Hydraulic retention time (HRT) for excellent performance of anaerobic bioreactor (Cruz-Salomón et al. 2018).

Chemical coagulation and flocculation have also been investigated to be an effective, simple, and low-cost way of treating coffee wastewater. No prior preparation is required for it (Novita et al. 2012). The studies revealed that, while color elimination was efficient, COD lessening was not owing to the technique inability to breakdown the compound binding present in the organic materials in effluent (Novita et al. 2012). Electrochemical coagulation can speed up the gravity-assisted settling of solid particles in wastewater. The electrochemical coagulation process is said to be effective in eliminating color, COD, and SS from various effluent (Panchangam and Janakiraman 2015). When a highly charged cation agent, like aluminum (Al) sulfate (SO_4) coagulant is utilized in the neutralization of negatively charged solids effluents, monitored by a high-mass polyelectrolyte, like acrylamide, the oxygen (O_2) demand and suspended solid are removed. The electrochemical approach involves connecting two conductive metal plates (cathode and anode) to an external power supply, where oxidation causes corrosion on anode substances while passivation causes corrosion on cathode (Sahana et al. 2018). Electroplating has various advantages above conventional methods for effluent treatment, including the capacity to eliminate impurities that are otherwise challenging to remove, economic efficient, eco-friendly, energy effectiveness, and safety (Sahana et al. 2018; Volpe et al. 2018).

6.2.2 Adsorption

Many agricultural by-products, including as corn stalk, chitin, rice husk, peat, chitosan, and wood, have been employed as adsorbents in the search for a less expensive and still effective method of removing organic matter. Activated carbon products have now been employed in industrial WWT due to their extraordinary surface area, hydrophobicity, and affinity. Activated carbon technique has outperformed several traditional chemical wastewater treatment techniques in terms of contaminant removal using a very cheap and easily available adsorbent. Despite the fact that just a few researches have used adsorption technology to regulate coffee wastewater, several studies have stated that the utilization of coffee by-products as efficient adsorbents in the elimination of heavy metals. However, researchers described the elimination of tannin through adsorption by coffee effluent, and the procedure efficiency was greatly dependent on pH (e.g., 5–6 for commercial activated carbon as well as 3–5 for activated coconut shell), although the method seems to have certain disadvantages, like higher costs due to the high energy necessary to activate the carbon (Shawwa et al. 2001).

7 Comparative Analysis of WWT Technologies

The production and emission of wastewater is rising, requiring the development of wastewater treatment methods. With rising population, industrialization and urbanization the number of pollutants in our environment increases, resulting in increased wastewater effluent discharge from both home and industrial sources. Because these effluents are formed in enormous quantities, they should be thoroughly treated before being discharged. As a result easy-to-maintain, cost-effective, and low-energy wastewater treatment methods are required (Yahya et al. 2020).

7.1 Activated Sludge Method (ASM)

ASM is made up of flocks of active-bacteria that takes in and eliminates aerobically biodegradable organic particulates effluent that has undergone traditional primary treatment. The cultivation of this organism in the aeration tanks results in the production of this sludge. In this method, the nanoparticles are clogged with bacteria, fungi, and protozoa therefore the system is called "active" (Seow et al. 2016). Whether it is gray water, fecal sludge, brown water, black water, or untreated wastewater from industry, all toxins treated through this process are generally biodegradable. This extensively utilized wastewater treatment technique is now employed by large cities and villages all over the world. It is an ideal option for isolated establishments like hotels or hospitals, subdivisions, and cluster situations. This WWT technology treats large quantities of wastewater from both domestic and industrial sources. Different species of microorganisms utilize the contaminants existing in the effluent as a basis of food in this sort of treatment. Being a method in which organisms are suspended in wastewater rather from being adhered to the media as in a biological contactor or trickling processes filter (Cheremisinoff 2019). This process is depends upon the activity of millions of different microorganisms, primarily aerobic as well as facultative heterotrophic bacteria, which are commonly found suspended in wastewater that passes from the aeration container. The aeration container is the place, in which the various biological reactions take place, and the aeration source is either from surfaces aerators, air diffusers. It also serves as a source of O_2 to the system, allowing the microorganisms to grow (Ho et al. 2018).

In comparison with, CWs and WSPs that are natural methods and seem to be the more viable choice as compared to the ASM, cost-effective, quality effluent, and requires less land. Furthermore, the AS system has the drawbacks of not being a flexible approach, having higher operating costs, and being sensitive to specific types of industrial waste, making it unsuitable for these organizations. Another drawback of this system is the sludge disposal issue, which is usually done on a large scale, the check and balance is mandatory, as well as the concentration of sludge that must be reverted, all of which necessitate trained management and expertise in WWT (Yahya et al. 2020).

7.2 Membrane Bioreactor Method

Membrane technology has recently gained popularity in the water industry as a means of improving fresh water output for both home and industrial needs, as compared to other WWT technologies. Membrane treatment is relatively analogous to conventional activated sludge treatment approaches in which bio-degradation, separation occurs instantaneously, with the exception of aeration steps and membrane modules, which are present in conventional activated sludge treatment (Dharupaneedi et al. 2019). Membrane separation can be achieved either by using a two-membrane vacuum driven membrane immersed in a bioreactor that operates in dead-end mode in submerged membranes or by pressure-driven filtration in side-stream membranes. The former is the most frequent one used in maximum WWT, in which wastewater pushed from the membrane module as well as then reverted to the bioreactor. Fine screening is a basic pre-treatment procedure that keeps particles out of waste streams that enter the membrane tank. This prevents solids from accumulating on the membrane and protects it from harmful debris and particles. It also extends the membrane life, lowers operational costs, improves sludge quality, and streamlines operations. Most membranes are chemically cleaned once or twice a week, and the process takes around half an hour. When the filtration seems to be no longer effective, a recovery cleaning is done once or twice a year. “Irrecoverable fouling” refers to a deposit that cannot be removed using current cleaning technologies. This form of fouling builds up throughout the years of operation, and it serves as an indicator of the membrane’s lifespan. Skilled people perform all of these operations and repairs (Iorhemen et al. 2016).

The merits of this procedure is that it reduces plant footprint by eliminating secondary as well as tertiary filtration operations, resulting in improved efficiency. Membranes are also linked to lower sludge generation and systems designed to delay sludge ageing. Aside from the merits listed above, MBR treatment technology is attracting the attention of many investigators in the field of wastewater engineering. High energy costs, high membrane operation, fouling, membrane complexity, and capital expenses are some of the drawbacks of these systems (Iorhemen et al. 2016).

8 Reuse in Future Cities

According to the 2030 Water Research Group, if current water consumption rates continue, worldwide yearly water demand in 2030 will be 6900 billion m^3, surpassing more than 64% of the total available and reliable water sources (4200 billion m^3). Climate change was thought to worsen the situation (Kellis et al. 2013). As a result, governments recognize the importance of discovering alternative resources, such as cleansed wastewater. The greater awareness of the benefits of reclaimed water among decision-makers and the general public is a crucial foundation for the growth of the water-reuse sector. As a result of existing policies, the water reuse sector is experiencing favorable conditions (Kellis et al. 2013).

9 Pledge

9.1 Cities of Europe

9.1.1 Cities of Spain

Spain is located at the western edge stage of a European Mediterranean area, that one side that faces the Atlantic Ocean and another facing a Mediterranean. Spain population is estimated to be around 50 million people. It has a Total Actual Renewable Water per capita (TARWR) of 2543 m^3year^{-1}, which is sufficient for food manufacture, and the Millenium Development Goal Water Indicator (MDGWI) was 29% in 2012. This country has no water shortage issues on the Atlantic side, however, on the Mediterranean side has the semi-arid and arid weather as the rest of the Mediterranean. The WWT system in Spain is excellent. Despite the fact that agricultural irrigation purpose is the primary reason of reuse treated wastewater. In Spain, legislation for the reuse of urban wastewater was established in 2007. The major guidelines are the Regulation for the Public Hydraulic Dominion (1986) as well as Spanish water law (1985). Reuse occurs primarily around the Mediterranean and adjacent coastal locations, and also in the areas surrounding Vitoria-Gaztei and Madrid. Spain has developed tertiary wastewater treatment capabilities and is aggressively promoting the treated wastewater's reuse in municipal settings. Treated raw sewage has been utilized in applications such as irrigation at Ginora, golf course, Spain, since July 2000. Municipal wastewaters are presently employed in wetlands restoration, landscape irrigation, road washing, and firefighting in Spain. For example, tertiary level drainage is often utilized for firefighting, irrigation of land, street cleaning, and boat washing in the Costa Brava. In 2004, Spain's total wastewater reused volume was 1,117,808 m^3d^{-1} (Xu et al. 2017).

9.1.2 Cities of France

The population of France is nearly 62 million people. Like Spain, it has both an Atlantic as well as Mediterranean coastline. This country has TARWR of 3,479 m^3year^{-1}, allowing it to maintain present food production levels. Furthermore, France's MDGWI, as of 2005, is 15%, indicating that the resources will be replenished that are withdrawn for the country. France, on the other hand, has two unique personalities: north side, where precipitation is abundant, and south side have pockets of semi-arid and arid Mediterranean climate. Guidelines for the reuse of wastewater were created in France in 1991 and are established on the guidelines of WHO, but they are tougher in terms of preventing people's exposure via contact. The limits are almost too rigorous, preventing reuse from spreading across the country (Yadav et al. 2002). In France, there are around 17,500 plants for wastewater treatment, the majority of which have a capacity of less than 2,000 PE. For almost a century, France has been re-utilizing wastewater for agriculture, particularly in the Paris side.

The reuse of purified wastewater is common throughout the Mediterranean region, especially in the south. In France, landscape irrigation is the utmost common use of recycled water now a days. The criteria, which are virtually too rigid, combined with overzealous local compliance regulations, which often go beyond the standards, limit the potential for widespread reuse of industrial and urban applications across the country. Applications in industrial operations in Renault factories and Annecy for toilet flushing are only a few examples of the reuse of municipal wastewater for urban and industrial usage. In 2004, France's total wastewater reused volume was 19,178 m^3d^{-1} (Yadav et al. 2002).

9.1.3 Cities of Monaco

Monaco has not identified autonomous wastewater effluents reuse as a state priority because of its small size and high population density. The conditions are identical to the Mediterranean area of France due to its geographical location inside France (Yadav et al. 2002).

9.1.4 Cities of Malta

Malta is an island nation with a population of slightly more than 425 thousand individuals. For a long time, the state has been suffering from serious water scarcity. Malta have TARWR of 132 m^3year^{-1} and an MDGWI of 72% in 2010 is experiencing the most acute natural wastewater scarcity among Mediterranean countries. The European Union has designated Malta as a drought-stricken area. There are no permanent rivers, lakes, or streams on the island of Malta. Law number 340 of 2001, which came into effect in 2004, governs wastewater reuse activities in Malta (Marchal et al. 2010). The issue of water supply has long been a top priority for the authorities in charge of these islands. As early as 1884, the reuse of wastewater for the purpose of irrigation was considered. The Sant' Antnin Sewage Treatment Plant (SASTP) was built in 1983 and it has been supplying irrigation water since that time. Because water is scarce on these islands, the SASTP was created to increase the irrigated area through 550 ha. Plant effluent began to be used for textile washing in the neighboring industrial park in 1990. SASTP is undergoing upgrades to boost up the reclaimed production of water from 7000 to 12,000 m^3day^{-1} (Marchal et al. 2010).

9.1.5 Cities of Italy

The population of Italy is more than 62 million people. Italy has a TARWR of 3175 m^3year^{-1}. Furthermore, the MDGWI for Italy last recorded in 2010 was 25%. Nonetheless, Italy has two separate geographies: north side contains the abundant amount of water, and south side is desert. Italy guidelines have been developing to incorporate an additional liberal set of boundaries, allowing for more reuse. Over 10,000 wastewater treatment plants are in operation in Italy, with a substantial proportion of them using tertiary treatment, mainly for treated wastewater discharge to rivers. Applications such as fire protection and gardening have been introduced in the effluent reuse planning phases on a small scale. The total volume of domestic sewage in Italy is around 2400 Mm^3 per year (Njuguna et al. 2019).

9.1.6 Cities of Albania, Bosnia, Montenegro, Croatia, Slovania, and Herzegovina

The aforementioned states have a higher TARWR (13,060, 9,552, 23,917 m^{3}year^{-1}), hence, they are not in desperate need of water protection. The majority are also recently established countries that are still experiencing instability in their establishment. In terms of wastewater treatment as well as recycling, those countries have made minimal progress. The capability of wastewater treatment as well as reuse has not been universally implemented since the conclusion of the war due to a lack of sewage network and basic infrastructure. There have been various attempts to set rules that have yet to flourish (Spina et al. 2012).

9.1.7 Cities of Greece

The population of Greece exceeds 12 million people. This country has TARWR of 6696 m^{3}year^{-1} and an MDGWI of 12%, according to data from 2005. This data reveals a low level of water stress. On the other hand, country is generally hilly with unequal water circulation. This imbalance causes water scarcity on the continent of Greece. The Greece islands, on the other hand, are frequently devoid of water supplies and rely on boats to bring water in during the summer months owing to tourists. Due to its unique geology, Greece experiences a significant level of water stress in only a few areas. Because Greece's water supply is unequal, with summertime shortages, reuse is appealing. In 2008, a governmental degree was used to establish wastewater reuse regulations, which were updated in 2011. The plants of wastewater treatment serve all towns with a population of more than 2050 people, or the treatment plants are being built in all towns with a population 2050 people. Wastewater rehabilitation and reuse is done in a few places (e.g., Chalkis, Thessalokiki, Cherssonisos) and mostly in guesthouses on islands (Tringe et al. 2005).

9.1.8 Cities of Cyprus

Cyprus is a Mediterranean island in the Mediterranean Sea with a population of over one million people. According to WHO guidelines, it has the TARWR of 725.6 m^{3}year^{-1}, qualifying it as a nation with no viable production of food. Furthermore, the MDGWI for Cyprus was 19.4% in 2010, indicating that the H_2O remote for use on the island could not be restored. The average annual precipitation in Cyprus is around 500 mm, with evapotranspiration accounting for roughly 80% of it. The European Union has designated Cyprus as a drought area. Reuse standards and guidelines were developed in 2005 with a focus on agriculture irrigation. Now, there are 25 recycling plants of wastewater operating in Cyprus. The current total H_2O use is 300 $\mathrm{Mm^3 year^{-1}}$, approximately 20% of it is going to use in applications other than irrigation. In the Limassol area, tertiary treated wastewater is utilized in golf courses. Wastewater is often utilized for the purpose of irrigation in landscape (Gupta et al. 2018).

9.2 Cities of Asia

9.2.1 Cities of Turkey

Turkey population is over 72 million people. It has TARWR of 3073 $\mathrm{m^3 year^{-1}}$, allowing the state to maintain current food production levels. Furthermore, Turkey's MDGWI, as of 2005, is 19%, indicating that the sources pulled for the state as a whole are being carefully replaced. In Turkey, strategies for the reuse of wastewater for the purpose of irrigation have recently been implemented. Turkey now has 130 plants in process, serving roughly 50% of the people of state. The majority of the WWT discharge is dumped into rivers or the sea. In Turkey, wastewater reuse for industrial and municipal use is under attention (Freitas et al. 2009).

9.2.2 Cities of Syria

The population of Syria is more than 20 million people. Syria has a little Mediterranean coast and is largely surrounded by the arid desert environments of the Middle East. It has TARWR of 837 $\mathrm{m^3 year^{-1}}$, bringing it dangerously close to the threshold of 750 $\mathrm{m^3 year^{-1}}$, below which the production of food cannot be sustained. It is, however, already "water stressed." Furthermore, Syria's MDGWI last recorded in 2010 was 99%, indicating that the majority of the country's water resources had been depleted. In Syria, guidelines for wastewater reuse, including in agriculture, are being studied (Hall 2007). In absolute terms, the wastewater volume reused in Syria is large because of the state serious water shortage conditions; nevertheless, the production value is still confined to primary and secondary treatment. The re-utilization of untreated wastewater for irrigation is a major health hazard. Various plants for wastewater treatment have been built, particularly in the larger cities (Damascus, Aleppo, and so on), with a total capacity of 400 $\mathrm{m^3\ year^{-1}}$ available for reuse, primarily for irrigation. The Syrian government is constructing a number of plants for wastewater treatment in response to the serious health problems created by a shortage of wastewater treatment (Hall 2007).

9.2.3 Cities of Lebanon

Lebanon's population exceeds 4 million people. With a considerable portion of the country bordering the Mediterranean Sea, Lebanon weather is more moderate, with only dry environments. It has TARWR of 1173 m^3year^{-1}, indicating that the country is in severe water shortage. Furthermore, state MDGWI was 19% at the time of the last assessment in 2005. According to Lenanese regulations, purified wastewater cannot be used to grow crops for human use. There are also regulations for the disposal of treated wastewater discharge into the surface waters. In Lebanon, new rules for wastewater reuse, including those in agriculture irrigation are being established (Gupta et al. 2018). The single wastewater treatment plant that produces irrigation water is Baalbak WWTP, which produces 12,000 m^3day^{-1}. Lebanon has a potential wastewater volume of 640.000 m^3, which will be dumped at sea. Because of the relative water shortage of country, wastewater treatment plant outflows are being investigated for reuse (Xu et al. 2017).

9.2.4 Cities of Palestine

The population of Palestine (West Bank and Gaza Strip) is over 4 million people. It has TARWR of 212 m^3year^{-1}, significantly below the 750 m^3year^{-1} threshold at which the production of food can no longer be sustained. In 2005, the MDGWI for the state was 50%, indicating that sources are being taken that could not be replaced. The government established strategies for wastewater treatment depending upon WHO proposed standards, though they have yet to be implemented or enforced. It is worth noting that certain Muslims in the West Africa and Middle East feel that treated wastewater use is filthy and thus prohibited by Islam (Zimmels et al. 2004).

Sewage treatment is limited to urban areas, with only roughly 35% of the Gaza and the West bank connected to the sewerage system. The first plan was to use treated effluents in agriculture in Gaza and Jabaliah City. However, due to a lack of funding and farmers' rejection of reuse of treated wastewater due to religious and cultural beliefs, implementation failed. Very few pilot small-scale plants for wastewater reuse have been built in Palestine (Zimmels et al. 2004).

9.2.5 Cities of Israel

Israel population is over 7 million people and the north side has the Mediterranean weather, whereas the south side has desert weather. It has TARWR of 245 m^3year^{-1}, significantly below the 750 m^3year^{-1} threshold at which the production of food can no longer be sustained. Furthermore, the MDGWI for Israel in 2005 was 101%, indicating a severe water deficit across the country as well as wastewater shortages. Since 1959, the legislation has defined treated wastewater as an essential component of the country's water sources. After a cholera outbreak in Jerusalem caused by the usage of untreated wastewater in the 1970s, that's why strategies for the reuse of treated wastewater were established. The Israeli rules are based on real-world applications and set an example for the rest of the globe. Guidelines and regulations are changed on a regular basis to reflect new research findings. However, Israel's desalination, wastewater recycling, and reuse initiatives serve as a model for the rest of the globe. The desert has been turned into lush land thanks to man's efforts. The treated wastewater is re-utilized in 75% of cases, largely for agricultural irrigation (Zhang and Reynolds 2019).

9.2.6 Cities of China

Regardless of the debate over precise discharge rules, China's wastewater sector will undoubtedly implement more stringent and global-scale H_2O pollution control in the future. Many provinces and cities, including Beijing, Zhejiang, Hunan, Tianjin, and Jiangsu, have already begun to impose stricter local WWTP effluent limits. Based on international development trends, the next phase will be to improve developing pollutant management and water reclamation, both of which are still in their development in China (Jhansi and Mishra 2013). As a result, the goal of wastewater management has shifted from pollution abatement to the reuse of water, resource recovery as well as water ecosystem restoration. The latest policy revolutions in China show this type of aim change (Jhansi and Mishra 2013).

9.3 Cities of Africa

9.3.1 Cities of Egypt

Egypt population is over 80 million people. Egypt northern border is with the Mediterranean, and the Nile River is the country primary source of water. With a TARWR of 718 m^3year^{-1} per capita in Egypt, it is clear that production of food cannot be sustained. Furthermore, Egypt's MDGWI was 113% in 2000, indicating that the majority of the country's resources are being drained. Even in the winter, the rains are rare and only occur in the north, as 96% of the country is desert. Oasis and dwellings are modest and unable to meet the demands of the surrounding areas. The Nile River is the country's main source of water. There are no reuse guidelines in Egypt. The martial law regulation of 1984, which is still being prepared, restricts the wastewater reuse unless it is treated according to set standards (Jamwal and Mittal 2010). Ministry of Housing is responsible for establishing public wastewater systems and issuing licenses regulating the wastewater release into community sewerage systems and into the surroundings, according to Law 93/1962. The regulatory standards are set by the Ministry of Health. Agreement with these water quality parameters, however, has yet to be implemented. The volume of treated wastewater is expected to be 4930 million m^3year^{-1}, with almost 22 plants of wastewater treatment in operation and another 150 under development, mostly in big cities like Alexandria and Cairo. The wastewater treatment plants have a combined capacity of roughly 1.752 billion m^3year^{-1} (Jamwal and Mittal 2010).

9.3.2 Cities of Libya

The population of Libya is more than 6 million people. It has the lowest TARWR per capita in the Mediterranean, at 95.8 m^3year^{-1}, and considerably below 750 m^3year^{-1}, indicating that production of food cannot be sustained. Furthermore, Libya's MDGWI, which was last published in 200, is 793%, indicating that the country has even now drained all the existing sources through its area. In spite of the state acute need for water, Libya has no rules for the reuse of wastewater. In this state, less than 15% of the wastewater produced (546 Mm^3year^{-1}) is processed (40 Mm^3year^{-1}) and is only used in limited agricultural uses (Yu et al. 2019).

9.3.3 Cities of Tunisia

Tunisia is a country with a population of over ten million people. Tunisia climate is semi-arid to arid. Tunisia has TARWR of 453 m^3year^{-1}, significantly below the global average of 750 m^3year^{-1}, indicating that the production of food cannot be sustained. Furthermore, Tunisia's MDGWI was 57% in 2001, indicating that the country will face serious water shortage difficulties in the coming years and decades. Tunisia was one of the first states in the side of Mediterranean region to develop and execute a wastewater reuse programmed. The farmers of state are not only prepared to reuse wastewater, but also to pay for it. As a result, certain operational costs are compensated. Golf course irrigation, hotel landscaping, and green belts are some of the other applications in Tunisia. Finally, wastewater is used to recharge storage reservoirs as well as coastal aquifers (Hansen et al. 2018).

9.3.4 Cities of Algeria

The population of Algeria is about 40 million and has TARWR of 344 m^3year^{-1}, significantly below the global average of 750 m^3year^{-1}, indicating that the production of food cannot be sustained. Furthermore, Algeria's MDGWI was 52% in 2000, indicating that the country will face serious water scarcity difficulties in the coming years. Algerian legislation forbids the use of wastewater for agricultural irrigation purpose that could be eaten materials, although it does allow irrigation of pasture, trees, and fodder crops. In 1985, the volume of sewage wastewater in Algeria was at 660 Mm^3, with 15 treatment plants projected to increase that to 1500 Mm^3 by 2010. Algerian government has launched a scheme that will see 28 treatment plants rehabilitated, as well as new wastewater treatment plants and wastewater stabilization ponds built. To protect water resources as well as the environment from the detrimental effects of pollution, an effective follow-up and periodic review is required for the program's effectiveness (Wang and Gong 2018).

9.3.5 Cities of Morocco

The population of Morocco is about 31 million and it is a semi-arid to arid state. It has TARWR of 926 $m^3 year^{-1}$ per capita, making it "water strained." Morocco's Atlantic Coast supplies some moisture, which helps to explain why the country is marginally better off than its neighbors. Morocco's MDGWI was 44% when it was last recorded in 2000. It is a semi-arid to arid country despite having plentiful precipitation due to its closeness to the Atlantic Ocean. In 1995, legislation establishing a national water policy was enacted. It has not until now defined wastewater reuse guidelines and instead relies on WHO suggestions (Lu et al. 2015). Morocco lacks a well-defined wastewater reuse policy. The majority of plants for wastewater treatment are secondary. In 1998, the amount of collected sewage wastewater was predicted to be 380 $Mm^3 year^{-1}$, 500 $Mm^3 year^{-1}$ in 2000, and 700 $Mm^3 year^{-1}$ by 2020. The treated wastewater is dumped into the sea in 58% of cases and rivers in 52% of cases. 85% of the wastewater produced does not receive treatment. Morocco has 69 WWT plants, but only 30 or 40% of them are operational, with the rest either out of service or incomplete. Agriculture and, to a lesser extent, industrial uses and landscaping, as well as cement, are the primary uses of treated wastewater today. Aquaculture, environmental reuse, agroforestry, and industrial reuse are all possible future applications (Lu et al. 2015).

10 Future Challenges of WWT and Reuse in Future Cities

To optimize water reclamation and reuse during the next decade, a variety of concerns and obstacles will need to be addressed. Important issues include (a) how to connect sea water desalination facilities with advanced wastewater treatment facilities, (b) the growth of more effective tools and procedures incorporating risk assessment to evaluate the human as well as environmental health impacts of wastewater constituents, and (c) the implementation of specific water reclamation as well as reuse legislation, applicable to a wide range of situations that both promote and regulate reuse. Furthermore, according to recent studies, users of recycled water are more concerned with the quality of the water than with the source of the water (Paranychianakis et al. 2015).

11 Conclusion

WWT is a major challenge for several countries due to rising levels of unwanted or unidentified contaminants that are hazardous to both health and the environment. As a result, this chapter investigates the strategies based on ongoing and prospective research. WWT includes very conventional methods such as primary, secondary, and tertiary treatment methods, but the use of advanced methods is continuously increases the potential of water quality. In this chapter, we discussed different methods for WWT, where the proposed model concept works on different kinds of wastewater effluents. The suggested method is helpful not just for WWT but also for the usage of solid waste material as fertilizer. The primary innovative outcome is really an effective method for WWT and further usage for potable water. It is also strongly advised to follow to the standard approaches as well as available guidance issued by WHO.

One of the twenty-first century rising concern is the freshwater crisis. Annually, approximately 330 km3 of municipal wastewater is produced worldwide. The above data gives a better understanding about why the utilization of treated water is critical to resolving the water crisis problems. The use of treated wastewater (municipal wastewater, or industrial or seawater) for irrigation has a positive outcome in terms of fulfilling water demands. Farmers in developing countries are currently utilizing wastewater directly for the irrigation that may pose a number of health risks to both farmers as well as consumers (vegetables or crops). As a result, it is important to incorporate both conventional and advanced methods for WWT. Even though most advanced and developing countries do not follow proper guidelines, a local evaluation of the environmental consequences of wastewater irrigation is required. As a result, it is essential to establish concrete practices and policies to promote safe water reuse in order to gain all of its positive benefits in agricultural production and also for farmers.

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Inorganic Nitrogen and Phosphate Removal from Port Water Using Microalgal Biotechnology Toward Sustainable Development

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1 Introduction

Water is one of the most potent and essential components for all living organisms. Water is a fundamental requirement for sustainable ecosystem maintenance and distribution. Water also plays a vital role in all known forms of life even though it is not providing calories. The green planet "Earth" is occupied with about 1.386 billion km^3 of water, of which 97.5% is saltwater and the remaining only 2.5% is fresh water. Out of 2.5% freshwater, only 1.2% of water is used for drinking purposes, and the rest 1.3% of water is locked up in glaciers, ice caps, and permafrost or buried deep in the ground. The imbalanced water cycle results in more frequent and extreme precipitation in some regions, while other regions leave dry. This may lead to trimming down the uniform distribution of fresh water on the Earth.

Moreover, due to the drastic increase of population, industrialization, and urbanization ensuing that increase the demand for drinking water, causing water scarcity issues. Water scarcity is a natural as well as a human-induced phenomenon. The World's freshwaters resources are continuously polluting with various emerging pollutants, including organic waste materials, agriculture waste, fertilizers, pesticides, and heavy metals (UNESCO and UN-Water, 2020). Water pollution by organic matter is increased by discharging untreated municipal wastewaters, industrial wastewaters, intensifying water usage in agriculture, and reducing river dilution due to runoff (Zandaryaa and Mateo-Sagasta 2018). Nitrates and Phosphates are the primary contaminants that originate from medical wastes, fertilizers such as urea and DAP, pesticides, and domestic wastewaters of humans ((Isiuku and Enyoh 2020).

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The continuous loading of nitrates and phosphates in freshwater resources limits access to safe water for drinking and practicing basic hygiene in house and health care facilities. Water scarcity is more concerned where the water demand exceeds water availability. Mainly, water scarcity is a primary problem in the arid and semi-arid areas. To overcome water scarcity, many government agencies, research institutes, and industries are working on sustainable wastewater treatment technologies to initiate water recycling and reuse of treated waters. The potential of water recyclability or reuse of treated wastewaters is not exploited very well in many areas (Voulvoulis 2018). Nowadays, water recycling or wastewater treatment projects are practiced in many regions suffering from water scarcity, like Australia, Italy, France, Florida, and other polluted European countries, including the UK, Germany, and India (Van der Bruggen 2010). This chapter focused on an environmental load of nitrate (NO_3^-) or nitrite (NO_3^-) and phosphate (PO_4^{3-}) and its reclamation techniques. Consequently, it also explores the recovery of anionic nutrients such as NO_{3-}/NO_{2-} and PO_4^{3-} from Veraval fisheries wastewaters using green microalgae *Chlorella* sp. During this recovery process, lipid biomass was produced which can be used in biofuel production for sustainable energy production.

1.1 Inorganic Nitrates and Phosphates Contamination

Nitrates and phosphates are essential nutrients for marine planktons and terrestrial plants that enter the environment from a wide range of human activities. Discharging of untreated organic manure, domestic, industrial wastewaters, and runoff the ammonium-based agricultural fertilizers wastes into freshwater resources, resulting in the overloading of nitrates, nitrites, and phosphates. A wide range of urbanization can generate a huge amount of wastewater that significantly increases organic matter pollution. For example, the top ten developed and developing countries such as China, Central and South America, Japan, Russia and urban areas of Asia and Europe discharge untreated wastewater into surface water bodies, increasing nutrient pollution with a high content of nitrates and phosphates (Fig. 1).

In urban areas, organic wastes (OWs) are dumped or discharged in open areas without treatment or other sewage connections till 2010. Due to natural disasters such as floods and heavy rains, the discharged or dumped OWs are runoff along with streams and enter into surface water banks like lakes, rivers, and other water resources. Organic wastes (OWs) are a significant source of many nutrients, including nitrates and phosphates. OWs pollution can increase nitrates and phosphates levels in water (van Puijenbroek et al. 2019). As per the CPCB (2005) report, developing countries like India, water pollution accrued from discharging untreated municipal wastewater industrial effluents into water banks (Fig. 2). Thus, Maharashtra, West Bengal, and Tamil Nadu states are contributed about 80% of the wastewater disposal into coastal water. Maharashtra is one of the biggest states in India had a tremendous industrial background in urban cities like Mumbai and Pune. From these urban areas,

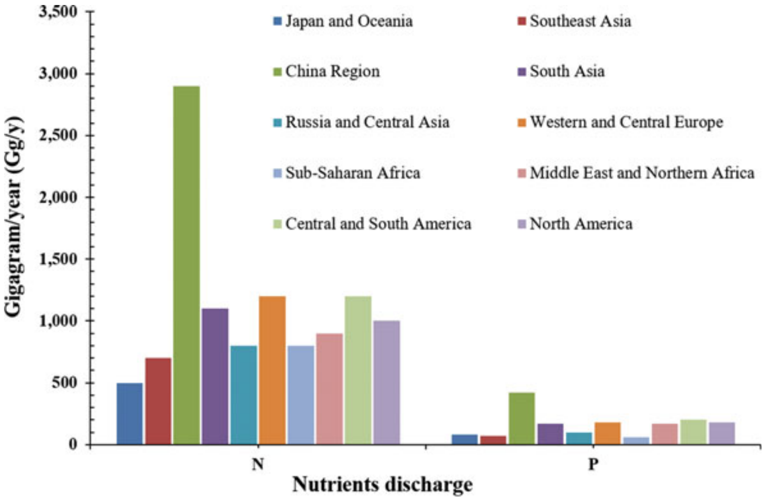

Fig. 1 Discharge of nutrients for a year into the surface water bodies in ten countries during the 2010 year. Data source van Puijenbroek et al. (2019)

47% (i.e., 2348.63 million liters per day) of untreated wastewater (47%) is disposed into coastal waters (CPCB 2005).

Nitrates are enriched into water directly or indirectly from the atmosphere, indirect process runoff of nitrate-containing fertilizers or oxidation of nitrogen, ammonia, and organic nitrogen compounds like amino acids and nucleic acids. In the indirect process, nitrates enter the water in the form of nitrogen-carrying compounds derived

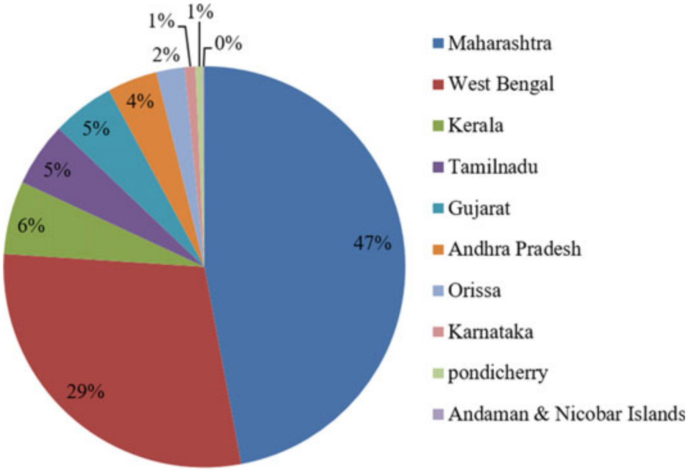

Fig. 2 Percentage of state and Union Territory of India disposed untreated municipal wastewater into coastal water (MLD: million liters per day)

from various industries and automobiles. The atmospheric nitrogen will be deposited in liquid nitrogen, nitric oxides, fuel, and gasoline. For example, in the United States, more than 3 million tons of nitrogen is deposited from the atmosphere either by chemical reactions or combustion of fossil fuels. Many research laboratories and field studies have found that the contamination of freshwater resources with inorganic nitrates and phosphates emanate from various wastewater sources (Table 1).

Table 1 Environmental pollution of inorganic nutrients from different wastewaters sources

S. No	Wastewater types	Nitrates/nitrites (mg L^{-1})	Phosphates (mg L^{-1})	Reference
1	Domestic waste, Tomato cannery industry, Pharmaceutical industry	85–90, 0.1–5.6, 5166	15–20, 0.3–7.4, ND	Abdelaziz et al. (2013)
2	Hog farming	1290–2430	264–324	Bradford et al. (2008)
3	Poultry farming	96–802	30–50	Bradford et al. (2008)
4	Dairy industry	58–115	9.7–28	Trevor et al. (2005)
5	Textile industry	42.7–161	9.4–27.9	Fongsatitkul et al. (2004)
6	Winery industry	67–71	7.0–8.5	Vlyssides et al. (2005)
7	Olive mill industry, Paper mill industry	532, 13	182, 4	Ammary (2004)
8	Tannery industry	273	21	Durai and Rajasimman (2011)
9	Carpet industry	32.6–45.9	26–49	Chinnasamy et al. (2010)
10	Laundry wastewater	–	94.65	Braga and Varesche (2014)
11	Dairy manure, Poultry manure, Swine slurry, Poultry litter, Dairy compost, Poultry compost	–	71, 75, 74, 57, 42, 34	Bowman et al. (2007) and Shah Maulin (2020)
12	Primary settled sewage water	20	3.68	Lau et al. (1996)
13	Piggery waste	590		Kamyab et al. (2019)

(continued)

Table 1 (continued)

S. No	Wastewater types	Nitrates/nitrites (mg L^{-1})	Phosphates (mg L^{-1})	Reference
14	Pickle industry wastewater	388.8	47.9	Wan et al. (2019)
15	Municipal Sewage water	27.7	1.59	Zhang et al. (2013)
16	Brewery wastewater	25–80	10–15	Amenorfenyo et al. (2019)
17	Animal wastewater	2600	120	Park et al. (2009)
18	Dairy wastewater	10.66–86.65	2.52–9.50	Daneshvar et al. (2019) and Shah Maulin (2021a, b)
19	Fish processing	46–50	2.7–10.7	Riaño et al. (2011)
20	Poultry (diluted) wastewater	76–152	6–12	Singh et al. (2011)
21	Veraval fisheries wastewater	1.86	0.78	Present work report

Note ND = not detected; mg L^{-1} = milligram per liter

1.2 Toxicity of Nitrates and Phosphates Pollution

Nitrates and phosphates are primarily essential nutrients for algae and plants growth which provide food and habitats for fish and many aquatic life forms. However, too much of these nutrients (nitrates and phosphates) in the water can cause algal to grow faster than normal conditions known as an algal bloom. Algal blooming is one of the most common natural and challenging environmental problems. It is caused by excess loading of nitrates and phosphates in the water. Overloading of these anionic nutrients leading bioaccumulation, bio-magnification, and eutrophication (Yang et al. 2008; Liu et al. 2010). The excess loading of phosphorus and nitrogen in water promotes algae growth, which are faster-growing micro planktons than terrestrial plants. Phosphate is one of the anionic components that stimulate the eutrophication process even at lower levels of 0.1–5.6 μg/L. In the eutrophication process, progressively enriched nutrients enhance the phytoplankton blooming in water bodies. Algal blooming reduces the freshwater quality oxygen levels, which is essential for the survival of fishes and other aquatic life and indirectly declines the productivity of an aquatic ecosystem (Bowman et al. 2007).

Algal blooms can affect human health because they produce more toxins and bacterial growth. The estimated cost of damage mediated by eutrophication in the United States alone is approximately $2.2 billion annually (Dodds et al. 2009). According to the Harmful Algae Event Database and Biodiversity Information System (HAEDBIS), the frequency of harmful algal bloom (HAB) events increased, and no uniform global trends in the number of occurred during 1985–2018 (Hallegraeff et al. 2021). Most HAB events reduce water quality and cause fish mortality in the bloom regions. For example, A bloom of the *Prymnesium polylepis* (prymnesiophyte) in the southwestern North Sea and the Wadden Sea coasts shellfish mortality has been liked to HAB events from 1991 to the end of 2019 (Karlson et al. 2021). Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) also mapped the evidence of the marine HABs and acute and chronic human health effects between 1985 and May 2019 (Young et al. 2020).

The hazard quantity (HQ) and index (HI) values for nitrate and phosphate are exceeded in water bodies such as Abadaba and Njaba rivers and Agulu, Oguta, and Nika lakes in South-Eastern Nigeria. Generally, humans are exposed to polluted waters in two ways via oral and dermal routes. Nutrient pollution index (NPI) and human health risks computational studies find that the domestic usages of nutrient polluted water pose non-carcinogenic health risks to uses via oral pathway (Isiuku and Enyoh 2020). The use of nutrient polluted waters may also cause severe health issues like cancer, immune system suppression, reproductive and developmental problems, and disruption of the nervous and endocrine system. Many chemicals, physical-biological, and nanotechnology methods have been developed to remove nutrients (nitrates and phosphates) from contaminated water (Rocca et al. 2007; Karthikeyan et al. 2020).

2 Method for the Removal of Nitrates and Phosphates

2.1 Chemical Methods

In the chemical process, inorganic metal-based chemicals compound are used to precipitate the nutrients by using salts like iron, aluminum, lime, calcium, and magnesium. In the chemical process, soluble cationic salts react with reagent ions like nitrates and phosphates, present in wastewaters to form precipitations (Ruzhitskaya and Gogina 2017; Jutidamrongphan et al. 2012). Di or tri valent metals such as calcium, magnesium, and aluminum-based salts can act as an effective regent for removing nitrates and phosphates (de-Bashan and Bashan 2004). For example, Aluminum hydroxide is a good absorber for orthophosphate. Aluminum hydroxide reacts with organic phosphates to form precipitate at pH 3.6 (Kumar and Viswanathan 2020; Manikam et al. 2019; Belkada et al. 2018). In contrast, the activated alumina can remove very low concentrations (1 mg/L) of phosphates. Magnesium salts are also used to remove nitrogen and phosphorus. About 83% of ammonia and 97% of phosphorus are removed simultaneously at pH 10.5 (Carvalho et al. 2007; Okano et al. 2015; Chakraborty et al. 2017). In comparison, chemicals like lime and metal salts remove 70–90% phosphorus (Sumathi and Vasudevan 2019).

2.2 Adsorbents

Inorganic nitrate or nitrites (N) and phosphates (P) are removed using different adsorbing agents. Generally, activated carbon materials such as charcoal, carbon powder, carbon cloth, carbon dots (CDs), and carbon nanotubes (CNTs) are commercially available. These carbon-based sorbents are natural materials. They are used in various water purification techniques to remove various toxic pollutants and recover N, P, and S from water bodies. Various inorganic anions such as fluoride, nitrites, bromate, and perchlorate were removed using carbon-based sorbents (Ji et al. 2020; Zheng et al. 2020).

The bamboo carbon powder was produced by calcining bamboo waste material at 900 °C in an electrical furnace. The charcoal from bamboo has more nitrates adsorption ability than activated carbon powder. Due to the high adsorption efficiency of Bamboo charcoal powder, it was used to remove nitrates from aquatic bodies (Li et al. 2020a, b; Rezvani et al. 2019; Yazdi et al. 2019). However, the nitrates removal efficiency of carbon nanotubes (CNTs) was higher than the activated carbon powder. CNTs can become more efficient at pH 5.0. Hydrous aluminosilicates such as clays or a mixture of finely grained clay minerals and other crystals like carbonate, quartz, and metal oxides are also employed to remove nitrates from contaminated water (Battas et al. 2019; Jaafari et al. 2019).

Furthermore, nanotechnology is an excellent alternative technique that can be used to remove nutrients from wastewater. The hybrid nanoconstructs of Fe/Ni and zeolite form bimetallic nanoparticles. These nanoparticles have been used to eliminate nitrates and phosphates concurrently from contaminated water (He et al. 2018). Ultrafiltration (UF) or nano-filtration (NF) and reverse osmosis (RO) membranes are have been in municipal wastewater treatments. Especially, selected inorganic nutrients like nitrates and phosphates are removal rate achieved 90% on a pilot scale using reverse osmosis and electro-dialysis techniques with the help of UF/NF/RO membranes (Aziz and Kasongo 2021). In conventional treatments, only 10–20% of phosphate is removed by primary treatment, 10–25% by activated sludge, and 8–12% by trickling filters and rotating biological contactors.

2.3 Bioremediation

Bioremediation is a biological process. In this process, the living organisms act as scavengers and may be photoautotrophic or heterotrophic. To grow and energy production, they uptake nutrients such as nitrates, phosphates, and other essential elements from their surroundings. Nutrient removal by physical and chemical processes is much more expensive, and they are pH and temperature-dependent and require post-treatment that may lead to byproduct formation. Photoautotrophic microorganisms like *Chlorella*, *Dunaliella*, *Nannochloropsis oculata*, *and Tetraselmis chuii* are photosynthetic green microalgae. Photosynthetic organisms use sunlight and water to create energy in sugars. They absorb nitrate (NO_3^-) or nitrite (NO_{2^-}) and phosphate (PO_4^{3-}) from their surroundings to grow, and also they accumulate high-energy molecules like lipids, pigments, and carbohydrates. Some heterotrophic microbes such as *micrococcus*, *pseudomonas*, *Bacillus* sp., and *Achromobacter* can perform the denitrification process (Lee et al. 2009; Nakarmi et al. 2020).

Microalgae are a diversified group of living organisms, photoautotrophic eukaryotic, and prokaryotic cyanobacteria, which are habituated into both marine and freshwater bodies (Lee 2008). There are about 200,000–800,000 species of microalgal diversity in which about 50,000 species are described (Starckx 2012). Algae are multitasking organisms. They can perform multiple roles such as bioremediation, carbon sequestration, and generation of valuable bio-molecules. Algal-based products have high demand and are used in the food industry, nutraceutical and pharmaceutical industries, and bio-feed and biofuel productions (Sood et al. 2012; Ali et al. 2013; Franchino et al. 2013; Richards and Mullins 2013). The growth of microalgae mainly depends on nitrate and phosphate availability as the wastewater contains a high nutrient load of nitrates and phosphate which can serve as a nutrient medium for algal cultivation. Employing microalgae for the nutrient removal process or wastewater treatment can be considered one of the most eco-friendly, simple, and cost-effective than other conventional wastewater treatment processes (Table 2).

Chlorella vulgaris is a photosynthetic eukaryotic microscopic green algae. Many researchers find that the *Chlorella* species are the most promising algae used for wastewater treatment (Chiu et al. 2015; Otondo et al. 2018; Shetty et al. 2019). *Chlorella* species can tolerate physic-chemical changes under nutrient limitation conditions. In addition, the synthesis and accumulation of high lipids as stored food

Table 2 Bioremediation of inorganic nitrates and/ or nitrites using microalgae

S. No	Name of the algae	Biomass (g L^{-1} d^{-1})	Nitrate and/or nitrite remediation (mg L^{-1} d^{-1})	References
1	*Scenedesmus accutus pvuw12*	0.25	6.26	Doria et al. (2012)
2	*Chlorella vulgaris*, *Dunaliella tertiolecta*	0.6 0.83	3.2, 3.1	Hulatt et al. (2012)
3	*Chlorella* sp.	ND	2.65	Wang et al. (2010)
4	*Chlamydomonas reinhardtii*, *Scendemus rubescence*, *Phormidium* sp.	ND ND ND	0.16, 0.13, 0.1 –	Garbayo et al. (2000)
5	*Neochloris oleoabundans*	0.63	150	Li et al. (2008)
6	*Coelastrella* sp.	ND	8.75	Gardner et al. (2011a)

(continued)

Table 2 (continued)

S. No	Name of the algae	Biomass (g L^{-1} d^{-1})	Nitrate and/or nitrite remediation (mg L^{-1} d^{-1})	References
7	*Scenedesmus obliquus*	0.17	6.56	Nuez et al. (2001)
8	*Chlorella sorokinia*, *Spirulina platensis*	ND ND	3.3, 7.2	Ogbonna et al. (2000)
9	*Palmaria palmate*, *Chondrus cripus*	ND ND	4.96, 11.21	Corey et al. (2013)
10	*Nannochloropsis gaditana*	ND	7.71	Ren and Ogden (2014)
11	*Heamatococcus pluvialis*	ND	8.48	Kang et al. (2006)
12	*Botryococcus braunii*	ND	22.21	Sydney et al. (2011)
13	*Neochloris oleobundans*	3.15	43.7	Wang and Lan (2011)
14	*Trentepohlia urea*	0.002	1.1	Abe et al. (2008)
15	*Chlorella kessleri*	ND	4.6	Lee and Lee (2001)
16	*Monoraphidium sp. 92*, *Scenedesmus sp. 131*	0.07, 0.09	49.5, 33	Eustance et al. (2013)
17	*Scenedesmus* , *Coelastrella saipanensis*	0.83, 0.71	0.83, 7.83	Gardner et al. (2011b)
18	*Chlorella sp.*	ND	8.5	Marcilhac et al. (2014)
19	*Muriellopis* sp., *P. subcapitata*	1.13, 1.02	47.5, 27.5	del Mar Morales-Amaral et al. (2015)
21	*Chlorella vulgaris*	0.195	9.8	Cabanelas et al. (2013)
22	*C. reinhardtii*	2	55.8	Kong et al. (2010)
23	*Chlorella sorokoniana*	0.13	18.5	Chen et al. (2018)
24	*Desmodesmus* sp. *PW1*	ND	36.47	Chen et al. (2020)
25	*Desmodesmus* sp. *EJ9-6*	0.029	4.542	Ji et al. (2014)
26	*Desmodesmus* sp.	0.026	0.0833	Ji et al. (2015)
27	*S. obliquus*	ND	77.5	Ruiz-Martínez et al. (2015)
28	*Chlorella vulgaris*, *Spirulina platensis*	ND ND	28.62, 25.46	Sayadi et al. (2016)
29	*Chlorella vulgaris*	ND	10.5	Malla et al. (2015)
30	*Chlorella* sp. CSIRCSMCRI	0.016	0.062	Present work report

Note g L^{-1} d^{-1} = gram per liter per day, mg L^{-1} d^{-1} = milligram per liter per day, ND—not determined material. *Chlorella* species also have high efficiency for removing nitrogen and phosphorus from wastewater (Chiu et al. 2015; Guldhe et al. 2017; Chen et al. 2018). Due to such unique characteristics, the genus *Chlorella* large scale cultivations are more attractive and play an important role in wastewater treatment, as well as they can regenerate bioenergy that can be used for sustainable energy development (Mussgnug et al. 2010; Collet et al. 2011; Mahdy et al. 2014; Klassen et al. 2016, 2017) (Table 3).

Table 3 Bioremediation of inorganic phosphates using different microalgae

Sr. No	Name of the algae	Biomass (g L^{-1} d^{-1})	Phosphates remediation (mg L^{-1} d^{-1})	References
1	*Monoraphidium minutum* sp., *Tetraselmis suecica* sp.	0.039, 0.034	0.49, 0.45	Patel et al. (2012)
2	*Muriellopis* sp., *P. subcapitata*	1.13, 1.02	3.8, 2.7	del Mar Morales-Amaral et al. (2015)
3	*N. gaditana*	ND	5.7	Sepúlveda et al. (2015)
4	*Chlorella vulgaris*	0.195	3.0	Cabanelas et al. (2013)
5	*C. reinhardtii*	2	17.4	Kong et al. (2010)
6	*Chlorella sorokoniana*,	0.13	3.63	Chen et al. (2018)
7	*Spirulina platensis*	1.67	18.12	Bahman et al. (2020)
8	*Scenedesmus dimorphus*	0.58	32.68	Lutzu et al. (2016)
9	*Desmodesmus* sp. *EJ9-6*	0.029	0.326	Ji et al. (2014)
10	*Desmodesmus* sp.	0.026	0.033	Ji et al. (2015)
11	*Phormidium bohneri*	ND	1.6–13.8	Whitton et al. (2015)
12	*Chlorella vulgaris*, *Spirulina platensis*	ND, ND	49.5, 45.83	Sayadi et al. (2016)
13	*Dunaliella salina*	ND	0.09–0.29	Eka Putri and Moh. Muhaemin (2010)
14	*Chlorella vulgaris*	0.6	2.0	Malla et al. (2015)
15	*Chlorella pyrenoidosa* CUF_808, *Chlorella vulgaris ACUF_808*	0.11, 0.08	5.1, 4.96	Osorio et al. (2020)
16	*Chlorella* sp. CSIRCSMCRI	0.016	0.026	Present work report

Note g L^{-1} d^{-1} = gram per liter per day, mg L^{-1} d^{-1} = milligram per liter per day, ND = not determined

3 Removal of Inorganic Nutrients Using *Chlorella* sp. CSIRCSMCRI

3.1 Collation of Wastewater and Nutrients Estimation

Fisheries wastewater samples were collected from the Veraval port location. After that, water samples were filtered with the help of Whatman filter paper grade No 40 to remove solid particles. Filtered water samples nutrients such as nitrate, nitrite, and phosphates were estimated by the spectrophotometric method (Isiuku and Enyoh 2020). Subsequently, the removal of trace metal was also determined using ICP-MS.

3.2 Experimental Design for the Removal of Inorganic Nutrients from Wastewater Using Chlorella *sp. CSIRCSMCRI*

The most dominant green microalgae *Chlorella* sp., was isolated from CSIRCSMCRI Salt farm (Lt: 21° 46′ 51.888″ N; Ln: 72° 7′ 33.7908″ E) Kumbharwada, Bhavnagar, and Gujarat. The axenic unialgal culture was developed by the standardized spread plate method. After that, unialgal cultures growth was optimized in the laboratory in BG11 media then high cell density seed cultures were developed for the experimental study (Fig. 4). To determine the nutrient removal capacity of *Chlorella* sp., the seed cultures were inoculated into three flasks (5 L) containing 2.5 L of filtered Veraval wastewater samples. The experimental flasks were kept in laboratory conditions at constant temperature (25 ± 1 °C) and light (1000 μmol s^{-1} m^{-2}) for 12 h and dark periods (12 h).

During the cultivation period, in every 5 days interval, 50 ml of cultures were taken, and cell density was measured using a spectrophotometer (EPOCH, BioTek, USA). At the same time, biomass was harvested by centrifugation of cultures. Then the fresh biomass pellets weights were taken, and specific biomass growth rates were determined. After that, biomass samples were treated with 75% methanol, and Chlorophyll content was determined using a spectrophotometer. The nutritional remediation of the *Chlorella* sp. was determined by nitrates, phosphates estimation protocols at the initial day (0 days) and end of the cultivation (30 days). Similarly, trace metal remediation was determined by ICP-MS.

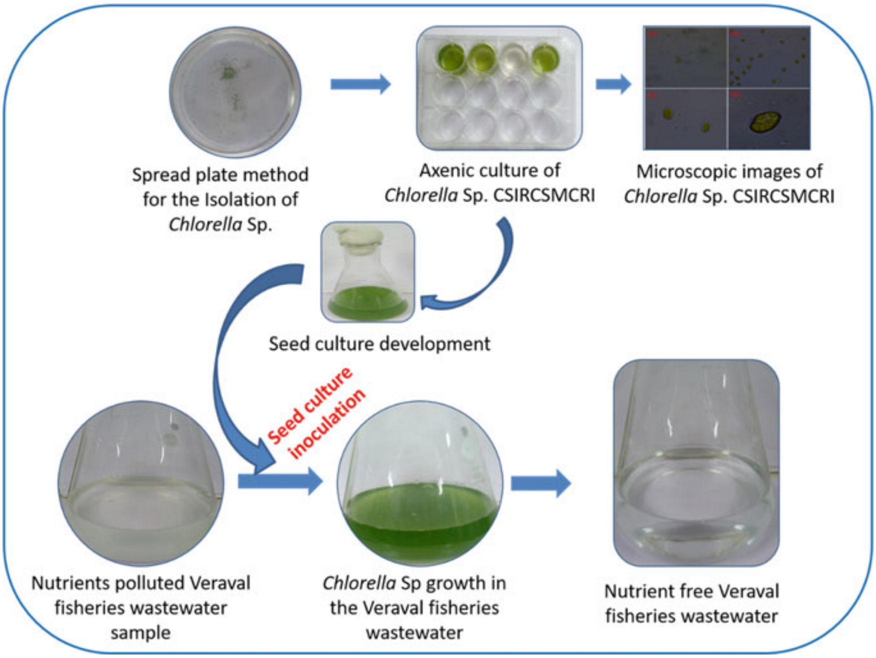

Fig. 4 Schematic flow chart of isolation and bioremediation of inorganic nutrients from wastewater using microalgae at laboratory scale

3.3 Nutrients Uptake and Growth of **Chlorella** *sp. CSIRCSMCRI*

Nitrates and phosphates concentrations were under WHO (World Health Organization) limits in Veraval fisheries wastewater in the monsoon session (July 2021). The remediation of nutrients such as nitrite, nitrate, phosphates, and other trace elements concentrations decreased after 30 days in *Chlorella* sp. in Veraval wastewaters. Especially, the nitrate (NO_3^-) and phosphates (PO_4^{3-}) concentrations were almost zero on the 30th day of cultivation (Fig. 5). Similarly, the cationic trace metals such as iron (Fe), manganese (Mn), copper (Cu), nickel (Ni), strontium (Sr), molybdenum (Mo), and silver (Ag) were also quantitatively decreased on the 30th day. It represents that the isolated algae *Chlorella* sp. were completely removed the inorganic anionic nutrients (NO_3^- and PO_4^{3-}) from wastewater samples. Whereas only 53% of Fe and 54% of Ag remediation were noticed, a few cationic trace metals (Fig. 6). Moreover, the Chlorophyll a (*Chlo a*) content was increased exponentially up to 25 days after that *Chlo a* content was decreased at the end of the cultivation (30 days) (Fig. 7a). Similarly, the cell density of cultures was also exponentially increased up to 25 days, followed by the same were declined at the end of the cultivation (Fig. 7b). From these observations, the available nutrients enhance the growth of *Chlorella* sp up to

25 days. After that, the same was declined. It may be due to a lack of nutrients in media.

During 30 days cultivation period, the *Chlorella* sp. biomass was exponentially increased from 0.22 to 0.77 g/L up to 15 days cultivation. After that, the biomass growth was reduced from 0.77 to 0.6 g/L, 0.57 and 0.48 g/L on 20, 25, and 30 days, respectively (Fig. 8a). However, the specific biomass growth rate (μ) was decreased

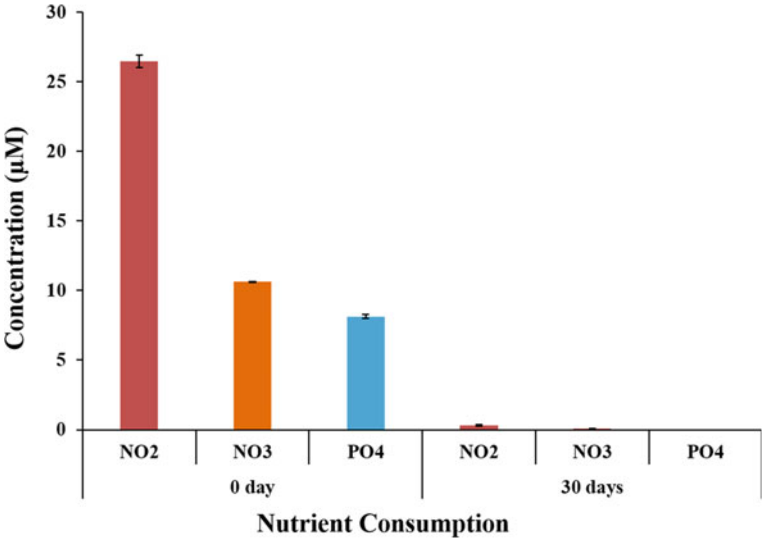

Fig. 5 Removal of anionic nutrients from the Veraval wastewater samples using *Chlorella* Sp. CSIRCSMCRI

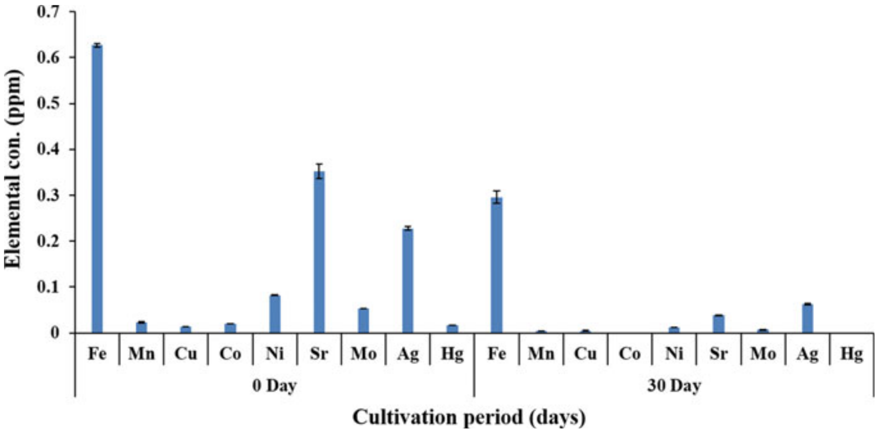

Fig. 6 Removal of trace elements from the Veraval wastewater using *Chlorella* sp. CSIRCSMCRI

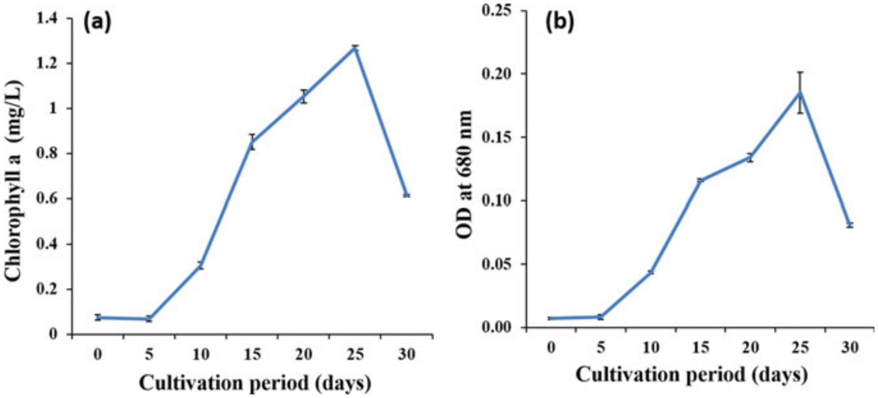

Fig. 7 Growth parameter of *Chlorella* sp. cultures in Veraval wastewater. **a** Chlorophyll a content. **b** Cell density curve

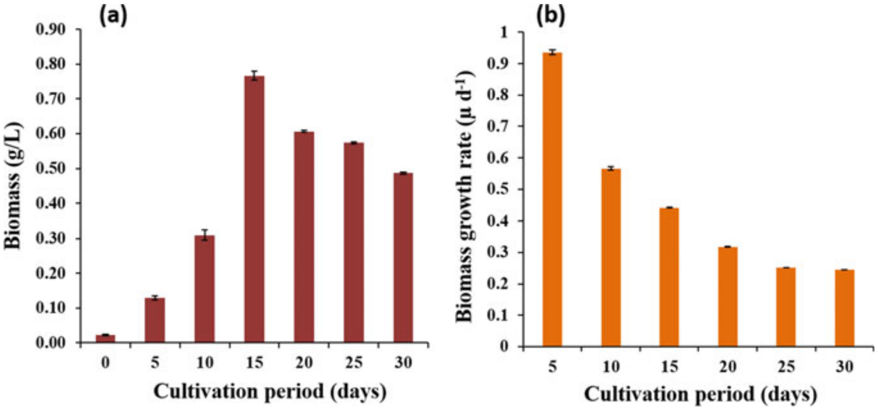

Fig. 8 Growth response of *Chlorella* sp. in the Veraval wastewater samples (**a**) Biomass production (**b**) specific biomass growth rate during 30 days cultivation

over time (Fig. 8b). These observations confirmed that due to the starvation of nutrients in water samples, *Chlorella* sp. biomass growth was stopped after the 15th day. After that, nutrient starvation and biomass growth were increased.

4 Conclusion

Nutrients like nitrates and phosphates are essential for life, but too much overloading of these anions in the environment might cause eutrophication and non-carcinogenic risks. In the present study, nitrates and phosphates concentration were under WHO limits in Veraval fishers wastewater in the monsoon session (July 2021). The isolated algae *Chlorella* sp. CSIRCSMCRI have great toxic resistance and nutrients up taking capacity. Especially, anionic essential nutrients such as nitrates and phosphates concentrations were almost zero in Veraval wastewater samples in the presence of *Chlorella* sp. at the end of the cultivation. It confirmed that the algae *Chlorella* sp. CSIRCSMCRI have better adaptation potential for various pollutants.

In conclusion, the inorganic nutrients were effectively removed from wastewaters using microalgae. The algae-treated wastewater samples were physically clear and nutrients free. The treated wastewater can be recommended to reuse for irrigation, recharge of aquifers, and toilet flushing.

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Bioremediation of Organic and Heavy Metal Co-contaminated Environments

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1 Introduction

Soil and water cleanness are very crucial for the sustainability of human life on the Earth. However, irresponsible industrial and agricultural activities have deteriorated many of the natural environments through the usage and the dumping of toxic chemicals and materials into the environment (Alabi et al. 2019). Thus, environmental contamination with such hazardous materials became a serious problem that affects both natural biodiversity and human health (Aparicio et al. 2018). The contaminants that reach the environment are either organic pollutants (OPs), inorganic metals/ions, radioactive isotopes, organometallic compounds, air pollutants, or nanoparticles that developed from the growth of their usage in different applications (Briffa et al. 2020). Moreover, it is known that the pollutants discharged to the environment as mixtures rather than a single contaminant. Among all these various types

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of contaminants, both the persistent organic pollutants and heavy metals (HMs) are the worst types of contaminants either in soil or in water bodies (Liu et al. 2015). HMs such as copper, nickel, cadmium, and cobalt are defined to be toxic to both humans and the environment including animals, plants, and microorganisms (Briffa et al. 2020; Sandrin and Hoffman 2007). However, the HMs-contaminated sites are usually co-contaminated with other OPs such as oil, and chlorinated solvents, pesticides, and herbicides (Sandrin and Hoffman 2007). The conventional remediation methods are less effective in these instances; thus, bioremediation is the most effective and low-cost tool for such environments, but co-contaminated sites require a better understanding of the remediation mechanisms, factors influencing the remediation process as well as the species engaged in the process. A selective group of microorganisms including bacterial, microalgal, and fungal species has been exposed to possess potential tackle to degrade numerous OPs and to eliminate HMs from various contaminated environments (Sun et al. 2021), and that likewise owing to their capacity to adapt to harsh environments (El Fantroussi and Agathos 2005). To this end, in this book chapter, we will give a brief introduction to different mechanisms that might be used for bioremediation of co-contaminated sites by utilizing different types of organisms coupled with other biotic or abiotic factors to tackle the pollution problem in co-contaminated sites.

2 Metal Speciation and Bioavailability

Metals are found in the environment in different forms called meal species as a result of a speciation phenomenon of metals. Speciation can be widely described as the identification and quantification of the various, defined forms, species, or phases anywhere an element occurs (Olaniran et al. 2013). The oxidation state, coordination, and physical state or establishment with other phases all commit to defining metal speciation (Reeder et al. 2006). At the same time, bioavailability is another term that describes a metal species. Bioavailability could be explained as the portion of the HMs that can be incorporated in structural or biological functions (de Paiva Magalhães et al. 2015). The integration of these metals could be described as either external or internal, the external, is stating the capability of metals to be liquefied and discharged from the soil matrix or other media while internal refers to the ability of metals to be utilized succeeding internally inside cells or tissues (Kim et al. 2015).

3 Heavy Metals Toxicity

Heavy metals are termed such because of their high density compared to pure water (Tchounwou et al. 2012a). Additionally, it is distinguished by incomplete d-orbital, thus it tends to bind to different biological molecules such as proteins, deoxyribonucleic acid (DNA), and cell wall components. For microbial cells, these metals are vital

Table 1 Toxicity of heavy metals to microorganisms (Fashola et al. 2016)

Heavy metals	Effects of microbe
Zinc	Death, decrease in biomass and growth-inhibitory
Nickel	Hinder enzyme activities oxidative stress, and distraught cell membrane
Chromium	Inhibits the growth, increases the lag phase, and reduces oxygen uptake
Copper	Interrupt cellular functions and reduce enzymatical activities
Mercury	Inhibits enzymes and disrupts membrane integrity
Lead	Inhibits transcription and damages nucleic acid
Selenium	Prohibits growth
Cadmium	Damages nucleic acid, denature protein, and slow down cell division and transcription

in low concentrations for cell integrity and physiology, but in elevated concentrations, these metals bind none-specifically to the biological molecules which hinder their function leading to toxic effects. In another word, the formation of strong complexes between the metal and the organic molecules interferes with the normal biological functions leading to the death of the cell (Tchounwou et al. 2012b). The uncontrolled disposal of HMs into soil and water is a serious health issue across the world since they cannot be degraded to non-toxic forms and so these metals have a long-term impact on the environment (Briffa et al. 2020). The toxic effects of these metals start when these metals infiltrate into the cells and then bind to the negatively charged molecules such as teichoic acid and lipopolysaccharides in Gram-positive and Gram-negative bacteria, respectively (Sandrin and Hoffman 2007). However, the binding to intracellular molecules is mainly based on the metal-binding affinity, for example, the lead, and cadmium tend to bind to sulfur-containing targets within the cell and they can bind as well to rhamnolipids produced by some microbes (Sandrin and Hoffman 2007; Singh and Cameotra 2004). Therefore, understanding the binding affinities of the HMs will assist in the selection of the toxicity reduction mechanism of the microbe.

Due to these metals' species, bioavailability and toxicity are controlled by the chemical structure and the pH value, which makes it not easy to be compared between studies due to the variation between the experimental settings and sampling procedures. Table 1 lists some of the HMs and their toxicities mechanisms on a different component of the microbial cells.

4 Bioremediation

Heavy metals in soil have a detrimental effect on soil microorganism respiration, metabolism, and activity, as well as the conversion of organic carbon to bio-carbon. Because of the harmful effects of HMs on ecosystems and creatures that rely on them, there is a pressing need to clear the environment of pollution caused by these poisonous substances (Kapahi and Sachdeva 2019).

Undoubtedly, bioremediation is gradually becoming the standard practice for restoring heavy metal contaminated soils as mentioned before because it is more environmentally friendly and cost-effective than traditional chemical and physical methods, which are often very expensive and ineffective when metal concentrations are low, as well as producing significant amounts of toxic sludge (Ayangbenro and Babalola 2017; Ekperusi and Aigbodion 2015). Moreover, because most heavy metal salts are water-soluble and dissolve in wastewater, physical separation methods cannot be used to separate them (Hussein et al. 2004).

5 Microbial-Based Bioremediation Mechanisms

Due to the non-biodegradability of metals, concern regarding their remediation is greater than that of OPs. However, the co-existence of OPs and metals in the same space may further complicate the overall remediation process since remediation of each substrate confer some slightly specific procedures (Chen et al. 2021). However, it transpired that tackling organic-metal co-contamination can be efficiently performed by various microorganisms. Primarily, there is a theory substantiating metal resistance capacity in microorganisms due to their emergence in the initial period of life whereby toxic metals were readily present in nature. Moreover, statistical evidence has inferred that the evolution of metal resistance and detoxification mechanisms in microorganisms is a consequence of rising environmental pollution from metals produced by various anthropogenic activities (Roane et al. 2015).

Innumerable efficient mechanisms are involved in bioremediation by diverse kinds of microorganisms which can be understood by a diversity of examples. Therefore, here, we are prioritizing explaining the microorganism's capacity in bioremediation through the various mechanisms they possessed namely biosorption, bioaccumulation, biotransformation, biomineralization, and bioleaching.

6 Biosorption

Biosorption is a passive, mostly reversible process that is metabolism independent, whereby particles get adsorbed to a biological matrix through physical interactions (e.g., electrostatic forces), chemical interactions (e.g., ion or proton displacement), complexation, or chelation. Due to that, the extracellular surface of microorganisms is negatively charged at neutral pH, this would attract positively charged HMs in the liquid phase (Diep et al. 2018). Among several strategies utilizing microorganisms to overcome the toxicity of HMs, the biosorption process has a distinguishing role in the bioremediation of HMs by their ability to sequester the dissolved HMs even from very dilute complex solutions with high competence (Ahemad and Kibret 2013).

By way of illustration, *Streptomyces rochei* ANH, a marine actinomycete isolate, was reported to demonstrate a good ability in removing metal pollutants like Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, and Cr^{6+} present in industrial wastewater. Besides, a 90% increase of *Lepidium sativum* seed germination was reported in the tannery wastewater treated with *S. rochei* ANH biomass than that in the untreated one, which may also indicate the removal of phytotoxin which affects the seed germination and plant growth (Hamdan et al. 2021). Moreover, *Shewanella putrefaciens* reached 86.5% biosorption of cadmium from the initial concentration of 20 mg/L and pH 5.0 (Yuan et al. 2019). Likewise, *Escherichia coli* is capable of absorbing more HMs than other bacterial species (Jin et al. 2018).

7 Bioaccumulation

Associated with the microorganisms' metabolism, bioaccumulation is the active uptake of heavy metal into the cytoplasm by utilizing importer complexes that form a translocation channel in the cell membrane. The heavy metals could then be sequestered by protein and peptide ligands in the cytoplasm (Diep et al. 2018). As reported by AbdElgawad et al. (2021), it was shown that arsenic bioaccumulation by barley and maize plants was decreased by inoculating the soil with a halophilic bacterium *Nocardiopsis lucentensis.* Moreover, in presence of metal pollutants, citric acid produced by some plant roots lower the pH of the rhizosphere that could serve as a chelating agent (de Sousa et al. 2019). Hence, higher arsenic retention in the soil was hypothesized to have been secreted by both barley and maize plants from the soil inoculated by *N. lucentensis,* this was an indication of higher arsenic uptake inhibition in barley and maize plants (AbdElgawad et al. 2021). Remarkable microbes have been reported by their active transport systems for HMs, for instance, *Pseudomonas putida and Bacillus cereus* for cadmium, *Thiobacillus ferrooxidans* for silver, *Citrobacter* sp. for both cadmium and lead, *Bacillus subtilis* for chromium, *Aspergillus niger* for thorium, *Rhizopus arrhizus* for mercury, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* for uranium, and finally *Micrococcus luteus* for strontium (Sun et al. 2021).

8 Biotransformation

As bio-remedying of metal pollutants does not result in degradation, in a process called biotransformation, metals' physical and chemical characteristics can, however, be modified resulting in conversions to a low water-soluble element or a low toxic water-soluble element (Emenike et al. 2018). This change is achieved by biotransformation agents utilizing their enzymes and metabolites. According to Vaidya et al. (2017), complete degradation performed by only one kind of organism may be hampered by the presence of xenobiotic compounds, which can be degraded and metabolized through synergistic interactions between more than one microorganism in a consortium. In the study of *Burkholderia sp.* ASDP2, *Rhodococcus sp.* ASDP3, and *Pseudomonas sp.* ASDP1, these bacteria were developed as a bacterial consortium called consortium PBR that could metabolize pyrene (a polycyclic aromatic hydrocarbon) efficiently as the sole source of carbon and energy via the phthalic acid pathway. Apart from pyrene, six different hydrocarbons could be degraded by this consortium without any nutrient supplementation in the Bushnell-Haas medium (Bushnell and Haas 1941).

In another study, five bacterial isolates developed could serve as an effective benzo(a)pyrene degradation system when being associated with *Melia azedarach* plant. All bacteria comprised in the consortium could help in the growth of the plant. Besides, *Paenibacillus sp.* S1I8 and *Bacillus flexus* S1I26 were known as biosurfactant-producing while *Bacillus subtilis* SR1, *Serratia marcescens* S2I7, *Staphylococcus arlettae* S1I1 were known for high cadmium resistance and benzo(a)pyrene (BaP) degrading capacity. After 60 days, higher degradation of benzo(a)pyrene was reported in soil spiked with BaP and Cd due to the plant-microbe association than that in bulk soil (Kotoky and Pandey 2020). Furthermore, the functional abundance of bacteria degrading several xenobiotic compounds like benzoate, ethylbenzene, toluene, naphthalene, PAHs, and xylene was reported to have increased upon the co-existence of cadmium and benzo(a)pyrene (Kotoky and Pandey 2020).

9 Biomineralization

In biomineralization, microorganisms can transform ionic metals to solid minerals in their cells or tissues in the presence of certain organic or inorganic compounds. For instance, the presence of calcium helps fungi to produce calcite minerals from microbial-induced carbonate precipitation (MICP)-like mechanism (Dhami et al. 2017; Zhang et al. 2020). In another hand and as reported by Dhami et al. (2017), a ureolytic fungi *Aspergillus* sp. UF5 and *Fusarium oxysporum* UF8 were capable of calcium carbonate biomineralization as well as lead and strontium co-precipitation. While both isolates could survive high metal concentrations, they demonstrated different capabilities in metal biomineralization. In only calcium medium calcium biomineralization by *Aspergillus* sp. UF3 and *Fusarium* sp. UF8 were 78 and 34%, respectively. Further heavy metal co-precipitation experiments showed lead and strontium precipitation by *Aspergillus* sp. UF3 and *Fusarium* sp. UF8 were 34 and 48% versus 54 and 31%, respectively. In the ureolytic pathway, calcifying fungi would hydrolyze urea to carbonate which would then spontaneously hydrolyze to ammonia and carbonic acid. Equilibration of these products in water resulted in bicarbonate formation resulting in pH increase upon the formations of ammonium and hydroxide ions. In such an alkaline environment, the presence of calcium as well as HMs and radionuclide would result in calcite formation and co-precipitation of lead and strontium. Overall, the biomineral precipitates reported were calcite, vaterite, aragonite as well as carbonates, and hydroxides of lead and strontium were found to be associated with fungal hyphae which could have been nucleation sites.

10 Bioleaching

Biomining explains both bioleaching, which involves the complexation processes, or mobilization of positive heavy metal ions from insoluble ores often by biological dissolution (Brunetti et al. 2012), and bio-oxidation (Rahman and Sathasivam 2015). Acidophilic microorganisms can utilize the microbial bioleaching process for enhancing the solubilization of solid-phase HMs from the sediment, targeting the fractions of HMs associated with iron or sulfur minerals (Sun et al. 2021). Bioleaching is performed by a diverse group of microorganisms that can affect the metal sulfide dissolution by Fe^{2+} (ion II) and S (sulfur compound) oxidation. In that way, Fe^{3+} (ion III) and protons, the metal sulfide-attacking agents, are available by the mediation of cell attachment as a result of the positive charge in Fe^{3+} (ion III) (Vera et al. 2013).

Major sulfide-dissolving microorganisms of sulfur-oxidizing bacteria as Proteobacteria (e.g., *Acidithiobacillus*, *Acidiphilium*, *Acidiferrobacter*, *Ferrovum*) are most regularly used in soil remediation with acidification to pH 4 and the optimal temperature within the range of 15–55 °C, depending on the strain (Deng et al. 2012), Nitrospirae (e.g., *Leptospirillum*), Firmicutes (e.g., *Alicyclobacillus*, *Sulfobacillus*), Actinobacteria (e.g., *Ferrimicrobium*, *Acidimicrobium*, *Ferrithrix*), and archaea such as Crenarchaeota (e.g., *Sulfolobus*, *Acidianus*, *Metallosphaera*, *Sulfurisphaera*) (Vera et al. 2013).

Likewise, fungi bioleaching has been considered as high potential for metal reclamation from contaminated solid wastes, electronic materials, soils, and low-quality ores (Yang et al. 2019). As reported by Johnson (2010) this method was successfully used to leach Cu (copper ion) from contaminated soils both in situ as on the heaps and ex-situ as in bioreactors, an instance of citric and gluconic acids, that produced by fungi and actinomycetes, mostly of the genus *Aspergillus*, (e.g., *A. niger*), besides other genera, including *Penicillium* and *Fusarium* (Deng et al. 2012; Valix et al. 2001). Cobalt (Co) recoveries were obtained by using a culture filtrate of *A. niger* with 24 and 60% from laterites and black shale deposits, respectively (Anjum et al. 2010; Biswas et al. 2013). In addition, *A. niger* magnificently recovers 97% Copper (Cu), 98% Nickel (Ni), 86% Cobalt (Co), 91% Manganese (Mn), and 36% Iron (Fe) from Indian Ocean manganese nodules (Mehta et al. 2010; Yang et al. 2019). The summary of different mechanisms of HMs remediation is shown in Fig. 1.

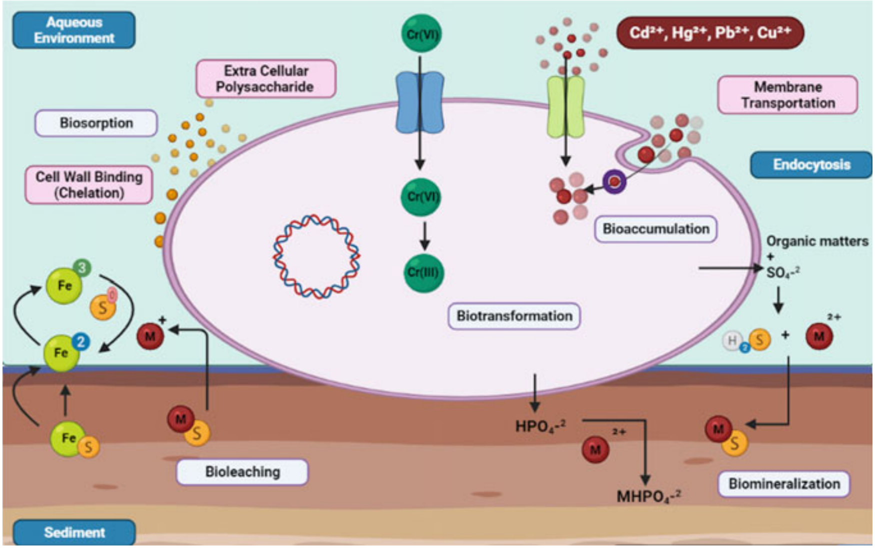

Fig. 1 Scheme of bioremediation mechanisms for heavy metals and other toxic compounds in co-contaminated sites

11 Where Does Metal Remediation Take Place?

The different mechanisms early summarized are only concerned about the remediation process itself, but where it happens is also important. This is very crucial to decide on the recovery methods of the metals remediated or to decide on the approach of reusing the microbial cells. For this sake, two general mechanisms are out there, intracellular sequestration and extracellular sequestration.

12 Intracellular Sequestration

Metal ions are complexed by different molecules in the cell cytoplasm, resulting in intracellular sequestration. The interaction of metals with surface ligands, followed by delayed transit within the cell, can result in a high concentration of metals within microbial cells (Igiri et al. 2018). Metal ions form a complex in the cytoplasm with the metal-binding peptides during intracellular sequestration. The capability of bacteria to collect metals intracellularly has been used in a variety of applications, most notably in wastewater treatment, for instance, *Pseudomonas putida* as a cadmium-tolerant strain, with the aid of low molecular weight cysteine-rich proteins, able to sequester an array of metals including cadmium, copper as well as zinc ions intracellularly (Higham et al. 1986). However, in *Rhizobium leguminosarum*, glutathione was shown to be engaged in the sequestration of cadmium intracellularly (Lima et al. 2006).

13 Extracellular Sequestration

Cellular components in the periplasm or outer membrane form complexes with metal ions in extracellular sequestration. Thus, metal ion complexation can result in the production of insoluble compounds (Igiri et al. 2018). Copper-inducible proteins are found to be synthesized by *Pseudomonas syringae*, a copper-resistant strain. These proteins bind to copper ions and collect them, turning bacterial colonies blue (Cha and Cooksey 1991). Extracellular sequestration of metals may take place as metal precipitation. As an example, iron-reducing bacteria, such as *Geobacter* spp., and sulfur-reducing bacteria, *Desulfuromonas* spp., can reduce dangerous metals into lesser toxic metals. Sulfate-reducing bacteria produce plenty of hydrogen sulfide, which causes metal cations to precipitate (Ayangbenro et al. 2018). It has been found that *Klebsiella planticola* under anaerobic conditions tends to produce hydrogen sulfide from the reduction of thiosulfate and finally precipitates cadmium ions in the form of sulfides (Sharma et al. 2000). Moreover, the soluble divalent lead was successfully separated by precipitation after the formation of an insoluble complex composed of a lead phosphate salt. This was accomplished by the *Vibrio harveyi* (Mire et al. 2004). In the extracellular environment, *Desulfovibrio desulfuricans* create hydrogen sulfide, which shields the host cell against heavy metal toxicity through metal ion precipitation (Yin et al. 2019).

14 Phytoremediation in Co-contaminated Sites

Phytoremediation is another encouraging approach for remediation of the contaminants released to the environment and encompassing the cultivation of the plant. The phytoremediation of OPs is described in different steps; the first one is the absorption of the pollutant and transforming it into an essential component of the plant. However, in the case of metals phytoremediation, it is describing the absorption and the accumulation of the metals inside different plant parts. The most important trait of the plant to be used is the ability to tolerate the toxicity of the contaminants. Phytoremediation tends to selectively uptake different HMs. Table 2 lists some examples of plants species and their respective HMs that could be uptake.

In the application of co-contaminated sites, phytoremediation was used for spent lubricant oil that has been released to the environment. It has been found that using castor oil plant seeds (*Ricinus communis L.*) was successful in the uptake of Mn, Ni, and Pb and transported them to the shoot system specifically on the leaves (Vwioko et al. 2006). Nevertheless, phytoremediation processes have advantages and limitations in the removal of HMs from tropical soil as documented by Meenambigai et al. (2016) and Susarla et al. (2002) and summarized in Table 3

Table 2 Selective plants for heavy metals phytoremediation

Plant species	Heavy metal	Reference
Ricinus communis L.	Mn^{2+}, Ni^{2+}, Pb^{2+}	Vwioko et al. (2006)
Populus canescens	Zn^{2+}	Bittsánszky et al. (2005)
Salix spp. (Salix viminalis, Salix fragilis) *Populus spp. (Populus deltoides, Populus nigra, Populus trichocarpa)*	Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}	Pulford and Watson (2003) and Ruttens et al. (2011)
Populus deltoides	Hg^{2+}	Che et al. (2003)
Jatropha (*Jatropha curcas* L.)	Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}	Abhilash et al. (2009) and Jamil et al. (2009)

Table 3 Advantages and limitations of the phytoremediation process

Advantages of phytoremediation	Limitations of phytoremediation
In situ applications decrease the amount of soil disorder compared to conventional methods	Restricted to sites with low contaminant absorption
In situ applications reduces the contaminant spread through air and water	Climatic states are limiting factors
Comparatively low-cost and low labor-intensive	Elongated remediation moment
Does not need expensive equipment or highly specialized workers	Introduction of nonnative species may affect natural microbial community structure
Reduces the amount of waste (up to 95%), can be further utilized as bio-ore of HMs	Utilization/operation of contaminated plant biomass is a cause of concern
Flexible to a variety of organic and inorganic compounds	Effect on human health by the accumulation of pollutants in fruit, vegetables, and other edible parts of the crop
In large-scale applications, prospective energy stores could be used to produce thermal energy	Restricted to sites with trivial contamination within the root zone of remediating plants

15 Phytoremediation Strategies

Phytoremediation encompasses many strategies in HMs removals, which could be through stabilization, extraction, volatilization, or root filtration.

16 Phyto-Stabilization

Phyto-stabilization relates to the utilization of plants having the capability of decreasing metal mobility or/and bioavailability either to avoid its leaching to groundwater or to avoid its access into the food chain by definite mechanisms involving roots adsorption, precipitation, complexation in the root region (Sarwar et al. 2017). In this case, plants species with high metal tolerant are more effective, while the metal accumulation may become a drawback to the environment (Meenambigai et al. 2016). As documented by Chatterjee et al. (2008), *Anthyllis vulneraria, Festuca arvernensis, Koeleria vallesiana, Armeria arenaria*, and *Lupinus albus* show great effect on reducing copper, zinc, chrome, lead, nickel, and cadmium bioavailability in the soil and environment.

17 Phytoextraction

In this approach, plants species should be tolerant in the case of a very high soil metal content, by their need to accumulate high metal concentrations in their aerial fragments (Meenambigai et al. 2016). For instance, Macek et al. (2000) have reported that *Viola baoshanensis* was used the phytoextraction method to remove metals pollutants such as cadmium, zinc, and lead as well as remove organics from soil particles by accumulating them in plant parts. Thus, phytoextraction is considered as the most important approach for the elimination of metals and metalloids from contaminated sediments, water, biosolids, and soils with adjusting several factors that impact their efficiency including soil properties, metal availability to plants, and metal speciation (Sarwar et al. 2017).

18 Phytovolatilization

It is an additional approach that concerns the metal conversion into volatile form and releasing it into the atmosphere via stomata. This technique is mainly useful for Hg as the mercuric ion is converted into a comparatively less toxic elemental form. The released form of volatile mercury Hg could be recycled back by precipitation method to the soil, accordingly, this detoxification technique offers a short-term problem solution (Sarwar et al. 2017). On the contrary, Ayotamuno and Kogbara (2007) reported that *Stanleya pinnata, Zea mays,* and *Brassica* sp. could also convert Selenium Se, 1,2-Dibromoethane or ethylene dibromide (EDB), Tetrachloromethane (CCl_4), and Trichloroethene (TCE) to their volatile phase.

19 Rhizo-Filtration

In rhizo-filtration, the plants' roots are used for metals absorption from contaminated solution or soil then the metal removal process is accomplished by harvesting all plant parts. In this approach tolerance and translocation are mainly unrelated (Meenambigai et al. 2016). Roots of *Helianthus annus* and *Brassica juncea* have a

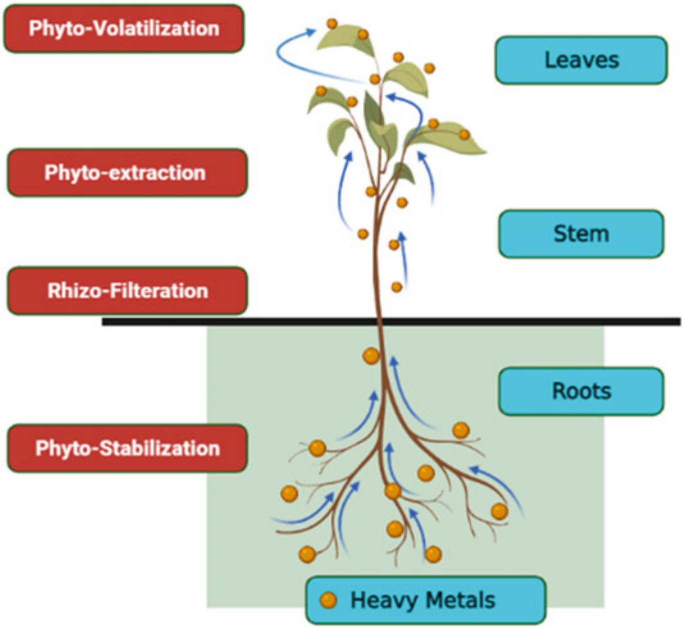

Fig. 2 Descriptive illustration of phytoremediation strategies

remarkable adsorption capability of pollutant metals, such as lead, zinc, cadmium, and arsenic from water and aqueous environment (Verma et al. 2006).

All the above-mentioned mechanisms of heavy metal removal will reduce the toxic effect of HMs on the microbial flora that is present on the soil to degrade the OPs (Fig. 2).

20 Mycoremediation

Owing to the robust morphology and diverse metabolic potential, fungi are the ultimate common soil and aquatic habitat-based ecosystems symbionts and decomposers that are frequently used as bio-sorbents for contaminants and metals removal with extraordinary capacities for recovery and metal adsorption (Yong-Qian 2012). Mycoremediation is a term of bioremediation method that applied by fungi (Devi et al. 2020). Nevertheless, other studies demonstrated that active and dead fungal cells have a significant role in the inorganic chemical's adhesion (Igiri et al. 2018).

Furthermore, the fungal mycelium was demonstrated in the solubilization of insoluble substrate during biodegradation by the effect of the extracellular enzyme in contrast with single-cell organisms. These benefits are assigned to their increased surface ratio of the cell and their ability to directly contact the environment either physically or enzymatically, as depends on the presence of variable extracellular and intracellular enzymes including cytochrome P450, laccase, oxidase, peroxidases, and, epoxide hydrolase, fungi have an extra advantage of standing for high concentrations of toxicity (Singh et al. 2021).

Wood-rot fungi have the capability of lignin degradation by producing laccases and peroxidases enzymes that are utilized in wastewater pollutants biodegradation and could also be used to detoxify/degrade a variety of recalcitrant toxic OPs such as phenols, fungicides, and industrial dyes by their high oxidation-reduction potential (Anastasi et al. 2010). On the contrary, due to the difficulty in the selection of organisms, they have high growth and degradation potential under variable and restrictive conditions of wastewaters generated through textile industries, its real application has not been explored effectively (Vanhulle et al. 2008).

Srivastava and Thakur (2006) have described that *Aspergillus* sp. has effectively removed 85% of chromium from tannery waste with adjusted pH 6 in a bioreactor system, compared to a 65% removal of the same from the tannery effluent, because of the presents of high OPs concentration that acts as growth inhibitors. Moreover, Sharma (2021) has reported that *Coprinopsis atramentaria* was the most effective mycoremediation by its ability to bioaccumulate about 76% of cadmium Cd^{2+}, at a concentration of 1 mg/l of Cd^{2+}, while it could bioaccumulate about 94.7% of lead Pb^{2+}, at a concentration of 800 mg/L of Pb^{2+}.

Various dead fungal biomass, such as *Penicillium chrysogenum, A. niger*, *Saccharomyces cerevisiae*, and *Rhizopus oryzae,* positively detoxify chromium metal from hexavalent ion to monoatomic tri-cation (Luna et al. 2016), in similarity he reported that *Candida sphaerica* could remove 79% of lead (Pb), 90% of Zinc (Zn), and 95% Fe (iron) by producing biosurfactants that could form metal ions-complexes before separated from the soil. In optimum metal ion concentration and (3–5) pH initial, *Candida* spp. effectively accumulate Copper Cu (52–68%) and Nickel Ni (57–71%) (Tarekegn et al. 2020).

21 Co-contaminations with Heavy Metals and Organic Hydrocarbons

Heavy metals reach the environment from many sources, either from a natural source such as volcanic eruption or rock weathering, but anthropogenic activities are the main cause of the leakage of HMs into the environment. This anthropogenic activity includes sewage discharge, oil spills, mining activities as well as waste and industrial effluent disposal (Briffa et al. 2020). As stated, before the HMs will not reach the environment as a sole contaminant as it is usually co-contaminating with OPs such as crude oil and its derivatives. The crude oil is composed of a heterogeneous mixture of long chains hydrocarbons, and it is used to produce internal combustion lubricating oils with other additives. The reckless disposal of the used lubricating oil accumulates different toxic HMs in the soil such as cadmium, zinc, lead, tin, and arsenic which might reach and contaminate the water bodies and groundwater

(Agarry et al. 2013; Stout et al. 2018). Because of the toxicity of the HMs in spent lubricant oil, the oil bioremediation is extremely difficult, and it is not sufficiently examined because of the HMs speciation that happens in situ and varies in response to the environmental factors and chemical structure. In a study carried out by Agarry et al. (2013), the effect of bio-stimulation and bioaugmentation on the restoration was studied. The result showed that the adding up of urea fertilizer (bio-stimulation) and adding exogenous microbes (bioaugmentation) from the genera of *Micrococcus*, *Aeromonas*, and *Serratia* had improved the oil removal and lead uptake (Agarry et al. 2013). The type of stimulation and the bioaugmentation suitable for successful environmental remedies depend on many factors that include the nature of the indigenous microbe community structure, nutrient availability, and the metal contaminant.

Over and above, hydrocarbon-degrading bacterial strains that can produce a biosurfactant are excellent for bioremediation of crude oil and its derivatives by reducing the surface tension (Qi et al. 2017). In that manner, two bacterial isolates belonging to the genera *Bacillus* and *Ochrobactrum* were isolated and found to be able to degrade the crude oil and both produce biosurfactant but, notably, both tolerated HMs differently. The growth of the strain identified as *Ochrobactrum* sp. P1 was inhibited at a Ni concentration of 0.1 mM; however, its hydrocarbon degradation capacity was increased. On the other hand, the growth of *Bacillus* sp. P19 was promoted by a low concentration of Ni, while 100 μM of Pb has improved the hydrocarbon degradation (Zhong et al. 2020). This supports the complexity and instability of microbial remediators in presence of different metal contaminate at different concentrations levels.

Due to the complexity of the responses of the microbial communities to contaminate, next generations sequencing was employed to address the change in the community profile. It has shown that the presence of Pb in the contaminated site has a dramatic effect on both natural attenuation and bio-stimulation, but it favored the existence of Pb-resist are bacteria belongs to the genera *Sphingopyxis* spp., *Thermomonas* spp., and *Nocardioides* spp. in natural attenuation approach (Khudur et al. 2019). However, under this co-contaminated condition, the abundance was from *Pseudomonas* spp. (Khudur et al. 2019). The selection of heavy metal-resistant bacteria in the site contaminated with diesel oil was also confirmed in the colder environment in Antarctica (Gran-Scheuch et al. 2020).

22 Factors Influencing the Microbial Remediation of Heavy Metal

It is noteworthy that abiotic factors may exert some roles in the efficacy of the remediation mechanisms, however, this may vary with the types of pollution discussed (Zhang et al. 2020). The capability of HMs to act either as inhibitory or as stimulatory to microorganisms is determined by the total concentrations of metal ions, chemical forms in other words the metal species, and associated factors such as oxidoreduction potential. Other factors are environmental, such as the temperature of the contaminated environment, pH value, presence of low molecular weight organic acids, as well as the presence of humic acids can modify the transformation, transportation, and HMs bioavailability to microorganisms (Tarekegn et al. 2020).

Heavy metals are inclined by forming a free ionic species at acidic pH, with more available protons to saturate sites of metal-binding, the adsorbent surface will be positively charged at elevated concentrations of hydrogen ion, which results in decreasing the electrostatic attraction between the metal cations and the adsorbents, therefore increasing the number of soluble metal ions cumulative its toxicity (Tarekegn et al. 2020). Temperature plays a vital role in HMs adsorption, the solubility of HMs increases with a temperature rise, which tends to improve HMs bioavailability (Bandowe et al. 2014). The metal-microbes complex stability relies on the cell wall configuration, sorption sites, and the ionization of chemical moiety on microbial cell walls. The outcome of the degradation method relies on the substrate and environmental factors in different (Tarekegn et al. 2020).

23 Microbial Tolerance and Removal Mechanisms

Microorganisms have developed remarkable defenses and even advanced several metabolic pathways for the use of HMs and their beneficial effects on cells (Sun et al. 2021). The removal mechanisms of HMs include extracellular barrier exclusion, in which the HMs are blocked from entering the cell, while the extracellular mechanisms are represented by binding the toxic metal to extracellular polymeric substances that are being produced by the microbial cell, for instance, extra polysaccharide, which has a great benefits in overcoming hostile environmental conditions (Arayes et al. 2022, 2021). On the other hand, intracellular sequestration is mediated by trapping the metal within the cytoplasm. Additionally, metals are exported from the intracellular environment via active transport employing efflux system. Enzymatic detoxification is similarly a well-established mechanism of tolerance which has been evolved by microbial evolution. However, the lengthy, unclear remediation pathways and the uncertainty of bioremediation efficiency are seen to be various kinds of limits in practical applications (Ahemad 2019).

24 Microalgae in Bioremediation or Phyco-Remediation

Because of its nonpathogenic nature, easy growth requirements, and the fact that microalgae have been found to operate on a wide spectrum of hazardous wastes, phyco-remediation is preferred over other biological remediation strategies (Mahmoud et al. 2022).

Microalgal cells have been shown to detoxify or convert inorganic and OPs (Mondal et al. 2019; El-Sheekh et al. 2021). Microalgae from the genera *Selenastrum*, *Scenedesmus*, and *Chlorella* are efficient in the breakdown of polycyclic aromatic hydrocarbons like naphthalene, phenanthrene, and pyrene, as well as the immobilization of metals (Ghosal et al. 2016; El-Sheekh et al. 2012; Dell' Anno et al. 2021). Principally, it depends on the development of exopolysaccharides (EPS). This EPS assists in the absorption of pollutants on the cell surface and/or the formation of less bioavailable complexes (Casillo et al. 2018; Deshmukh et al. 2016). Exopolysaccharides adhering to the membrane or cell wall exopolysaccharides can either stay adherent or be absorbed and chelated by phytochelatin molecules. In addition, the metal-binding capabilities of microalgae were found to be rather high. This characteristic of microalgae was linked to the presence of polysaccharides, proteins, or lipids on the surface of their cell walls, which included functional groups, such as amino, hydroxyl, carboxyl, and sulfate, which can act as metal-binding sites (Yu et al. 1999). This makes them an excellent source of complex multifunctional polymers that are used to sequester a variety of metals via adsorption or ion exchange. Microalgae with exceptional biological traits such as high photosynthetic efficiency and a simple structure may thrive in adverse environmental circumstances, such as heavy metal contamination, high salinity, nutritional stress, and extreme temperature (Leong and Chang 2020). For instance, *Chlorella* sp. can remove Al, Fe, Mg, and Mn. *Chlorella pyrenoidosa*, which found to have the capability to remove a variety of HMs, including copper, mercury, zinc, nickel, lead, arsenic, chromium, and cadmium (El-Naggar and El-Sheekh 1998; Yan and Pan, 2002; Yao et al. 2012) *Spirogyra* has also been demonstrated to be efficient in the elimination of HMs as Pb, Co, Cd, Hg, As, and Ni (II) (Singh 2007). Cyanobacteria such as *Spirulina platensis* are capable of removing lead in addition to copper and mercury (Al-Homaidan et al. 2016; Garnikar 2002), *Nostoc muscorum* and *Anabaena subcylindrical* capable of removing heavy metals from wastewater (El-Sheekh et al. 2005).

25 Enhanced Bioremediation of Co-contaminated Sites

Parallel advances in science and engineering extant an opportunity to further explanation of bioremediation mechanisms and enhance their efficiency. The integration of biotechnology into microbial processes promotes the bioremediation of harmful HMs, reduces the toxic effects, and offers a promising prospect to understand the mysterious properties of microorganisms (Sun et al. 2021).

26 Engineered Bacteria

Biotechnology was developed on a specific microorganism that genetically altered its DNA to generate an efficient strain with a new character that was used for bioremediation of soil, water, and activated sludge by exhibiting enhanced degrading capabilities against a wide range of chemical contaminants (Sayler and Ripp 2000).

Table 4 Genetically modified bacteria in heavy metal removal

GM bacteria	Gene(s)	Heavy metals	Reference
E. coli JM109	Metallo-regulatory protein ArsR, Hg^{2+} transporter	Ar, Hg^{2+}	Kostal et al. (2004) and Zhao et al. (2005)
Methylococcus capsulatus	CrR	Cr^{6+}	Hasin et al. (2010)
Deinococcus radiodurans and *Ralstonia eutropha*	*merA*	Cd^{2+}, Hg^{2+}	Brim et al. (2000) and Valls et al. (2000)
P. putida	Chromate reductase (ChrR)	Cr^{6+}	Ackerley et al. (2004)
Pseudomonas K-62	Organomercurial lyase	Hg^{2+}	Kiyono and Pan-Hou (2006)
Pseudomonas fluorescens 4F39	Phytochelatin synthase (PCS)	Ni^{2+}	López et al. (2002)

For instance, a genetically modified *Cupriavidus metallidurans* MSR33 of mercury resistance bacteria transformed with pTP6 plasmid. The pTP6 plasmid has the genes: *merB* and *merG* to modify the Hg biodegradation along with lyase protein (MerB) and mercuric reductase (MerA) (Rojas et al. 2014). *Escherichia coli* and *Moraxella sp.* have been accumulating 25 times of mercury (Hg^{2+}) or cadmium (Cd^{2+}) on the surfaces of cells more than single strains by expressing phytochelatin 20 (Bae et al. 2001). Nevertheless (Dixit et al. 2015), mentioned that because of the limited bacterial strains applied in molecular approaches, other microorganisms should be considered to investigate their specific bioremediation application through molecular intervention as a promising approach (Table 4).

27 Engineered Plants

Appropriate plants could be engineered for promoting their biomass production, rhizo-secretions, and thus increasing the phytoaccumulation of the pollutants that lead to rising the microbial activity in the rhizosphere which in sequence enhance the biodegradation of OPs and the phytoaccumulation of HMs (Tripathi et al. 2015).

Natural attenuation, bioaugmentation (increasing the viable microbial counts), bio-stimulation, and phytoremediation are instances of in situ biological remediation strategies that could be utilized in the remediation of co-contaminated soils and waters (Padhan et al. 2021). Natural attenuation consists of the use of natural processes perhaps (biodegradation, sorption, volatilization, dispersion, biochemical stabilization) to reduce the absorption of pollutants at contaminated sites (EPA 1999; Mulligan and Yong 2004; Padhan et al. 2021).

To improve the phytoremediation in co-contaminated environments, the introduction of exogenous microbes might be of great help to complete the process. For instance, a study was conducted to investigate the effect of the bacterium *Mycobacterium* sp. N12 into phytoremediation systems involving two different plants from species *Echinacea purpurea* and a mixture of *Festuca* spp. on the remediation of polycyclic aromatic hydrocarbons (PAHs) and Cd. The study results indicated that there is a kind of collaboration between the plant and the added microbe in tackling the two contaminants. Cd was removed by the plant while the PAHs were removed by the degrading microbes and their interaction with the associated plant species. Moreover, the combination between *Mycobacterium* sp. N12 and *Festuca* spp. was more efficient in bioremediation by the removal of 76.29% of the PAHs after 150 days experiment (N. Li 2021). It was also found that the addition of urea to the soil has improved the remediation efficiency of willow for cadmium in presence of pyrene (Y. Li 2021). In a recent study, the phytoremediation of the famous explosive trinitrotoluene (TNT) and cobalt co-contaminated soil was improved by genetically modified *Arabidopsis thaliana* (Gao et al. 2021). The modification was made by the transformation of single-strand DNA-binding (SSB) protein from the bacterium *Acidithiobacillus ferrooxidans* which was previously reported to be resistant to contaminants (Park et al. 2007). The SSB is recognized to be involved in the DNA repairing mechanism (Chen et al. 2016). The result of this study revealed that the transformed *Arabidopsis* showed enhanced phytoremediation activity for both contaminants when compared to the wild-type plant and the control in 15 days experiment (Gao et al. 2021). This investigation has put light on the usage of genetically modified plants and their potential application to be a successful tool in the bioremediation of co-contaminated soils.

28 Bio-attenuation

Natural attenuation uses the ability of the soil intrinsic microbial communities to decompose the contaminant. The remediation process comprises the catabolic activity of microorganisms converting a variety of organic compounds into less or entirely non-toxic deposits, depending on the plasmid-encoded catabolic processes and chromosomal genes for bacterial degradation or by extracellular enzymatic activity for the fungal degradation process (Pandey and Jain 2002). Similarly, indigenous prokaryotic microorganisms contribute to redox reactions and change the valence of HMs, in that way changing their activity, affecting their mobility or toxicity. On the contrary, Padhan et al. (2021) mentioned that this strategy requires a long time compared to others, which is required to increase the relative population density of intrinsic degrading microbes.

29 Bio-stimulation

This method takes place by boosting the activity of native microbes to adjust the physical and chemical parameters of the soil (Hamdi et al. 2007). Bio-stimulation strategy usually comprises the addition of crucial nutrients, such as phosphorus, nitrogen, oxygen, or carbon and aeration to support the native microorganisms to activate the metabolic pathways and generate the required enzymes needed to transform or degrade different contaminants (Atif et al. 2009). Some biotic and abiotic factors may inhibit the activity of bio-stimulation strategy in soil for heavy metal removals such as nutrients concentration, pH, temperature, moisture, oxygen, soil properties, and contaminant toxicity type and concentration (Emenike et al. 2018). As mentioned by Dhami et al. (2017) different urease enzyme-producing microorganisms exhibited varying toxicity tolerance to different metals such as strontium (Sr) and lead (Pb), due to physiological adaptation and metal accumulation mechanisms of different microorganisms (Li et al. 2015). As reported by Chen and Achal (2019), the bio-stimulator substrate used supported MICP in situ (De Jong et al. 2009) and successfully accelerated the precipitation of $CaCO_3$ in acid rain-treated soil based on urease production by ureolytic bacteria. Alternatively, the use of Iron (Fe) as a stimulator supplement enhances the production of biosurfactant glycolipid as high as 80% by *Nocardiopsis* MSA13A, to overcome the limitation of biosurfactant technology-based bioremediation (Sun et al. 2021).

30 Bioaugmentation

The bioaugmentation method has arisen as the most effective, and sustainable remediated method used for pesticides contaminated soil, based on its ability to increase the efficiency of the biodegradative capacities of contaminated environments with the integration of the desired catalytic capabilities of single strains or consortia of microorganisms (Agnello et al. 2016). As a free or immobilized inoculum when the indigenous microbes able of degrading contaminants are not adequately large. The effective bioaugmentation process depends on the competitive microbial interaction between exogenous and indigenous organisms, mainly for nutrients. As reported by Alvarez et al. (2011), actinomycetes have been broadly used to remediate soils contaminated with petroleum and hydrocarbon derivatives. Several bacterial strains have been used in the bioaugmentation technology process such as genera *Alcaligenes, Pichia, Pseudomonas, Rhodococcus, Arthrobacter, Bacillus, Catellibacterium, Serratia, Sphingomonas, Stenotrophomonas, Brucella, Burkholderia, Streptomyces,* and *Verticillium*, based on their applicability to bioremediate of pesticides (Cycoń et al. 2017). Bioaugmentation technology also can promote plant growth and prove a doubling effect on plant biomass by introducing *P. aeruginosa* (Agnello et al. 2016) because of its ability to increase the solubilization of phosphorus (P) and production of indole acetic acid (Oves et al. 2013). Similarly, plant growth-promoting rhizobacteria may promote plant growth directly by assisting in resource acquisition in polluted soils mitigating phytotoxicity (Khan et al. 2013) and controlling plant hormone levels, or indirectly by reducing the inhibitory effects of pathogens (Ahemad and Kibret 2014).

31 Conclusion and Future Aspects

Heavy metals contamination represents a severe problem as the HMs are not degradable. The situation is even worse in sites that are co-contaminated with organic pollutants (OPs). The heavy metals toxicity typically makes the degradation of OPs extremely difficult. Therefore, the practical use of scientific knowledge to overcome this challenging situation is a must. The combination of different remediators remains the key point for the successful restoration of our environment. This can be accomplished by different proposed scenarios, for instance, the success of using the naturally resistant microorganism or genetically modified microorganism to degrade the OPs in co-contaminated sites. Further, increasing the plant tolerance to higher metal concentration through the proper introduction of the resistance genes might help in the uptake of efficiency of the toxic metals and promote the soil microbes or added microbes to degrade the degradable wastes. In addition, understanding the dynamics or different metals species will help to use the alternative of remediation strategy or mechanism, like using a certain microbe to produce acids locally to leach the metal and make it bioavailable to the plant. Despite all these possible scenarios being very promising and having grabbed scientists' interests to further explore, the realistic problem inevitably has other restrictions which come from the uniqueness of each environment being remediated. Each environment is composed of a heterogeneous mixture of biotic and abiotic factors, and the successful remediation process involves precisely selection of the remediation strategy and depends upon exceptional tuning to accomplish the desired aims in both cost and time-efficient manners.

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Algal Microbial Symbiotic System-From a Biological Process to Biorefinery

Sarveshwaran Saravanabhupathy, Rajlakshmi, Sayantan Santra, Mohan Das, Subhara Dey, Subhodeep Banerjee, Reddhy Mahle, and Rintu Banerjee

1 Introduction

Treatment of wastewater (WW) is necessary to improve the quality of water while also tackling the issue of water shortage (Karimi-Maleh et al. 2020). To eliminate hazardous pollutants from the environment, various physical, chemical, and biological methods were employed, for example, including membrane filtration, reverse osmosis treatment (Pavithra et al. 2020), flocculation, ion-exchange, flotation, electrochemical, adsorption, biosorption, activated sludge process, solvent extraction, chemical precipitation, etc., (Rathi et al. 2021; Joshiba et al. 2021; Prasannamedha et al. 2021). The demand of water for various utilities has increased as a result of population growth and urbanization. For optimum water reuse and recycling, pathogens, nutrient concentrations, and other organic compounds in WW should be eliminated (Fuentes et al. 2016).

The ability of algae to deplete accessible inorganic chemicals earned a lot of attention. Biological wastewater treatment (WWT) using algae have been effectively explored in recent years to get around the drawbacks of frequently used water treatment procedures. Algae require huge amounts of nitrogen and phosphorus to

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thrive, they may easily absorb it from WW for its growth (Goncalves et al. 2017). Algae's strong nutrient restoration capability contributes to its use in the treatment of WW (de Wilt et al. 2016). Algae can remove nutrients, heavy metals, organic, and inorganic hazardous contaminants and can collect radioactive substances and pesticides through absorption. The co-culture concept is explored recently for the identification of highly beneficial consortium and is possible by combining algae with any heterotrophic organism. Algae-algae co-cultivation is also found useful for a specific application. In fungal and microalgal co-cultivation with glycerol and potassium nitrate as the most suitable carbon and nitrogen sources, fungi helps in the harvesting with the formation of biofilm, thus increases the biomass and lipid production at an early stage of growth compared to monocultures. The Fatty Acid Methyl Ester (FAME) profile of the lipid generated from co-cultures matched that of microalgal components more closely, although both algal and fungal equivalents contributed to FAME profiles in co-culture, making it useful for biodiesel generation (Dash and Banerjee 2017).

Biorefineries use biomass feedstocks to produce a range of products thus in that way they are similar to petroleum refineries (Cherubini 2010). The idea of biorefinery entails extracting the most value from a particular biomass type to reduce waste discharge into the environment while simultaneously increasing bioproduct economics and expected to create an economically self-sustaining framework and new career opportunities (Mata et al. 2010; King 2010). Industrial biorefineries have been identified as a viable option for reducing the effects of human-caused climate change, as well as fulfilling the limitless need for fuel, energy, materials, and chemicals (King 2010).

This chapter will briefly discuss the potential of algae in WWT, followed by the requisite and examples for forming an algal consortium. This also provides a quick explanation of the theory underlying algae's pollution removal process. This includes a review of the factors that can influence the algal symbiosis, as well as the obstacles and progress in the algal biorefinery process along with the application.

2 Potential of Microalgae in WWT

2.1 Sources and Composition of WW for Microalgae Cultivation

The global population is expected to reach 10 billion by 2050, and this ever-increasing population is proportionate to urbanization, industrialization, and massive demand for food production (https://www.worldbank.org/en/topic/water-in-agriculture#1). All such activities consume fresh water and release toxic WW. Compilation of region-specific available data revealed that the current annual volume of global WW is 380 trillion litres, which is approximately five times the annual water volume of Niagara Falls, taking into account flow rate of water is 2407 m^3 per second through Niagara

Falls. Regionally, Asia is the largest producer of WW with 159 billion m^3, and by the end of 2030, it is expected to account for 44% of global WW (Qadir et al. 2020). Therefore, there is a growing interest in recycling this huge volume of WW and resource recovery to establish a sustainable system. Microalgae are one of the oldest and diverse oxygen-generating photosynthetic groups of organisms with an excellent nutrient removal capacity from WW. The crucial role of microalgae in WWT was first revealed in the 1950s (Johansen et al. 2012). The rationale of using microalgae in WWT is that; they will utilize the nutrients for growth, and as a result, the concentration of carbon, nitrogen, and micronutrients will be decreased, and also biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values will be decreased. To accumulate 1 g of algal biomass, 0.063 g nitrogen and 0.009 g phosphorous are absorbed from WW (Li et al. 2019). Ideal microalgae strains for WWT should have some characteristics like—high growth rate, adaptability to grow in a diverse environment, high nutrient accumulation rate, and high biomass productivity. A comparative nutritional profile of WW from a few major sources is presented in (Table 1) and sources of different WWs are presented in (Fig. 1).

2.2 Microalgae Cultivation Systems

Design and operation of microalgae cultivation plants aim to optimize light supply, carbon source, nitrogen source, and adequate mixing with minimum construction and operation cost. Microalgae can naturally grow in freshwater, saline water, and WW, but such ecosystem-level growth is not enough for commercial-scale biomass harvesting (Vandamme et al. 2013). Major modes of microalgal growth metabolism are photoautotrophic (light source as energy and Carbon-di-oxide (CO_2 as carbon source), heterotrophic (both carbon and energy from organic compounds), mixotrophic (primary energy source photosynthesis and organic/inorganic compounds as carbon source), and photoheterotropic (both sugar and a light source needed). The metabolic pattern is closely linked to available nutrients and adversities, but photoautotrophic is most commonly used to maximize photosynthesis (Kotasthane 2017; Cesário et al. 2018). Depending on the microalgal strain, WW type, and investment capacity, different cultivation systems are designed, which are generally categorized into two types—open system and closed system. Essentially, open systems are established in ponds, tanks, deep channels, whereas closed systems are indoor with significant control over the culture conditions.

2.2.1 Open Systems

Due to low construction and operation costs, open systems are in practice for a long time and contribute 95% of total microalgal biomass generation. But it suffers from several disadvantages like water evaporation from medium, large land requirements,

Table 1 Comparison of WW nutritional profile from different sources

Parameters	Municipal	Textile industry (average)	Petroleum refinery (average)	Palm oil mill (average)	Poultry slaughter-house (average)	Aqua-culture	Landfill leachate (average)	Pharmaceutical (average)
pH	9.3 ± 0.00	8.25	8.46	4.7	7.36	8.34 ± 0.008	7.4	7.2
TDS	95.0 ± 7.1	5738.33	–	40,500	84	–	2027	40
BOD	–	713.33	327	25,000	96.2	15.63 ± 0.033	1500	–
COD	–	2125.00	1209	50,000	578	42.63 ± 0.085	10,400	2500
TN	25.5 ± 0.2	–	89	750	18.5	–	–	–
Ammonia nitrogen	–	–	43	35	1.28	0.916 ± 0.001	1803	200
TP	2.8 ± 0.2	–	1.70	180	5.0	3.85 ± 0.007	–	16
TOC	23.2 ± 0.0	–	201	–	–	–	–	–
TVA	–	–	–	34,000	–	–	–	750
Oil and grease	–	–	73	4000	–	–	–	–
Na^+	62.9 ± 0.0	1672.00	–	–	–	–	3710	2000
K^+	12.3 ± 0.1	14.00	–	2270	–	–	1675	18
Ca^{2+}	17.3 ± 3.4	19.00	–	439	14	–	400	–
Mg^{2+}	1.78 ± 0.0	21.25	–	615	2	–	–	40
Cl^-	95.2 ± 0.3	1697.50	–	–	0.14	–	660	900
F^-	3.90 ± 0.1	1.25	–	–	–	–	–	–
SO_4^{3-}	50.5 ± 1.2	509.67	45	–	–	–	40	300
Bicarbonate	–	913.25	–	–	488	–	–	750
Cu^{2+}	–	0.07	–	0.89	–	–	0.151	0.1

(continued)

Table 1 (continued)

Parameters	Municipal	Textile industry (average)	Petroleum refinery (average)	Palm oil mill (average)	Poultry slaughter-house (average)	Aqua-culture	Landfill leachate (average)	Pharmaceutical (average)
Zn^{2+}	0.02 ± 0.0	–	–	2.3	–	–	3	0.09
Ni^{2+}	–	–	–	–	–	–	1.339	–
Pb^{2+}	–	0.03	–	–	–	–	0.3	0.35
Cd^{2+}	–	–	–	–	–	–	0.035	0.10
Fe^{2+}	–	0.018	–	46.5	–	–	11.16	0.45
Cr^{6-}	–	1.37	–	–	–	–	0.021	0.3
As^{6-}	–	–	–	–	–	–	–	0.25
Reference	Ryu et al. (2014)	Hussain et al. (1970)	Ishak and Malakahmad (2013)	Singh et al. (2010)	Meiraamkulova et al. (2019)	Sonia et al. (2015)	Naveen et al. (2017)	Wang et al. (2004)

Unit: mg/L (except for pH). Abbreviation used: TDS (total dissolved solids), BOD (biochemical oxygen demand), COD (chemical oxygen demand), TN (total nitrogen), TP (total phosphate), TOC (total organic carbon), TVA (total volatile acids)

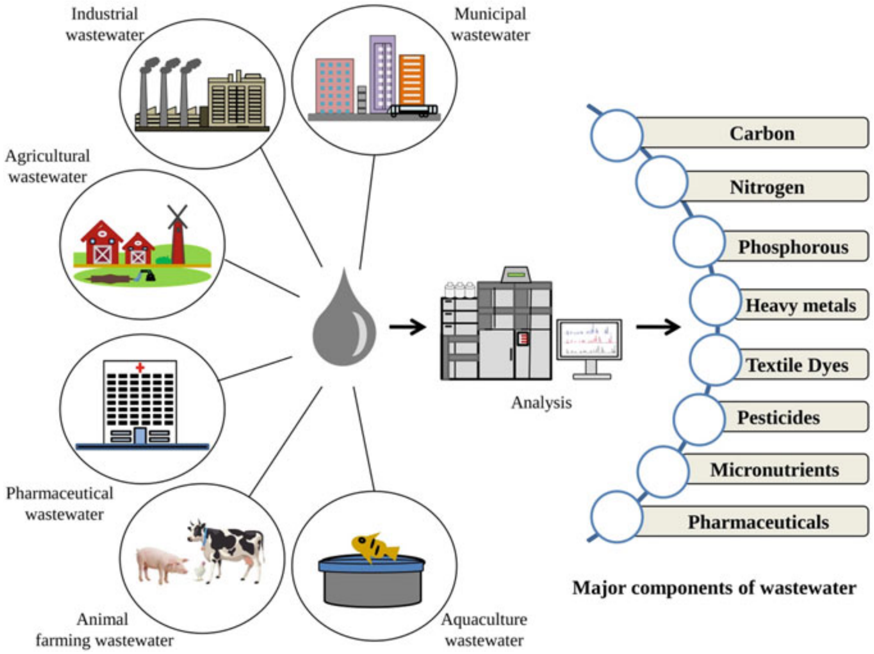

Fig. 1 A common source of WW and its major constituents

contamination by undesired microbes, and CO_2 release to the environment. For large-scale cultivation, unstirred ponds, race-type ponds, and circular ponds are used. The characteristic features, differences and pictorial representation are depicted in Table 2 and Fig. 2.

2.2.2 Closed Systems

Closed systems can resolve most of the problems associated with open systems and biomass yield capacity is superior to the former one. Closed systems don't allow mass transfer between environment and culture medium and are mostly referred to as photobioreactors (PBR). It maintains an aseptic condition and no water loss by evaporation. Although it offers a significant yield but to maintain a controlled state, capital, and operation cost increases few folds. Desirable characteristics of an ideal photobioreactor are the ability to cultivate microalgae universally, the high mass transfer rate of CO_2 and O_2, minimize fouling to maintain light transmittance, and should work under intense foaming conditions. This microalgal system is particularly suitable for WWT coupled with extraction of value-added products from algal biomass (Tsoglin et al. 1996). Characteristic features and pictorial representation of commonly used closed microalgae cultivation systems are presented in Table 3 and Fig. 3, respectively.

Table 2 Types of open pond microalgae cultivation systems

1. Unstirred ponds	2. Race-type ponds	3. Circular ponds
• Simplest form of microalgae cultivation is generally set in natural water bodies (lakes or lagoon ponds) with shallow depth (less than 1 m) for light permeability • Devoid of any mixing/stirring equipment (Ting et al. 2017) • Few microalgae like *Dunaliella salina* are still commercially produced by this process (Borowitzka and Borowitzka 1990) • Disadvantages of these ponds are—limited biomass yield, slow nutrient diffusion, and suboptimal light penetration in the absence of a mixing facility	• Extensively used for the mass production of microalgae with system depth of 15–50 cms only • Mixing is done by paddlewheel to provide sufficient aeration and maintain a uniform density • Common microalgae like *Spirulina, Dunaliella, Chlorella,* and *Haematococcus* are reported to produce enough biomass (60–100 mg DW/L d) in this system (Razzak et al. 2017; Saha and Murray 2018) • Low operational budget (Ting et al. 2017)	• The diameter of this type of pond is 40–60 m and the depth is 20–30 cm (El-Baz and Baky 2018; Szwaja et al. 2016) • Widely used in Asia for the large-scale production of *Chlorella* sp. • Mixing of culture done by a central rotator • More efficient than unstirred ponds, but major disadvantages are the inefficacy to maintain temperature and contamination by other microbes

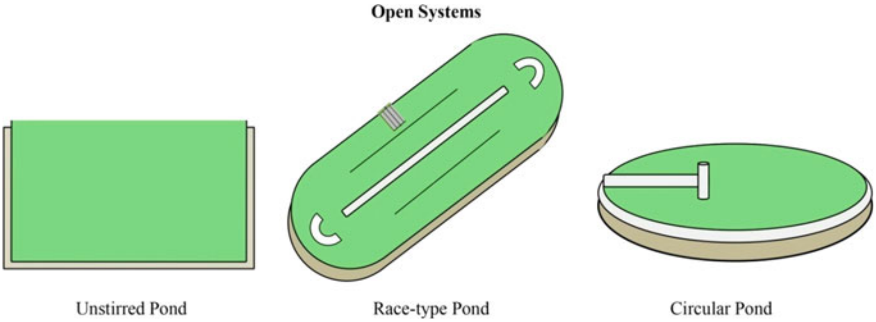

Fig. 2 Open microalgae cultivation systems

2.3 *Integration of New Techniques in Microalgae Cultivation*

2.3.1 Algal Turf Scrubber (ATS)

It is a solar-driven water treatment process designed to support periphyton (autotrophic and heterotrophic organisms) growth and removal of inorganic compounds. Here, an attached algal community is grown in shallow water. Then the target WW is dumped out and flown over the algal turf. Algae capture nutrients and increase biomass. After completing the raceway, WW is pumped back for the

Table 3 Types of close microalgae cultivation systems

1. Flat-panel photobioreactor (FP PBR)	2. Tubular photobioreactor
• Made up of transparent or semitransparent materials and rectangular appearance • Operated both indoors with an artificial light source or outdoor with sunlight • Agitation is done by air bubbles generated from the air sparger or by rotating mechanically (Ekin 2020) • Advantages include a high surface-to-volume ratio, convenient maintenance, and a short light path for easy penetration to medium • Limitation of flat panel PBR includes; expensiveness, hydrodynamic stress on algal species, and biofilm formation near the internal surface (Ting et al. 2017) • An improved version of FP PBR- alveolar Plexiglas sheets by Tredici and Materassi (1992) termed as vertical alveolar panel PBR (VAP) • Biomass productivity of outdoor cultivated *Anabaena azollae* using VAP was up to 16 g/m^2 per day	• Made up of an array of transparent, small diameter (generally <0.1 m) tubes in different patterns (spiral, straight, tilted) • Tube diameter is a crucial factor because too narrow tubes may cause photoinhibition by excessive radiant energy whereas width may also inhibit light penetration • Depending on the arrangements, tubular PBR is two types vertical and horizontal tubular PBR • Advantages include low capital cost, homologous culture, better heat and mass transfer, and a high surface/volume ratio • The major limitation is high power consumption (~2000 W m^{-3}) which is expected to maintain high linear liquid velocity (Posten 2009; Richmond 1987)

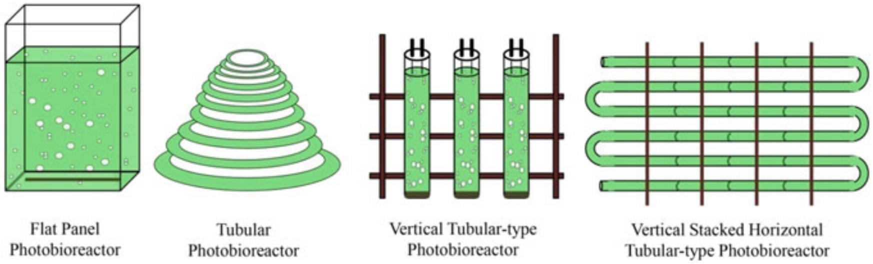

Fig. 3 Closed microalgae cultivation systems

next cycle. Generally, algae are harvested once a week (Leong et al. 2021; de Souza et al. 2020). The periphytic community of ATS can effectively remove nutrients, uptake heavy metals, and increase Dissolved Oxygen (DO). The overall process is the result of complex ecological cooperation and metabolic pathways. ATS is influenced by flow rate, temperature, WW composition, and sunlight. In a pilot-scale study, ATS (1.2 m × 234 m, 0.5% declining grade) removed 16% phosphate, 49% nitrate, 19% nitrite, and 41% ammonium from citrus orchard runoff (D'Aiuto et al. 2015, Shah, 2020). Ray et al. (2015) checked the efficacy of ATS for nutrient removal from oyster aquaculture WW. The experimental scale of 1m^2 ATS has reported to remove 12.2 g nitrogen and 0.25 g phosphorous per day. It has also shown attractive results from urban WWT. With average biomass production of 34.8 g DW m^2 d^{-1}, it removed nitrogen 2.52 g m^2 d^{-1}, -phosphorous 1.25 g m^2 d^{-1} (Marella et al. 2019). Major bottlenecks of this technique are low WW handling capacity and the need for continuous monitoring.

2.3.2 Membrane Photobioreactor (MPBR)

A microfiltration (MF) membrane is placed in a MPBR, which acts as a solid–liquid separator enabling complete isolation of algal mass from effluent. This technique allows to control solid retention time (SRT) and hydraulic retention time (HRT) independently, and algal cell concentration can be free from hydraulic loading (Bilad et al. 2014a). Decoupling of SRT and HRT increases nutrient load and avoids biomass washout. Hence, higher biomass can be produced effectively. Bilad et al. (2014b) found that MPBR produces nine times higher biomass of *Chlorella vulgaris* compared to PBR. Same microalgae were cultured in continuous flow mode using MPBR to treat domestic secondary effluent. Maximum growth biomass was 1.724 g/L, which was 1.64 times higher than PBR. It effectively removed nitrogen (87.7%), phosphorous (76.7%) and other metal ions. Gonzalez-Camejo et al. (2020) optimized MPBR performance and found that reduction of the light path significantly increases photosynthetic capacity, biomass production, and nutrient recovery. All such results indicated MPBR as a promising alternative to overcome the drawbacks of conventional PBR.

3 A Framework of Algal Consortium

The algal–bacterial consortium provides a potent opportunity to overcome the economic and technical issues faced by a single microalgal system including flocculation, exploitability, harvesting, and biomass accumulation, etc. Even though microalgae show prominent nutrients removal efficiency from WW, it is still difficult to continue the single culture system of microalgae during the process as a single microorganism may not fulfil certain functions more efficiently than in the co-culture (Mujtaba and Lee 2017). Algal growth is affected by several factors including its surrounding microenvironment (phycosphere), interaction with other microorganisms, and the available nutrient content (Johnson et al. 2020). The major benefits of using algal–bacterial consortia are strengthening each other from environmental instabilities, reduction in aeration demand, prevention from invading species, and enhanced nutrients utilization (Subashchandrabose et al. 2011). Further, co-cultivation also helps in replacing systems that require multistep to perform the function (de Godos et al. 2010).

Successful application of algal–bacterial symbiont has been depicted in Table 4 for effective removal of pollutants, toxic chemicals, and herbicides from both industries and domestic water systems. Several co-culture systems are studied including both the species in suspension (Subasankari et al. 2020; Rajapitamahuni et al. 2019;

Toyama et al. 2018), embedded in carriers (Lubarsky et al. 2010), species separated by a membrane (de-Bashan and Bashan 2008; Nagy et al. 2014), or immobilizing one microorganism in biofilms and other in suspension (Rivas et al. 2010; Abinandan et al. 2018). These interactions help in the establishment of cooperative systems via the metabolites exchange causing enhanced biomass production and thus efficient utilization of nutrients. The co-cultivation also results in the removal of secondary metabolites (allelochemicals, harm the cultivated microorganisms). However similar to bacteria, fungi and yeast also form a symbiotic association with microalgae thereby promoting the WWT methods. Kouzuma and Watanabe (2015) grouped this association into three types: signal transduction, gene transfer, and nutrient exchange depending on the kind of exchange, carried out by the symbionts.

3.1 Microalgal-Bacterial Consortium

Microalgae bacteria co-culture system has been utilized for industrial and municipal WWT. Most WWs contain nitrates, phosphorus, ammonium, and pathogenic organisms that are primarily targeted for removal. This algal–bacterial co-culture system effectively removes nutrients, pathogen, and also produce valuable biomass. Van Den Hende et al. (2011) used the co-culture of *Phormidium* sp., *Pediastrum* sp., *Chlorella* sp., *Scenedesmus* sp., and activated sludge for the remediation of municipal WW demonstrating the removal of 30.2–56.8% of PO_4^{3-}-P and 61.2% of total nitrogen (TN). Microalgae assimilate CO_2 generated by the bacterial respiration process and the inorganic nutrients (PO_4^{3-}, NH_4^+, NO_3^-, NO^-) from the WW. It also assimilates photons in its chloroplast cell during photosynthesis to synthesize its biomass and release O_2 into the atmosphere. This uptake process is illustrated in Fig. 4. The oxygen released is sufficient to acquire desired aerobic requirement for catabolic metabolism of residual organic substances by bacteria. Buhr and Miller (1983) experimentally validated the algal–bacterial pond model for high-rate WWT. The high rate algal pond (HRAP) model and the waste stabilization pond system (WSPs) are the most currently utilized techniques for microalgal-based WWT. HRAPs is a paddle wheel-mixed, shallow, and open raceway pond (ORP) where microalgae growth is very rapid producing extensive oxygen, which can be utilized for driving aerobic treatment of pollutants. WSPs are an open system where the HB and microalgae form a symbiotic association, promoting each other's growth and also fulfilling the nutrient requirement (Jia and Yuan 2016). This model not only offers an efficient removal rate of the pharmaceuticals and sewage treatment but also increases biofuel and biomass production with minimum energy utilization and synthetic chemicals usage (Wollmann et al. 2019; Johnson et al. 2020).

Microalgal-bacterial interaction varies from species to species (Park et al. 2008). Liang et al. (2013) observed that *C. vulgaris* inhibited bacterial growth due to competition for resources. Ji et al. (2018) noted that *Bacillus licheniformis*-*C. vulgaris* co-culture can efficiently remove N, P, and COD from synthetic water than the

Table 4 Eradication of pollutants from different WW by algal–bacterial consortia (TP—Total phosphate; TOC—Total organic carbon; TN—Total nitrogen)

Microalgae	Bacteria/microalgae	Water source	Nutrient and its removal efficiency	References
Chlorella vulgaris	*Exiguobacterium profundum*	Synthetic (metal containing WW)	79% (Cu) 80% (Ni) 56.4% (Cr)	Shahid et al. (2020)
Chlorella sorokiniana	*Dechloromonas* & *Nitrosomonas* sp.	Synthetic municipal WW	Nitrogen 98% COD 88% (COD) Phosphorus 96%	
Chlorella sp., *Pediastrum* sp., *Scenedesmus sp.*	*Gloeothece* sp.	Agricultural WW	Anti-inflammatory compounds 61%, Galaxolide 68%, Tonalide 73%,	García-Galán et al. (2018)
Pseudokirchneriella subcapitata	*Synechocystis salina*	Synthetic medium	Nitrogen 72.0% (45 mg l^{-1} NO_3-N) Phosphorus 91.8% (410 mg l^{-1} PO_4-P)	Gonçalves et al. (2016)
Microcystis aeruginosa	*Synechocystis salina*	Synthetic medium	Nitrogen 77.7% (45 mg l^{-1} NO_3-N) Phosphorus 97.2% (10 mg l^{-1} $PO_4{}^{-}$P)	
C. vulgaris	*Synechocystis salina*	Synthetic medium	Nitrogen 84.5% (45 NO_3-N) Phosphorus 85.9% (10 mg l^{-1} PO_4-P)	
Chlorella sp., *Scenedesmus spp.*	*Chlorella zofingiensis*	Dairy WW	Nitrogen 87.0–91.0% (176 mg l^{-1} TN) Phosphorus 91.2–96.0% (39.6 mg l^{-1} TP)	Qin et al. (2016)
Centrate WW native algal	Activated sludge bacteria	Synthetic domestic WW	Nitrogen 99.8% (50 mg l^{-1} NH_4-N) Phosphorus 99.8% (7 mg l^{-1} PO_4-P) Carbon 100% (300 mg l^{-1} TOC)	Alcántara et al. (2015)
Scenedesmus sp.	Anaerobic sludge bacteria	Starch WW	Nitrogen 88.7% (30–50 mg l^{-1} NH_4-N) Phosphorus 80.1% (~54 mg l^{-1} PO_4-P) Carbon 80.5% (1800–10,000 mg l^{-1} TOC)	Ren et al. (2015)

(continued)

Table 4 (continued)

Microalgae	Bacteria/microalgae	Water source	Nutrient and its removal efficiency	References
Centrate WW native algal	Activated sludge bacteria	Domestic WW	Nitrogen 70% (91 mg l^{-1} TN) Phosphorus 85% (7 mg l^{-1} PO_4-P) Carbon 90% (181 mg l^{-1} TOC)	Posadas et al. (2013)
C. vulgaris	*Bacillus licheniformis*	Synthetic medium	Nitrogen 78% (20 mg l^{-1}TN) Phosphorus 92% (4 mg l^{-1} TP)	Liang et al. (2013)
C. sarokiniana	Activated sludge bacteria	Pretreated piggery WW	TOC 47% (550 mg l^{-1}) phosphorous 54% (19.4 mg l^{-1}) $NH^{+}4$ 21% (350 mg l^{-1})	de Godos et al. (2010)
Euglena viridis	Activated sludge bacteria	Piggery WW	TOC 51% (450 mg l^{-1}) phosphorous 53% (19.4 mg l^{-1}) NH_4 34% (320 mg l^{-1})	de Godos et al. (2010)
C. vulgaris	*A. brasilense*	Synthetic WW	Phosphorous 31.5% (50 mg l^{-1}) nitrogen 22% (50 mg l^{-1})	Perez-Garcia et al. (2010)

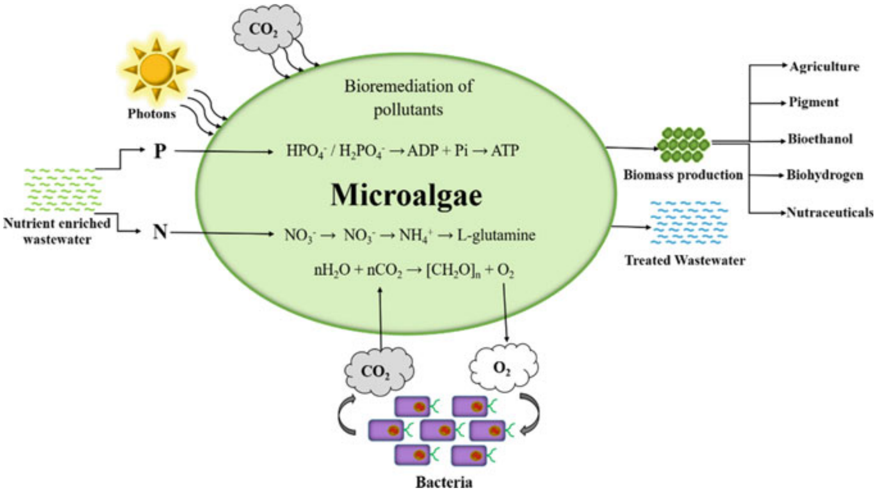

Fig. 4 Demonstration of microalgal-bacterial symbiotic association in bioremediation of pollutants from WW

B. licheniformis-Microcystis aeruginosa co-culture, concluding that the interaction between species varies with different bacterial and microalgal cultures.

WW also contains various microorganisms such as *E. coli* that can potentially lead to several diseases. Interaction of activated sludge bacteria and *C. vulgaris* not only enhance nutrient elimination rate but also remove pathogen and COD, enhancing the algal harvest by sedimentation (Unnithan et al. 2014). The pH of the culture and the Dissolved Oxygen (DO) level is mainly used to determine the pathogen removal (Posadas et al. 2015, 2017). High pH and temperature lower the survival of the pathogen and high DO levels stimulate photo-oxidative damage of cells leading to the removal of a pathogen. Microalgae excrete inhibitory metabolites for pathogen removal. Mezrioui et al. (1994) noted that the toxins produced by *Chlorella sp.* cause the efficient removal of *Vibrio cholera* from WW. The omics studies and next-generation sequence (NGS) provide the opportunity to understand in depth the microalgae-bacterial co-culture (Ramanan et al. 2016).

3.2 Microalgal-Fungal Consortium

Microalgal and fungi interaction is gaining attention due to their harvesting efficiency and ability to form a complete symbiotic relation without the external carbon source requirement (Jiang et al. 2019). Microalgae associate with fungi in the form of lichens, benefiting both partners (Shahid et al. 2020). Ummalyma et al. (2017) proposed that this co-culture can provide a self-sufficient organization that enhances the overall performance at a large scale in the microalgae industry. Similar to bacteria, fungi also assist the cultivation of microalgae as *C. vulgaris* and many other algal species have been shown to form a symbiotic association with several fungal strains such as *Aspergilius niger and Aspergilius oryzae* (Zhou et al. 2013; Zhang and Hu 2012; Luo et al. 2013; Wrede et al. 2014). The detailed characteristic and functional mechanism of the algal and fungal mechanism remains unclear. However, many literatures suggested that the presence of phosphodiester, phosphoric, proton–active carboxylic, amine, and hydroxyl functional groups imparts negative surface charge (−23.7 mV) on algae surfaces. The negative charge interacts with the positive charge (+46.1 mV) on the fungal hyphae due to the presence of polysaccharides. This leads to the neutralization of the negative charges present on algal cells allowing attachment to the fungal hyphae (Gultom and Hu 2013; Grima et al. 2003).

Muradov et al. (2015) cultured algal-fungal pellet on swine WW and found that this association increased WWT efficiency, lipid yield, and total biomass production. Jiang et al. (2019) reported the algae-fungal system *Chlorella variabilis* NC64A and *Ganoderma lucidum* for efficient assimilation of nitrogen, carbon, and phosphorous which showed appropriate nutrient reduction even under low nutrient concentrations. Wrede et al. (2014) reported the efficiency of *A. fumigatus/T. chui* and *A. fumigatus/Thraustochytrid* sp. (*Af/Thr*) pellet to remove nutrients (PO_4^{-3}-P and NH_4^+-N) from swine lagoon WW. This interaction shows significant potential to solve many challenges facing the commercialization of algal process including easy harvesting, cost-effective and efficient nutrient supply via recovery of primary nutrients, however, still, only a few studies have been performed on the morphological characteristic, detailed mechanism, and the biomass production by the microalgal-fungal interaction.

3.3 Microalgal-Yeast Consortium

Yoshizawa (1978, 1981) studied yeast for the WWT process at the end of the 1970s. Since then yeast has gained attention for the treatment of various WW systems. Dynowska (1997) suggested several yeast species including *Candida* sp., *Rhodotorula* sp., *Trichosporon* sp., and *Cryptococcus* sp. as potential candidates for WWT. Yeast and microalgae cultures are studied to amplify microbial lipid production using WW. It has been found that yeast-microalgae co-culture enhances the yield of high-value products leading to a high growth rate and biomass production (Cai et al. 2007). Iasimone et al. (2018b) suggested that yeast inoculum added during the exponential phase of microalgae can increase the assimilation of organic substances from urban WW and can enhance lipid accumulation. The interaction includes the O_2 production by microalgae that are utilized by yeast for heterotrophic metabolism and the consumption of CO_2 by microalgae for photosynthesis. For example, in the *C. vulgaris* and *S. platensis* co-culture systems, the COD removal efficiency and biomass concentration reach 73% and 1.6 g/L, respectively, on monosodium WW (Xue et al. 2010).

3.4 Microalgal—Microalgal Consortia

To overcome the challenge linked with microalgal cultivation, microalgal-microalgal co-culture cultivation was analysed in several literatures. Stockenreiter et al. (2016) studied the cultivation of multiple algal species to promote the synergistic growth of one another and also increase the pollutant removal rate as compared to monoculture. Huy et al. (2018) reported that *Chlorella* sp. and *Scenedesmus* sp. co-culture could eradicate 481 mgL^{-1} of nitrogen and 31 mgL^{-1} of phosphorus under photoautotrophic conditions from textile WW. Chinnasamy et al. (2010) found 15 native algal strain polycultures that efficiently remove more than 96% of nutrients and 9.2–17.8 tonnes $year^{-1}$ ha^{-1} of biomass productivity. Furthermore, the microalgae species that can readily flocculate can be mixed with other microalgae species to increase the sedimentation velocity. Microalgae-microalgae co-culture is not commonly used for WWT but is preferred for enhanced bio-manufacturing. To promote the growth of microalgae and efficient removal of contamination, external aeration is required which increases the cost of the total process.

4 Principle of Pollutant Removal by Algal Consortia

The use of algal–bacterial co-culture is an environment-friendly approach for WWT due to internal O_2/CO_2 exchange, as described earlier. Algal–bacterial consortium exchanges nutrients via suspended flocs formation or biofilm formation. For effective WW remediation, it is crucial to understand the mechanism behind carbon, nitrogen, and phosphorus removal. The basic mechanism (depicted in Fig. 4) lies in the photosynthetic oxygenation where algae produce oxygen that acts as an electron acceptor promoting the growth of heterotrophic organisms. HB utilizes dissolved organic carbon produced by algae and release CO_2 which is further utilized by nitrifying algae and bacteria. Algal and bacterial biofilm showed eminent capabilities in treating WW containing heavy metals like Zn, Pb, Cu, Mn, and Co; organic compounds including lipids, proteins, mono- and polysaccharides, aromatic hydrocarbons, organic acids, etc., (Abinandan et al. 2018). Gerhardt et al. (1991) reported that algae and anaerobic bacteria grown in high-rate pond systems efficiently eradicate heavy metals like selenium (94%–100%) along with nitrate from agricultural WW.

Several pathways, including photodegradation, biosorption, and volatilization, have been reported for pollutant removal by the microalgal-based system. Bioremediation via volatilization mainly converts micropollutants into liquid phases in the atmosphere. However, this process doesn't remove the micropollutant structure from the atmosphere and can cause pollution if no off-gas is collected (Liu et al. 2021). Hom-Diaz et al. (2017) stated that biosorption is a highly efficient process to eradicate micropollutants in dark conditions. Microalgae-based bioadsorption greatly depends on the targeted antibiotic structure including hydrophobicity and functional group availability for chemisorption (Norvill et al. 2016). Hyper-adsorbancy by the microalgal-bacterial co-culture can be accomplished by optimizing the factors influencing the bioadsorption process including initial adsorbate concentration, pH, temperature, contact time, and biosorbent dose (Sutherland and Ralph 2019). Biodegradation in combination with the biosorption process is seen to remove the micropollutant with much greater efficiency. Gao et al. (2011) studied this combination of approaches, stating the mechanism of nonylphenol removal by three different *Chlorella* species. Firstly, the nonylphenol pollutant was exposed to *C. vulgaris* for removal by adsorption, and then the adsorbed nonylphenol was further successfully biodegraded by *Chlorella* sp.

4.1 Algal–bacterial Biofilms

Biofilms are complex structures with microorganism communities growing on the matrix of extracellular polymeric substance (EPS) applied in sewage treatment. Upon availability of substrate, algae grow on bacteria with substrate resulting in biofilm formation. However, an additional nutrient supply (C:N:P) may be required for tertiary WWT to enhance the treatment. The algal productivity increases with the rise in nutrient loading rate corresponding to high P and N uptake. However, this process ceases upon reaching the maximum uptake capacity (Boelee et al. 2011).

Lupini et al. (2011) studied the film formation and suggested that bacteria grow underneath algae and seek surfaces to adhere to and consume oxygen generated by microalgae during the photosynthetic process; this is also referred to as the granulation or immobilization process. Researchers found that the granulation period varies based on nutrients availability, pH, surrounding environment, temperature, and biofilms carrier chemical and physical compositions (Yu and Mu 2006; Lutpi et al. 2015). The algal community forms the basis of the biofilm formation as upon anaerobic condition, the algal–bacterial biofilm gets completely covered by layers of algae. Heterobacteria is located nearby to the algae because of their ability to grow rapidly and with a high affinity to oxygen than phosphorus-accumulating organisms and nitrifying bacteria. Zhang et al. (2018b) suggested that cavities and pores assist the transfer of oxygen and metabolic products in the biofilm. Such an approach efficiently deals with the microalgae harvesting problem and further mitigates a load of conventional settling tanks in WWT.

Microalgal biofilm technologies are widely been approached for nutrient removal and its recovery in the form of biomass by physiological processes (Kesaano and Sims 2014; Mulbry et al. 2005). Biofilms can be cultivated upon several surfaces such as PVC sheetings, polystyrene, concretes, and nylon membranes and can be designed in horizontal and vertical setups (Sukačová et al. 2017; Posadas et al. 2013). “Twin layer” photobioreactor is an example of a vertical system that uses filter paper fastens to a glass plate as a support system for microalgal growth. Shi et al. (2014) reported a 79% reduction in phosphorus from municipal WW using nylon membrane after a two-day cycle.

4.2 Photodegradation of Pollutants

Photodegradation is an acknowledged process for micropollutant removal classified into direct and indirect photodegradation based on the component utilized. Micropollutants absorb light and cause direct photolysis, and in contrast, if they cannot absorb light, indirect photodegradation takes place in the presence of photosensitizers. WW generally contains photosensitizers like dissolved organic matter, nitrate, iron, and carbonate, making the indirect photolytic process more favourable. Yang et al. (2018) reported that organic molecules like proteins, polysaccharides excreted by the microalgae also act as a photosensitizer that helps in the breakdown of micropollutants (Zhang et al. 2014). The rate of tetracycline photolysis by indirect degradation was found 7 times higher than that for control (Norvill et al. 2017). Although the degradation mechanism is lower than the catalytic oxidation, still the strong solar radiation creates gradual degradation and structural alteration of the organic pollutants in aquatic WW (Wang et al. 2016; Li et al. 2014).

The photodegradation mechanism relies upon the environmental condition and molecular structure of micropollutants. The rate of photodegradation was influenced by the intensity of solar irradiance with both season and latitude. For instance, Gruchlik et al. (2018) showed the difference in removal rate of ketoprofen and diclofenac during summer (282 Wm^{-2}) and winter 74 Wm^{-2}. Mathon et al. (2019) analysed the photodegradation of 23 organic micropollutants at different depths and found that the photodegradation is more efficient in the initial 10 cm of the water depth. Further, the disadvantages related to the removal process involve huge reliance on the chemical properties of the contaminants and the incomplete photodegradation of the micropollutants. This phenomenon predominates at the epipelagic zone of the water depth. Also, the identification of the intermediate products is important as the possibility of intermediate being more noxious than the parent compound exists (Rivera-Utrilla et al. 2013).

4.3 Biodegradation

Microalgae-bacterial systems can directly or indirectly participate in pollutant removal. Biodegradation is a complex process where the organic matter breaks down either by complete mineralization to H_2O and CO_2 or through biotransformation that leads to the formation of intermediate metabolites (Alvarino et al. 2018). Biodegradation is the only pathway for removing kepone, benzotriazole, aspirin, bupropion, ketoprofen, and other hormone-active substances (Liu et al. 2021). In the first case, both microalgae and bacteria directly break down the micropollutants and provide metabolites for supporting each other's growth. For example, Shi et al. (2010) observed the efficient removal of active hormone substances only in the presence of microalgae-bacteria co-culture. In another study, Ibuprofen biodegradation by bacteria in lagoon water was found to be possible in the presence of microalgae. However, in the indirect approach, the microbes don't directly play a role in pollutant removal but provide a suitable niche for each other's growth. Xie et al. (2020) reported this indirect approach in the case of sulfamethoxazole eradication by microalgae providing oxygen release and organic matter to enhance the adaptation time and performance of the bacteria. The biodegradation mechanism can be further classified into two classes: (1) metabolic degradation, where the antibiotics serve as electron donor/acceptor or solo carbon source for microalgae; (2) co-metabolism, in which external organic substrates behave as an electron donor for the non-growth substrate and as a substrate for biomass formation (Leng et al. 2020; Tran et al. 2013).

4.4 Phytoremediation

Phytoremediation not only does the metal mining but also applicable for biofortification. In general, the liquid WW carry a high percentage of heavy metal that can be easily separated through several processes including agronomical practices such as conventional plant breeding and/or modern biotechnology. Phytoremediation is a green plant-assisted reclamation that employs micro and/or macroalgae (algae) for treating WW (Wang et al. 2010). The algae, as photosynthetic phytoplankton, rely on sunlight for photosynthetic activity and carbon and inorganic nutrients (nitrogen, phosphate, magnesium, and silicates) as well as other micronutrients for growth. In reality, microalgae have a lot of treatment potential and might remove numerous nutrients from water more effectively than an activated sludge.

Five types of phytoremediation methods have been widely used: phytostabilization (Mendez and Maier 2008), phytodegradation (Zazouli et al. 2014), rhizofiltration (Tiwari et al. 2019), phytoextraction (Clabeaux et al. 2013; Sekabira et al. 2011), and phytovolatilization (Guarino et al. 2020). Bioremediation using variety of microbes is a promising technique for waste removal (ul Hassan et al. 2017). Improvement of water quality along with nitrogen removal and biomass production using sewage utilizing consortia of native filamentous strain of microalgal (MC2: *Phormidium* sp., *Anabaena* sp., *Limnothrix* sp., etc.,), unicellular native microalgal (MC3: *Chlorella* sp., *Chlorococcum* sp., *Scenedesmus* sp., etc.,), and chosen germplasm microalgae (MC1: *Calothrix* sp., *Ulothrix* sp., *Lyngbya* sp., etc.,) was investigated (Renuka et al. 2013). MC2-inoculated sewage water the highest reduction of NO_3 (90%) and PO_4 (97.8%) was achieved. On the sixth day, the biggest drop in TDS from 1120 to 806 mg L^{-1} and the highest increase in DO from 0.4 to 9.0 mg L^{-1} were achieved using MC2-treated sewage water. MC2 (1.07 g L^{-1}) produced the most biomass, followed by MC1 and MC3 (0.90 and 0.94 g L^{-1}), respectively. In another study by Beigbeder et al. (2019), the potential to flourish on bark hydrolysate for simultaneous biomass generation and phytoremediation performances of 12 green microalgae strains tested and formulated as consortia were investigated. *Acutodesmus obliquus, C. vulgaris, Scenedesmus obliquus, and Chlorella sorokiniana* strains as a consortia were able to consume up to 70% and 60% of C5 and C6 sugars, respectively. In addition 55 mg/L/d of carbohydrates, 41 mg/L/d of lipids and 26 mg/L/d of proteins were produced by the microalgal community.

The adoption of phytoremediation as a mandatory treatment in wastewater treatment plants (WWTPs) can provide several benefits such as (Rajhi et al. 2020),

1. Phytoremediation technique can totally eliminate infectious bacteria that pose a major risk to public health.
2. Phytoremediation of WW can be used to irrigate agricultural lands, particularly those that are deficient in essential nutrients like phosphorus and nitrogen, poses a social remuneration of the low-cost valorization of WWs for irrigation.
3. A socio-economic gain results from the reuse of treated water for irrigation, which can help farmers improve their financial status by saving money on artificial fertilizers while employing nutrient-loaded WW.
4. The necessity to employ phytoremediation treatment as the suitable biological treatment that enables the whole elimination of heavy metals, resulting in an ecological improvement. After the treatment, chromium and cadmium heavy metals were removed completely.

5. The fact that phytoremediation technique may produce a high proportion of lipids needed for biodiesel generation results in considerable bioenergy benefits.

4.5 *Quorum Sensing (QS)*

QS, a mechanism of cell to cell interaction by exchanging signals to perform several biological functions, including EPS distribution, biofilm formation, population density observation, virulence, secondary metabolite production (Wu et al. 2016). The bacterial QS signal molecule provides a growth development signal to microalgal, helping nutrient processing and biofilm formation. The effect of QS on biofilm formation was first explained by Davies et al. (1998) in *Pseudomonas aeruginosa*; however, further studies revealed that the effect of QS on biofilm formation was dependent upon experimental conditions, and multiple QS circuits were employed to regulate biofilm formation (Shrout and Nerenberg 2012).The several stages of QS process and the mature biofilm formation have been depicted in Fig. 5.

QS's signal molecules are aromatic Autoinducer signals (AIs) and Acyl-Homoserine Lactones (AHLs). Gram-positive bacteria utilize the LuxS/AI-2 system depending on peptides for interspecies interaction; however, gram-negative bacteria use the LuxR system to respond to AHL molecules. Zhang et al. (2018a) revealed that the AHL-based QS supported the hydrophobic protein formation enhancing the algal–bacterial granular sludge development based on 3D-excitation-emission matrix (3D-EEM) fluorescence spectra analysis and EPS measurement.

QS helps in the formation and stabilization of Aerobic Granular Sludges (AGS), (a spherical biofilm formed of self-immobilized cells without a carrier) by aggregating the small particles into large complexes leading to AGS maturation (Ding et al. 2015).

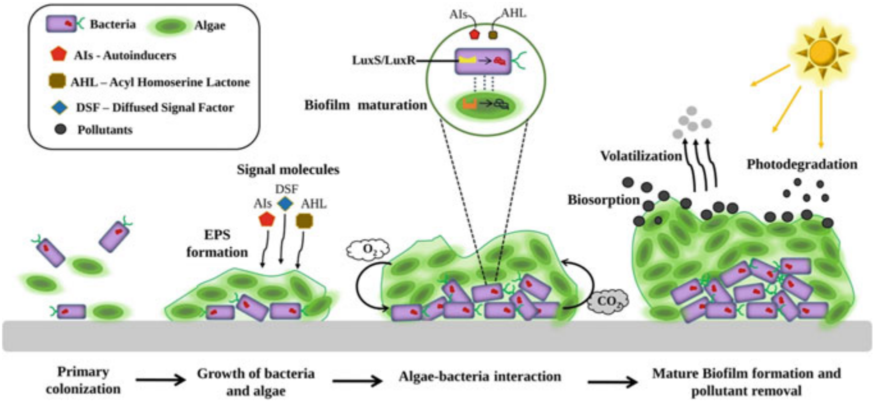

Fig. 5 The stages of mature biofilm formation mediated by quorum sensing. 1. Primary colonization and attachment to the substrate 2. Growth and development of algae and bacteria with response to signal molecule and EPS formation. 3. Algae-bacteria interaction 4. Mature biofilm formation leading to pollutant removal by the various processes

This is found to be an effective treatment for high-strength toxic aromatic pollutants including pyridine, toluene, textile dyes, adsorption of heavy metals, and organic WW. Sludge floc formation can absorb algal cells and prevent the algal cell from washing away, thus maintaining the communication between the algal–bacterial co-cultures. However, the QS of Anaerobic Granular Sludge (AnGS) is relatively more complex than the AGS and has not yet formed an accomplished regulatory process. Several researchers focused specifically on AHL, AI-2, and diffusing signal factor (DSF) in AnGS (Wu et al. 2016). Li et al. (2014) suggested that the performance of AnGS can be enhanced by the introduction of specific AHLs which ultimately increase the removal efficiency of organic matters in AnGS, also regulating the microbial consortium and EPS formation. However, the complexity of this system hinders the complete conclusion on the formation and mechanism adopted by the microbial consortia for the process (Daufresne et al. 2008).

Microalgae also develop a defence mechanism to suppress the excess biofilm formation (biofouling) on their surface by Quorum Quenching (QQ) process (discussed below). Studies revealed that microalgae excrete several secondary metabolites and specific chemicals that inhibit QS (Jha et al. 2013; Shah 2021; Natrah et al. 2011a).These studies suggested complex signalling interactions in phycosphere.

5 Factors Affecting Symbiotic Relationship

A list of factors which affect symbiotic relationships is described in detail below and has been depicted schematically in Fig. 6.

5.1 Dissolved Oxygen (DO)

Organic matter is a more significant component that affects the substrate utilization, growth of microbes, and performance of ASP (activated sludge process). DO is responsible for the oxidation of organics using microbes through BOD. Organic matter and DO affect the algal–bacterial consortium and cause algae decomposition (Goffin et al. 2018).

Maintaining a constant pH and DO% is critical while growing algae (Dineshkumar et al. 2015). The microalgae grew well under the increased DO content in the medium (Taguchi factorial level combination), the pH of the medium remained around neutral. The increase in DO and pH was ascribed to the absorption of CO_2 and the release of O_2during photosynthesis in all cases (Karemore and Sen 2015).

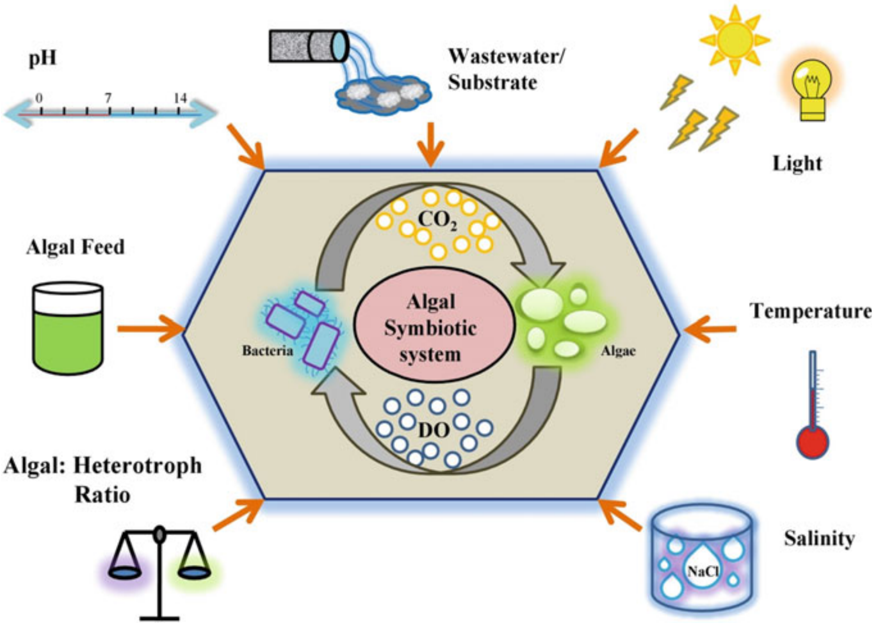

Fig. 6 Schematics of various factors having an important effect on the growth and symbiosis of algae

5.2 Substrate Concentration

Substrate is important for channelling available sugars to lipid production. C:N:P (carbon:nitrogen:phosphorus) is important for the activity of biomass for COD removal. Phosphate concentration limits biomass growth in symbiosis. C/N usually ranges from 10 to 20 for heterotrophs while for algae systems it is 12 to 15 (Cromar and Fallowfield 1997). Sulphides are toxic to algae (~0.055 mM) and bacteria (~0.28 mM) (Küster et al. 2005). Ammonia is the most important inorganic nitrogen source used for the promotion of growth, a very high concentration of ammonia leads to ammonia toxicity (~5–10 mM) but a very low concentration is not suitable for biomass growth (Konig et al. 1987).

5.3 Carbon-di-Oxide (CO_2)

Considering the economic aspect of the WW treatment process, the use of flue gas for the supplement of CO_2 is more economically beneficial than CO_2 in pure form. A higher concentration of CO_2 promotes biomass, improved lipid content, improved organic matter removal, and phosphate/nitrogen removal (Posadas et al. 2015).

The features of microbial strains, including SO_X and NO_X, their temperature tolerance, and the CO_2 contained in the flue gas all have a significant impact on biological CO_2 fixation. Additional parameters like light availability, pH, O_2 removal, photobioreactor design, culture density, and appropriate reactor agitation, among others, will have a major impact on the CO_2 sequestration process. The CO_2 sequestration method necessitates a thorough understanding of flue gas and cell biology. Temperature, CO_2 mass transfer, pH, O_2buildup, SOx and NOx, critical CO_2 concentration, culture strain, light, and culture density are all variables that might influence this process. Hence, limited concentration of CO_2 is not suitable for higher biomass production. For CO_2 sequestration, the airlift reactor appears to be the most promising. The use of an integrated reactor, on the other hand, will aid with scalability (Kumar et al. 2011). Microalgae tolerance to NOx and SOx varies among species; *Nannchloris* can grow in concentrations as low as 100 ppm of NOx (Yoshihara et al. 1996), while *Dunaliella tertiolecta* may thrive as high as 1000 ppm of NOx concentration (Nagase et al. 1998).

5.4 pH

Algal growth is affected by pH, suitable pH for growth is 7–9 with few exceptional species (e.g.: *Spirulina platensis*–alkalophilic with a suitable pH range of 9–10). The concentration of CO_2 has an impact on the pH, it forms acids thus decreases pH and when the CO_2 consumption is more the pH increases. The alteration of pH affects the physiological behaviour of algae (Yen et al. 2019; Goncalves et al. 2017). The pH shift induced by CO_2 has only a modest effect on algal growth, but the significant pH change caused by SOx prevented overall growth. When compared to lower SOx concentrations, buffered medium avoided pH drops maintaining growth rates (Maeda et al. 1995).The major element influencing the development of fungi–algae pellets were pH, which could be adjusted by changing the glucose content and the number of fungal spores injected. When the culture pH varied from 5 to 6, fungal pellets were easily created, and when the pH approached 4, *Aspergillus* sp. UMN F02, filamentous fungi could help produce bigger pellets, implying that pH adjustment during cell development plays a major role in *Aspergillus* sp. pellet production (Zhou et al. 2012). Due to some unknown acid emission, the pH of the culture decreased drastically throughout fungal growth (Xia et al. 2010).

5.5 Light Exposure

Light helps in the maintenance of homeostasis, proper function of photosynthesis, metabolic pathway, and quality biomass production (Iasimone et al. 2018a). Nutrient removal efficiency of algae is proportional to the amount of light exposure, more exposure to light results in more oxygen production and reduction in phenolic compounds (Gonzalez-Camejo et al. 2018; Al-Dahhan et al. 2018). Light has promoted higher PUFA in lipid profile and has no direct effect on bacterial or other heterotrophic organisms. Lower light intensities promote algae motility for adjustment to the change by moving along the depth (Meng et al. 2019). Light, in excess, is also detrimental to the growth and uptake of nutrients. The intensity of saturation light ranges between 30 and 45 W/m^2 (140–210 μEm^{-2} s^{-1}), light intensity of *Scendesmus* sp. and *Chlorella* sp. is about 200 μEm^{-2} s^{-1} (Hanagata et al. 1992).

5.6 Algal Volume

Algal growth depends majorly on the availability of nutrients, water, light, and CO_2 in media as discussed earlier. At higher cell concentration, the cells lying inside will get masked from the light exposure and CO_2 fixation also won't be efficient by those underlying cells thus optimal light and proper mixing improves the higher biomass production and nutrient removal from WW (Hoh et al. 2016; Judd et al. 2015).

5.7 Temperature

Temperature requirement is species-specific, increase in temperature can improve chlorophyll, biomass productivity and activates intracellular enzyme but a lower temperature is not favourable for the growth. Exceptionally, some species can grow even at a low temperature of 10 °C (Judd et al. 2015; Goncalves et al. 2017).The ratio of O_2 to CO_2 solubility raises with a temperature rise, resulting in substantial O_2 fixation by RuBisCO's oxygenase activity. In addition, when the temperature rises, RuBisCO's affinity for CO_2 diminishes (Kumar et al. 2011). Removal of organic matter by algal-assisted WW treatment involves mostly enzymes, and they are highly active at a higher temperature of 25–30 °C, but lower temperature have adverse effect in the consortium (Matamoros et al. 2015).

5.8 Salinity

Salinity has an adverse effect on the growth of algae and is a species-specific requirement. Salinity can be modified by dilution during rainfall and by evaporation during summer. Change in salinity can be avoided by adding freshwater or by mixing with other WWs (Mata et al. 2010).Some microalgae have been shown to thrive in severe environments, such as high salinity (*Dunaliella*), high pH (*Spirulina*), and open ponds (*Chlorella*) (Schenk et al. 2008). In a 19-day analysis, the effect of salinity (15, 25, 35, 45, and 55) on *Nannochloropsis oculata* CS 179 growth, biochemical composition, and lipid production was studied under controlled culture. The results show that the dry biomass of *N. oculata* was the greatest among the treatments in the first 10-day culture at a salinity of 25% ($P > 0.05$). At a salinity of 35% ($P > 0.05$), the dry biomass productivity was greatest over days 14–19 (Gu et al. 2012).

5.9 Algal–Bacterial Symbiosis

The selection of algal species depends upon the characteristics of WW and the ability of the strain for the effective removal of the nutrients. To improve nutrient absorption, algal species are allowed to starve before being exposed to WW. Few strains are reported to have a negative effect while starvation (Ruiz-Martinez et al. 2012; Kube et al. 2018; Solovchenko et al. 2016). The ratio of N to P is another important factor to be considered while dealing with *Klebsormidium*, since the increase in N/P ratio with fixed PO_4 concentration decreases the P removal efficiency (Kube et al. 2018; Liu and Vyverman 2015). Though the algal–bacterial symbiosis improves biomass production of algae, at a limited nutrient availability there found a fight for survival thus chitinases, cellulases, and glucosidases were produced by bacteria which lyses the algal cell wall and utilizes the algal lysate for its growth (Ma et al. 2014; Fuentes et al. 2016). In a study by Gutzeit, for removal of nutrients higher algal incorporation into algal–bacterial consortia are favoured by the following reaction conditions including continuous agitating, HRT of 2–3 days, biomass TSS1-1.5 g/L in a reactor depth of 0.3–0.5 m with sludge age of 20–25 days (Gutzeit et al. 2005).

5.9.1 Ratio (Algal: Fungal/Algal: Bacterial)

Ratio of microorganisms is a crucial factor as increase in one organism's concentration will not favour the growth of other and thus a glitch in the mutualism. Ji et al. (2018) in their study have investigated the effect of the ratio of algal (*C. vulgaris* and *M. aeruginosa*) and bacterial (*B. licheniformis*) in a culture condition for the removal of TDN, TDP, and COD in synthetic WW. *C. vulgaris-B. licheniformis* at the ratio of 1:3 at 10 days is effective in nearly 80% removal but the same ratio is only 60% effective with *M. aeruginosa.* To estimate the effective ratio, a range of ratios (1:0–3:1) were evaluated. *Aspergillus* sp.: *Chlorella* sp. having ratio of 1:2–1:5 in a dry weight basis with an optimum ratio of 1 spores/L:100 cells/L, cultured for 120 h resulted in 4.2 g/L biomass yield (Yang et al. 2019).

5.9.2 Hydraulic Retention Time (HRT)

Generally, HRT determines the time of contact of microbes in the consortium. Usually preferred HRT ranges from 4 to 7 days. Higher residence time improves the more stable algal–bacterial floc formation thus helps in efficient nitrogen removal. Hence, high HRT of cyanobacteria with heterotrophic organisms improves settling properties and nitrification in algal pond whereas lower HRT results in the biomass washout, and hence, poor performance (Medina and Neis 2007; Cromar and Fallowfield 1997). Investigation of the effects of HRT on biogas generation and nutrient removal with microalgae obtained from freshwater source in combination with activated sludge bacteria from untreated primary WW source in a semi-continuous PBR shows that 6 and 4 days HRT are best for TN elimination linked with microalgae, whereas 2 days HRT is not best for increasing microalgae development owing to light constraint in untreated periodical WW (Anbalagan et al. 2016).

5.9.3 Quorum Quenching (QQ)

QS, an important factor that has a huge impact on the stabilization of symbiosis. Its role in stabilizing co-culture has been discussed previously in this chapter. QQ (mechanisms that inactivate QS) is used by a variety of microorganisms to prevent hostile bacteria (luc Rolland et al. 2016).QQ metabolites produced by microalgae can defend aquaculture from pathogens (Natrah et al. 2011b). Dobretsov et al. (2011) found that cyanobacteria from marine sources exhibited QQ against *Vibrio* spp., which has the potential to cause significant diseases in aquaculture. Meyer et al. (2016) found that cyanobacteria of the genus *Lyngbya* are especially potent emitters of substances such as peptides, amides, and lipids responsible for QQ. Tumonoic acids are produced by the cyanobacterium *Blennothrix cantharidosmum*, which mildly suppress the *Vibrio harveyi*'s bioluminescence, without harming its growth (Harrigan et al. 1999). Identification and elimination of unfavourable conditions resulting QQ helps maintain symbiosis.

6 Applications of Harvested Biomass

Depending on growth conditions composition of microalgal biomass can vary, but the ratio of major constituents (i.e., protein, lipid, carbohydrate) remains unaltered. Several species accumulate a variety of value-added products like phycocyanin, lutein, β-carotene, etc. As we discussed earlier, microalgae can be cultivated using different techniques and they are prone to contamination by unwanted species. Also, they can store toxic compounds within themselves. Therefore, on a need basis, pretreatment is done before utilizing this biomass.

6.1 Biodiesel

Biofuel generation from WW is gaining attention because it serves the dual purpose of reducing dependencies on fossil fuel and sustainable waste management. Containing a high concentration of lipid and sugar in algal biomass, it is projected as a promising alternative for bioenergy reservoirs (Behera et al. 2015). Microalgal biomass can be converted to an energy source via different routes; lipid transesterification generates biodiesel, carbohydrate fermentation generates ethanol, and biohydrogen and co-digestion generate biomethane (Sharma et al. 2018). Depending on the abundance of fatty acids microalgae-derived biomass is utilized for a variety of biofuels like biodiesel, biohydrogen, biomethane, and bioelectricity. Under optimum condition, microalgal growth can be as high as 50–100 tonnes DW/hector/year, and up to 50% of this biomass contain lipid (Shuba and Kifle 2018).

6.2 Biohydrogen

Microalgae-based biohydrogen (third-generation) is one of the most favoured new generation clean energy (Demirbas 2009). Microalgae are the natural producer of biohydrogen that splits water into H^+ and O_2in the presence of light. Then oxygen-sensitive hydrogenase enzymes convert H^+ into hydrogen (direct protolysis), often leading to a low production rate (Nath and Das 2004). Alternatively, a two-step indirect protolysis process is utilized where by photosynthesis cells accumulate glucose, and oxygen is released. While in the second stage, cell anaerobically degrades organic compounds with hydrogen production. The later stage is either in the presence (photo-fermentation) or absence (dark fermentation) of light. Three important enzymes of this pathway are; hydrogenase (converts proton into hydrogen with help of PS II, PS I, ferredoxin), nitrogenase (cyanobacterial enzyme converts nitrogen into ammonium and produce hydrogen), and uptake hydrogenase (oxygen resistant, present in heterocysts) (Guan et al. 2004; Rashid et al. 2013). Mu et al. (2020) deployed cost-effective biohydrogen production scheme (dark fermentation) using duckweed as feedstock. The highest production (169.30 mL g^{-1} DW) was achieved at 35 °C and 1% H_2SO_4 pretreatment.

6.3 Biogas

Anaerobic digestion of microalgae biomass produces biogas. Hydrolysis followed by fermentation of biomass produces volatile fatty acids (propionic, butyric, and valeric acids). Finally, via acetoclastic methanogenesis, biomethane is formed (Uggetti et al. 2017). Carbohydrate and protein-rich microalgal biomass from piggery WW produced 106–146 and 171 ml methane/g COD, respectively, (Molinuevo-Salces et al. 2016). Toledo-Cervantes et al. (2016) reported a biogas up-gradation system linking HRAP to external Co_2-H_2S absorption column. Using this system >99% CO_2 and H_2S were removed and produced methane fulfils EU legislation for injecting into natural gas grids.

6.4 Biochar and Biofertilizer

Biochar is a solid material obtained from thermal degradation of different biomass (plant, lignocellulosic waste, and algal biomass) under oxygen-less conditions (Nair et al. 2017). It is widely used in agriculture as a plant nutrient source, stabilizer of organic matter in the soil, and also for soil carbon sequestration. Biochar can increase nitrogen, phosphorous, total carbon, microbial biomass, and rhizobial nodulation (Biderman and Harpole 2013).

Biofertilizer is considered a pollution-free, green, and sustainable alternative to chemical fertilizer (Dineshkumar et al. 2020). The efficacy of microalgae-based biofertilizers to improve soil nutritional status is well-documented. Some algal species can add upto 25–40 kg N/ha (Hussain et al. 2021). Secreted exopolysaccharide also improves soil microaggregates stability. Additionally, it produces plant growth hormones, metabolites, and polysaccharides and protects against pathogenic microorganisms. Algae-based biofertilizers are generally three types. First, slow-release biofertilizer (algal biomass after dehydration and pasteurization applied to the soil which is slowly degraded by microbial community example *Proteobacteria*, *Acidobacteria*, and *Bacteroidetes* and releases a large amount of nitrogen),second is living algal cell (directly grown blue-green algae *Nostoc* and *Anabaena* genera which fixes atmospheric nitrogen) and third, algal liquid biofertilizer (algal extract containing nutrients and minerals) works as biostimulant (Guo et al. 2020). Few microalgal biomass-based biofertilizers and their efficacy are summarized in Table 5.

7 Algal Biorefinery: Influential Factors, Challenges, and Advancements

7.1 Algal Symbiosis: Momentous Factors

Molecular mechanisms behind the symbiotic interactions which bring about the mutualistic relationship are not fully understood. According to researchers, potential sources for symbiosis are mainly due to primary metabolites exchange (Bai et al. 2014), cofactors, and hormones exchange (Bajguz and Piotrowska-Niczyporuk 2013), physical niches in the ecosystem (de-Bashan et al. 2008). Primary metabolite exchange has many examples like CO_2 and O_2 exchange, substrate exchange, etc. Algal-Algal symbiosis can also be possible through cofactor exchange (Higgins et al. 2016). Croft in his study investigated the vitamin cofactor exchange between bacteria and algae (Croft et al. 2005).

Table 5 Microalgae-based biofertilizer

WW source	Algal-microbial strains	Treated plant(s)	Result	References
Dairy (synthetic)	Aq. Cell extracts of *Chlorella variabilis* and *Scenedesmus obliquus*	Corn (*Zea mays*) and soybean (*Glycine max*)	Improved growth rate, increased phenolic and flavonoid content, higher antioxidant activity	Gatamaneni Loganathan et al. (2020)
Domestic	De-oiled biomass of *Chlorella* sp. and *Scenedesmus* sp.	Tomato (*Solanum lycopersicum*)	Improved shoot length, root length, dry and fresh weight, macro/micro-nutrients, and higher tomato yield	Silambarasan et al. (2021)
Domestic	Selenium-enriched biomass and extract	Beans (*Phaseolus vulgaris*)	Se-biofortified bean production, enhanced germination rate, increased seedling length, vigour index	Li et al. (2021)
Sewage	Consortium of *Chlorella*, *Scenedesmus*, *Chlorococcum*, *Chroococcus*, *Phormidium*, *Westiellopsis*, *Spirogyra*	Wheat (*Triticum aestivum* L. HD2967)	Increased macronutrient content in the root, shoot, and grain, increased plant dry weight, positive correlation between soil nutrient and biometrical parameters of plant, Improved yield	Renuka et al. (2018)

(continued)

7.2 *Challenges*

Algae for WW treatment are gaining popularity because of its environmentally favourable and long-term benefits, but it comes with a set of limitations and flaws. The adoption of cost-effective technology with better yield is one of the key difficulties. The criteria required at various level of biorefinery construction for algal consortium has been listed (Table 6).One of the biggest drawbacks of the cost-effective algal WW treatment is biomass collection. Challenges associated with the selection

Table 5 (continued)

WW source	Algal-microbial strains	Treated plant(s)	Result	References
Brewery	*Scenedesmus obliquus* culture and biomass	Barley (*Hordeum vulgare*) and Wheat (*Triticum aestivum*) seeds	Enhanced germination and growth of both barley and wheat	Ferreira et al. (2019)
Domestic (with coal-fired flue gas)	De-oiled biomass of *Scenedesmus* sp.	Rice (*Oryza sativa*)	Improved plant height, tiller number, grain yield, panicle weight, and 1000-grain weight	Nayak et al. (2019)

of harvesting technology include: higher growth rate, negatively charged microbial surface, and the small size of the cell (Milledge and Heaven 2013). Physical, chemical, and biological-based methods are employed. Gravitational force-based sedimentation methods driven by density difference (Collet et al. 2011) and centrifugation (Rawat et al. 2013) are commonly employed physical methods. Negative surface charge is neutralized using synthetic polymer and electrolytes to achieve flocculation (aluminium sulphate and ferric chloride) (Sims and Christenson 2011; Udom et al. 2013) but chemical flocculants obstruct downstream processing and increase product recovery costs. Thus, natural flocculation under elevated pH, change in nutrient or DO concentration is desirable but still unreliable (Uduman et al. 2010; Schenk et al. 2008). Electrophoresis also improves flocculation with the advantage of the chemical-free method but involves higher operational cost (Sims and Christenson 2011). Floating by decompression of pressurized fluid promotes algal mat formation for easy harvesting; this method is an effective but energy-intensive method (Hanotu et al. 2012; Zhang et al. 2012). Filtration gives 90% recovery of biomass but the selection of the filter medium is influenced by the size of the algal biomass (Shen et al. 2009). The immobilization method of cell separation onto the polymeric bead is conventional and cost-effective (Blank et al. 2016). The biological method of biomass harvest includes the use of filter feeders, algal biomass removal using filter feeders in laboratory-scale systems (Kawasaki et al. 1982) and mezocosms were possible and experimented (Kim et al. 2003; Jung et al. 2009). The above-discussed methods come with their own cost, thus cost-effective biomass harvesting is a bottleneck in algal WW treatment and it can be addressed by adaptation of co-culture technique as discussed earlier in this chapter.

Table 6 Algal cultivation in WW with influencing criteria and parameters

Substrate Level	WW	Criteria for WW	1. Nutrient Concentration: Chemicals, Trace elements, organic carbon, and COD 2. N/P ratio: TP and TN content 3. Availability and accessibility	Selection of WW is very important and depends on the versatility of the organism under study. Biomass growth and product production highly depend on the nutrients in WW	Liu et al. (2020), Yadav et al. (2021)
		Types of WW	1. Pharmaceutical Industry 2. Metal processing/Mines 3. Agro-Industrial 4. Anaerobic digestion 5. Municipal water 6. Textile/Leather 7. Centrate WW	Algal treatment of WW helps reduces pollution by reducing COD and organics in it. Nutrient recovery and product production are two in one advantage and it depends on the type of WW	Udaiyappan et al. (2017), Villegas et al. (2017)
	Pretreatment	Criteria for pretreatment	1. Process Cost 2. Suitability and Applicability 3. Scale-up Broadly the pretreatment methods were classified into, physical, chemical, and biological E.g.: Autoclaving, dilution, filtration and UV treatment, ultrasonic, enzymatic, microwave, acidic, bead milling, basic, steam explosion, etc.,	Sterilization adds additional cost to the process (20–30%) but it is necessary to avoid contamination and helps in the breakdown of a complex compound into a simple compound for easy assimilation. Pretreatment suits for WW as well as for post cultured algal biomass pretreatment to produce methane or to use it as manure	Passos et al. (2013), Kumar et al. (2018), Juárez et al. (2018)

(continued)

Table 6 (continued)

Microalgal level	Strain selection	Criteria for selection	1. Growth rate—In severe environmental conditions 2. Large-scale adaptability 3. Tolerance (CO_2; NH_4^+; Heavy metals like Cadmium, Copper, Chromium, and lead; Organic matter like acetate, butyrate, and propionate) 4. Incubation time 5. Harvesting 6. Product—quantity, and quality	Selection of strain for WW treatment and product production is a crucial step. Organism must satisfy the below-listed criteria to be considered as a potential strain for large-scale industrial applications	Yadav et al. (2021), Wang et al. (2016)
	Cultivation	Methods	1. Photoautotrophic 2. Photoheterotrophic 3. Mixotrophic	Cultivation mode is important for the consideration of co-cultivation for efficient WW purification	Yu et al. (2020), Chojnacka and Noworyta (2004)
Cell Harvesting			1. Centrifugation 2. Electrophoresis 3. Filtration 4. Floating 5. Flocculation 6. Sedimentation 7. Ultrafiltration 8. Electro-coagulation Criteria: Selection of harvesting method is based on the final moisture content required, >85% of the moisture is detrimental	Downstream processing starts with cell harvesting. Costlier the method of harvesting higher will be the operational cost Recently fungal assisted flocculation is gaining more importance	Milledge and Heaven (2013), Grima et al. (2003)

(continued)

Table 6 (continued)

Bioreactor Design (Photobioreactors)	Tubular reactors	1. Vertical Tubular (Airlift/Bubble column) 2. Horizontal (Diaphragm/mechanical pumps) 3. Helical (centrifugal pumps) 4. α–shaped reactor (Airlift) Criteria: Volume of sample, physiology, and requirement of microbes, amount of light required, and aeration for growth	Generally with high S/V, good mixing with efficient CO_2 supply, unidirectional flow rate. For some of the listed types requires heat exchangers	Dasgupta et al. (2010)
	Flat plate reactor	1. Flat plate pivoted at the center 2. Flat plate bubbled at bottom 3. Floating type 4. Induced diffused PBR 5. Torus shaped 6. Fermentor type with lighting (internal/external)	For flat panel pivoted at center type the scale-up is difficult. Generally, flat plate reactors are having a medium S/V ratio. Heat exchange coils are required to maintain temperature for most of the reactors	

7.3 Advancements

7.3.1 Bio-Physicochemical Coupled System

Combining physical and/or chemical processes along with biological methods (bioremediation by algal WWT) to treat intractable compounds present in the WW. This adds additional techno-economic burdens like high capital cost, energy consumption and may have higher pollutant generation. In a study by Fernandes et al. (2014), nearly 68% of the COD removal from pulp mill WW effluent was achieved by yeast. A new technique called solar fenton is coupled with it to remove nearly 90% of the dissolved organic carbon, hence, the water is suitable to be released into natural water bodies. Advanced Oxidation Process (AOP) coupled with immobilized biological reactor have been used to treat winery WW having Dissolved Oxygen Concentration (DOC) of 882 mg/L resulted in 10 mg/L DOC after treatment for 28 days (Souza et al. 2013). When the temperature of the experimental solution (6×10^{-7} M Cd^{2+}) was dropped from 20 °C to 2 °C, Cd accumulation by *Selenastrum capricornutum* fell by 50% (Eckhardt and Buckhout 2000). When the temperature was reduced from 12 °C to 2 °C, Boisson et al. (1997) detected a reduction in internalized Cd by *Fucus vesiculosus*.

7.3.2 ANN an Artificial Intelligence Approach

Artificial Neural Network (ANN) is another promising technology used recently in all the fields for better modelling and to obtain logical output based on the previous experience gained over time. The basic architecture of ANN includes input neurons connected to the output neuron through hidden layers, hence, it is referred to as the black-box model. Data collection, pre-processing, network training, and validation are the important stages of ANN network construction. Various researchers have attempted to bring the AI into the application of WWT (Mjalli et al. 2007) some of the selected examples were listed (Table 7).

Temperature, pH, EC, DO, NO_3, and $PO_4{}^{3-}$ were the six inputs to predict the microalgae DCW using a three-layer Feed-forward BackpropagatioN (FBN) ANN. The greatest prediction performance was attained using the optimized ANN design (6–10–1) with the Levenberg–Marquardt training method withR^2 of 0.983(Ansari et al. 2021). Model by Noguchi et al. (2019) aimed to estimate the growth of polyculture microalgae in a semi-continuous ORP using a multilayer backpropagation neural network. Another example involving the growth of microalgae *Karlodinium veneficum* using FBN neural network was used for predicting the growth dynamics with 25 different components in the culture medium as input. Training with nearly 420 batch experiments containing different media compositions were performed. Cell concentration and culture duration are the output parameter tested. The output showed that vitamins and microelements have a relative impact on growth compared

Table 7 AI in the application for WW treatment

Algorithm	Organism	General outcomes	WW	Reference
Monte Carlo simulation with a linear regression model	NA	To calculate the efficiencies of biomass generation, chemical oxygen demand (COD), total nitrogen and phosphate (TN and TP) removal	Piggery WW	Ambat et al. (2019)
Linear Regression	*Chlorella zofingiensis*	Using a tubular bubble column PBR, COD values ranging from 400 to 3500 mg/L were evaluated	NA	Zhu et al. (2013)
Logistic regression applied with Luedeking–Piret model + Spearman's rank correlation analysis	*Rhodopseudomonas palustris*	Carotenoids biosynthesis dynamics simulation. 1 L PBRs in parallel	Acidic food industry effluent high in volatile fatty acids (COD 3350 mg/L and TP, TN with 34 mg/L, 200 mg/L respectively)	Liu et al. (2016)
Decision tree—supervised learning algorithm	*Dunaliella salina*	To assess *D. Salina* abundance: the number of sunshine hours, salt concentrations, and DO levels had a positive effect	Meighen wetland (hypersaline wetland)	Zarkami et al. (2020)

to that of the macronutrients. This model helps predict the cell concentration with previously untested conditions (García-Camacho et al. 2016).

7.3.3 Internet of Things (IoT)

IoT is an emerging field with the integration of new digital technology for the easy monitoring and mitigation of online problems without causing delay. IoT is useful in the water quality measurement, with a dimensionless water quality index, few measurable parameters are measured online and data log maintained in the database for future reference. BOD, COD, DO, Electrical Conductivity (EC), Oxidation–Reduction Potential (ORP), pH, salinity, temperature, TN, Total Phosphate (TP), and turbidity are the measurable parameters to access the quality of water, these can be achieved by the use of highly advanced sensors. The architecture includes a microcontroller (processes the signal), sensor (detects the signal), and communication setup (transmit the signal from onsite to over the internet). IoT has become more famous due to its ability of automated real-time monitoring, adaptive, and responsive system and cheaper than traditional measurement (Zin et al. 2019; Dogo et al. 2019). Availability of open-source platforms for IoT like Raspberry Pi, Arduino, BeagleBone, and ESP8266 which supports both short-range (Bluetooth and Wi-Fi) and long-range communication (LoRA, 3/4G, UMTS, and GPRS) methods of data transmission (Singh and Kapoor 2017; Marques et al. 2019). Few examples of use of IoT are as follow,

Saravanan et al. (2018) in his study conducted at a water pumping station in Tamil Nadu, India has measured (turbidity, temperature, and colour) parameters using a Supervisory Control and Data Acquisition system (SCADA) enabled with IoT. GSM module has used for data transfer in wireless mode. Zin et al. (2019) in their study conducted at surface water in Curtin Lake, northern Sarawak in the Borneo Island has measured (turbidity, temperature, pH, water level, and CO_2) parameters using a Field Programmable Gate Array (FPGA) enabled with IoT. Zigbee wireless communication module used for data transfer in wireless mode. Liu et al. (2019) in his study on drinking water quality conducted at a water pumping station in Yangtze river in Yangzhou, China has measured (Ammonia, Conductivity, COD, DO, pH, turbidity, temperature) parameters incorporated the Long Short Term Memory (LSTM) deep learning neural network-enabled with IoT. Hence, IoT can be used for monitoring the quality of water as well as can be customized to measure parameters of our interest in WWTPs for timely identification and elimination of contaminations.

8 Conclusion

Microalgae-assisted WW reclamation called phycoremediation is a promising method for the achievement of environment-friendly treatment and energy production as a two in one benefit. Apart from the reduction of pollutants in the WW and making it suitable for release into water bodies, the algal biorefinery is also famous for its ability in CO_2 sequestration, thereby contributing to the curtailment of greenhouse gas emissions. Though having high values, algal-based lipid production and treatments are not yet commercialized due to their less yield and revenue involved in the operation. This has laid a path for the researchers to explore various opportunities to make it reach industrial scale. Hence, this book chapter took an opportunity to emphasize the advancements in the algal biorefinery process for improved biomass and lipid accumulation. Co-culturing by establishing a symbiotic benefit between microalgae with any oleaginous microbes combination has to be explored to achieve a higher yield, because symbiosis help achieves operational issues with ease and also in a cost-effective way. Algal biomass has high lipid content, making it suitable for the synthesis of biodiesel, while the long-chain fatty acids, proteins, and pigments found in it have its own medicinal and nutraceutical benefits. As a conclusion, microalgal biorefinery methods should be investigated further. To establish the market viability of transforming microalgae constituents into bioenergy (biodiesel, biohydrogen and biogas) and other beneficial value-added products (biochar and biofertilizers) can help improve the revenue out of it. As a future direction, it is also crucial to look at cost–benefit life cycle analysis and techno-economic feasibility of the processes and have to explore more organisms for symbiosis relation to get a high yielding low operation cost combination suitable for scale-up into commercial operation.

Conflict of Interest The authors have declared no conflict of interest.

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Emphasizes the Role of Nanotechnology in Bioremediation of Pollutants

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1 Introduction

As a consequence of the industrial revolution, economic progress has been accompanied by a rise in pollution. Recent research has shown that bioremediation methods are a novel and successful strategy for removing contaminants from varied settings, as well as a highly adaptable and scalable management alternative. In recent years, this has been developed to boost the efficiency of pollution removal. Biostimulation and bioaugmentation have been found to be effective ways of expediting the decontamination of a polluted region (Tanzadeh et al. 2020). Despite the fact that bioremediation is a fantastic and adaptable technology for recovering a variety of contaminants, it is unsuccessful when dealing with high concentrations of pollutants, xenobiotics or refractory compounds, resulting in unsustainable treatment efficiencies and recovery durations.

Biostimulation and bioaugmentation were shown to be efficient strategies for speeding the decontamination of a contaminated site with low ecological damage. When dealing with large quantities of contaminants, xenobiotics or refractory chemicals, bioremediation is ineffective, generating unsustainable treatment efficiencies and recovery times. A new technique for bioremediation is being developed by integrating nanotechnology and the use of nanomaterials, defined as particles having at least one dimension of less than 100 nm. Nanoparticles, defined as particles with

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two or three dimensions of less than 1 nm, are especially important. This combined approach can include a broader range of potential applications with reduced costs and minimal negative environmental impacts in the treatment of pollutants in groundwater and wastewater sediments polluted with heavy metals and hydrocarbons or organic or inorganic compounds in soil. Heavy metals are mostly derived from sediments. Atmospheric deposition, soil erosion, rain leaching, wastewater discharge, and scouring are just some of the ways pollutants get in, Mercury (Hg), lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), zinc (Zn), copper (Cu), and nickel (Ni) are some of the prominent trace metals found in sediments (Chang et al. 2022). Heavy metals returned to the uppermost layer of water in reaction to changes in the physicochemical parameters of the water, resulting in secondary contamination. Heavy metals may affect plants and animals and reach humans through the food chain due to a variety of toxicological concerns, bioaccumulation, and persistence in the environment.

Phenols and their derivatives are among the most hazardous pollutants discovered on Earth and are ubiquitous in nature. Halogenated phenols, for example, are anthropogenic pollutants that have been documented to be carcinogenic, mutagenic, and teratogenic (Nešvera et al. 2015). Various industrial effluents, home wastewater, chemical spills, and agricultural runoff have a significant impact on several aquatic bodies. Furthermore, solid waste is made up of dangerous poisonous inorganic and organic substances that are produced by home and industrial garbage. They contaminate the environment, and the majority of the litter is non-biodegradable. These wastes have a negative impact on soil structure, liveliness, and fertility, as well as animal and human health. Fly ash is a significant cause of pollution in the vicinity of the industrial region. Chemical pesticides and fertilizers, on the other hand, are used in excess on crops to increase production while poisoning the land and water. The fertilizer, insecticide, pesticide, and pharmaceutical industries generate a significant amount of solid and liquid waste. Toxic chemicals enter the food supply through a variety of methods, including bioaccumulation, biomagnification, and bioconcentration.

Bioremediation is gaining popularity as a green and long-term solution to contaminated environments (Azubuike et al. 2016). Bioremediation, on the other hand, is a time-consuming process that may or may not be effective in the treatment of severely polluted locations. Efficiency, cost-effectiveness, complexity, dangers, resource availability, and time-consumed are all important factors to consider when choosing an acceptable plan for contaminated site clean-up.

With this in mind, the use of nanotechnology in biological processes has been proven to be helpful. The process is known as nanobioremediation. It is now being researched extensively in a number of polluted areas. Environmental pollutants are made more amenable to biodegradation by biological processes using the nanobioremediation technology. Combining biotechnology and nanotechnology for environmental clean-up is a promising technique. Nanomaterials can either be employed alone or in conjunction with bacteria or microbial enzymes. Microbes or microbial enzymes might be immobilized on nanoparticles or employed in combination with nanoparticles.

2 Nanotechnology—A New Frontier in Science and Technology

Frontiers in nanotechnology covers cutting-edge basic and practical research on innovative nanofabrication technologies, as well as the synthesis, assembly, and characterization of nanostructures with biomedical, energy, and environmental applications.

Nanotechnology is already being employed in industrial areas such as information and communications, as well as in food technology, energy technology, and some medicinal items and treatments. Nanomaterials may potentially open up new avenues for environmental pollution reduction. Nanotechnology plays a frontier role in different sources, they are-

2.1 A Frontier in Food Science

The global food system has the potential to be revolutionized by nanotechnology. Novel agricultural and food security systems, disease-treatment delivery methods, molecular and cellular biology tools, pathogen detection sensors, environmental protection, and public and future workforce education are all examples of the significant impact nanotechnology could have on agriculture and food systems science and engineering (Nasrollahzadeh et al. 2019). Some instances are as follows:

- Nano-sensors for pathogen and contaminant detection might improve the safety of food manufacture, processing, and shipping.
- Specific nano-devices might allow for precise tracking and recording of a product's environmental conditions and transportation history.

Development of novel functional materials, micro- and nanoscale processing, product development, and creation of procedures and instruments for food safety and biosecurity are the four primary areas in the food business where nanotechnology will most likely have a substantial impact (Maksimović et al. 2019). The figure depicts the possible uses of nanotechnology in the food business, organized by target area.

Agriculture and agricultural research will bring some of the benefits of nanotechnology to the food industry. The development of new molecular and cellular biology tools will lead to significant advances in reproductive science and technology, as well as the conversion of agricultural and food wastes into energy and useful by-products via enzymatic nano-bioprocessing, and disease prevention and treatment in plants and animals. New materials with unique nanoscale features, such as self-assembly and self-healing qualities, or the ability to detect pathogens and contaminants, might be game changers in the agriculture and food industries in the near future.

2.2 A Frontier in Agriculture

Nanotechnology is at the forefront of the most recent technical developments. It may be used at all stages of agricultural product production, processing, storage, packing, and transportation. Precision farming will be achieved by reducing the usage of herbicides, pesticides, and fertilizers while increasing efficiency, controlling release, and targeting delivery. The dream of fully automated and centrally managed agriculture is now a possibility. Because traditional agriculture will not be able to feed an ever-increasing population in the face of changing climates, decreasing resources, and limited landscapes, modern agriculture is urgently needed. However, the use of nanomaterials in the agri-food sector must be examined for public approval so that it does not encounter the same problems that GMOs had in the past (Gómez-Llorente et al. 2022).

The use of nanotechnology in agriculture is gaining traction because of the potential benefits, which include increased food value, lower agricultural inputs, greater nutritional content, and extended shelf life. The agriculture industry is the backbone of the economy in developing nations, with more than 60% of the people relying on agriculture for a living, either directly or indirectly. However, it still needs to cope with challenges such as the sustainable use of natural resources, the depletion of nutrients in soil, and environmental issues such as runoff and the build-up of fertilizers and pesticides in the twenty-first century. The key to adopting such technology is that it has the potential to shape modern agriculture in a more productive manner, leading to precision farming in a cost-effective manner with the delivery of just the correct quantity of input at the appropriate moment. Nanotechnology, among the most recent technical advancements in the area of agriculture, has a prominent place in reshaping agriculture and food production to meet demand in an efficient and cost-effective manner (Prasad et al. 2014).

Nanotechnology promises that future food will be tailored to the preferences of consumers, with improved flavour, texture, nutrient content, and shelf life. It has the potential to be a compelling value proposition and the “next big thing” in future agriculture. The food will be packaged in “smart” packaging that can detect impurities and spoiling agents.

2.3 A Frontier in Medicine

Nanotechnology is clinically viable and has the potential to aid in the diagnosis of both general and microbiological illnesses. It is crucial to detect harmful bacteria quickly at the point of care. Nanoparticles allow for the direct detection of infectious diseases in tiny sample quantities in a sensitive, specific, and fast manner at lower costs than currently available methods. It is clinically viable and has the potential to aid in the diagnosis of both general and microbiological illnesses. It is crucial to detect harmful bacteria quickly at the point of care. Nanoparticles allow for the direct detection of infectious diseases in tiny sample quantities in a sensitive, specific, and fast manner at lower costs than currently available methods. Controlling physiologically relevant structures with molecular accuracy is what nanotechnology entails. Nanomedicine is looking at how carbon buckyballs, dendrimers, and other skilfully made nanoparticles may be used in new drugs to fight viruses, germs, cancer, and drug delivery (Shunin et al. 2018). Medical nanorobots will be the size of a microbe, self-replicating, and equipped with sensors, processors, manipulators, pumps, pressure tanks, and power supply aboard. Molecular manufacturing will need to use massively parallel assembly lines in nanofactories to build such complex molecular machine systems.

3 Principles of Bioremediation

Biological degradation of organic waste under controlled circumstances to a benign state or concentration levels below regulatory limits is what is meant by the term "bioremediation" (Giri et al. 2021). Environmental pollutants may be used as a food source by microorganisms because they have enzymes that make this possible. When it comes to bioremediation, the goal is to get organisms to degrade or detoxify harmful pollutants by providing them with the right amount of nutrients and other chemicals they need to thrive. Enzymes have a role in each and every one of these processes. It is part of the family of oxidoreductases, hydrolases, and lyase transferases. This is related to the fact that many enzymes may degrade a large variety of substrates, both specific and non-specific. There must be enzymatic action on the contaminants in order for bioremediation to be successful. Because bioremediation can only be successful in environments that enable microbial growth and activity, it is often used in conjunction with environmental parameter modification to expedite microbial growth and destruction (Kour et al. 2021).

i. Bioremediation works by encouraging the development of bacteria that feed on pollutants such as oil, solvents, and pesticides for food and energy.
ii. The pollutants are consumed by these bacteria, which convert them to little quantities of water and harmless gases like carbon dioxide.
iii. A combination of the correct temperature, nutrition, and food is required for effective bioremediation; otherwise, the removal of toxins may take significantly longer.
iv. If the environment is not conducive to bioremediation, it can be enhanced by adding "amendments" such as molasses, vegetable oil, or simply air.
v. These changes provide the ideal environment for bacteria to thrive and finish the bioremediation process.
vi. Bioremediation might take anything from a few months to many years to complete.

vii. The duration required is determined by factors such as the size of the polluted region, the concentration of toxins, environmental factors such as temperature and soil density, and whether bioremediation will be done in situ or ex situ.

3.1 Types of Bioremediations

3.1.1 Ex Situ Bioremediation

These methods entail removing pollutants from contaminated areas and delivering them to a treatment facility. The treatment cost, depth of pollution, the pollutant, the degree of pollution, and the geology of the contaminated site are considered when ex situ bioremediation approaches. Ex situ bioremediation strategies are selected based on performance parameters.

Biopile

Biopile-facilitated bioremediation is heaping the ground with toxic soil, followed by nutrient augmentation and aeration to enhance microbial activity and refining bioremediation. The major components of this system include aeration, irrigation, nutrient and leachate collection systems, along with a treatment bed. The engagement of such ex situ technology is fetching popularity owing to its beneficial features of cost-effectiveness and skills of achieving excellent biodegradation under ambient management of nutrition, temperature, and aeration. Using biopile in polluted expanses can minimize volatilization of low molecular weight (LMW) contaminants, together with efficient restoration of contaminated settings in cold and remote locations. In line with this, Gomez and Sartaj (2014) used response surface methodology (RSM) based on factorial design of experiment (DoE) for examining at lower temperatures the effects of microbial consortia application rate (3 and 6 ml/m^3) as well as mature compost (5 and 10%) on reduction of total petroleum hydrocarbon (TPH) in field-scale biopiles (Gomez and Sartaj 2014).

The bioaugmented and biostimulated set-up showed 90.7% decrease of TPH in 94 days compared to the controls with 48% TPH elimination. This was attributed to a coactive interaction between bioaugmentation and biostimulation, revealing biopiles versatility for environmental remediation. Dias et al. (2015) found a 71% reduction in the total hydrocarbon content along with a shift in bacterial structure after pre-treating polluted soil prior to biopile formation and subsequent biostimulation for 50 days using fishmeal. Biopiles may bioremediate a variety of soil samples, including clay and sandy soil (Akbari and Ghoshal 2014). Their versatility provides for a quicker remediation since addition of a heating system in the design may promote microbial action and pollutant accessibility, ensuing faster degradation rates (Azubuike et al. 2016). Furthermore, incorporating warm air into biopile design provides with both air and heat for further easing bioremediation. Sanscartier et al. (2009) reported humidified biopiles with lower ultimate TPH concentration in comparison with heated and passive ones due to appropriate moisture content, decreased leaching, and little volatilization of less degradable pollutants.

In addition, biopile were said to be able to cure a huge amount of dirty soil without requiring much space. The biopile arrangement may simply be scaled up for pilot setting to produce comparable results as in laboratory experiments. Prior to processing, contaminated soil must be sieved and aerated, which is critical to the biopile's performance. To improve the remediation process in a biopile, bulkier components such as straw, bark, sawdust, wood chips, and other organic materials are used.

Biopile setting save space in comparison with other ex situ bioremediation methodologies, say land farming and robust engineering. However, higher maintenance and operation cost, and deficiency of power supply predominantly at inaccessible locations, which would otherwise permit uniform air distribution in contaminated piled soil, are challenges that must be figured out. Air heating results drying of bioremediation soil and inhibition of microbial activity, promoting volatilization rather than biodegradation (Zheng et al. 2021).

Windrows

Windrows depend on frequent rotation of polluted soil piles to upsurge bioremediation by triggering the degradation potential of indigenous and/or transitory hydrocarbonoclastic polluted soil bacteria. Periodically churning the polluted soil, along with the water addition, endorses aeration, even dispersal of pollutants, nutrients, and microbial degradative actions, to hasten bioremediation through assimilation, biotransformation, and mineralization. In comparison with biopile treatment, windrows have superior hydrocarbon removal rate; however, this is further effective due to the much friable soil type. However, frequent turning involved with windrow treatment may not be suitable for remediating soil polluted with toxic volatile contaminants. The application of this technology has been related to production of greenhouse gas like CH_4 owing to development of an anaerobic zone inside contaminated soil piles on inhibition of proper aeration (Tran et al. 2021).

Bioreactor

Biological activities are carried out in a bioreactor to transform raw ingredients into a desired end product. Batch, fed batch, sequencing batch, continuous, and multistage bioreactors all have their own unique modes of operation. The status of the economy and the amount of money invested decide the style of operation to be used. By mimicking and conserving their natural environment, the bioreactor's settings support normal cell activities, resulting in ideal growth conditions. Using a bioreactor to treat contaminated soil gives several advantages over traditional ex situ bioremediation processes. Because of a bioreactor's flexibility in regulating and adjusting process parameters, bioremediation processes may be expedited. Bioreactor-based bioremediation is important because it may effectively create controlled bioaugmentation, nutrient addition, increased pollutant bioavailability, and mass transfer (contact between pollution and bacteria), which are among the limiting features of the bioremediation process. Benzene, toluene, ethylbenzene, and xylenes, among other VOCs, are removed from soil and water with this method (BTEX). Because of the use of different bioreactors, bioremediation has been able to remove a wide range of toxins (Benedek et al. 2021).

With their flexibility, bioreactor designs enable maximum biological degradation while decreasing biogenic waste. By monitoring changes in the microbial population dynamics, it is possible to identify the core bacterial populations that play a role in bioremediation processes in a short- or long-term bioreactor operation. As a biostimulant or bioenhancing agent, sewage sludge may be used in a number of ways. Bioreactors may also utilize genetically modified microorganisms (GEM) for bioaugmentation before killing them and returning treated soils to the field for landfilling (Yadav and Chandra 2020).

Land Farming

Land farming is the process of treating soil for pollutant degradation or transformation using a mix of volatilization and indigenous microbes (Effendi et al. 2022). A popular approach is to install the soil as a shallow layer within a beamed and lined treatment cell or biocell, supplement the soil with nutrients and water on occasion to encourage biodegradation, and aerate the soil on a regular basis to mix and help pollutant volatilization.

3.1.2 In Situ System

This approach involves treating contaminated substances at their font. Devoid of any excavation, this has minimal disruption of soil structure. Theoretically, these actions are less luxurious in comparison to ex situ bioremediation techniques being devoid of excavation; nonetheless, the expenses involved towards designing and installing of multifaceted equipment's to upsurge microbial activity during bioremediation are a huge concern. Certain in situ bioremediation actions such as bioventing, biosparging, and phytoremediation need improvement and technicality, while others such as intrinsic bioremediation or natural attenuation could be left alone (Maitra 2018).

Bioventing

This approach involves providing oxygen to the unsaturated (vadose) zone of the airflow in order to promote bioremediation through enhanced the indigenous microbial activity. Bioventing facilitates bioremediation by decisively promoting microbial conversion of contaminants to a harmless product with continuous input of nutrients, moisture, and oxygen in a regulated manner. This is done through wells to the polluted soil in the bioventing bioremediation method, to encourage microbial activity. In this approach, very little oxygen is given to contaminated soil at low air flow rates, which is insufficient for successful microbial biodegradation and to reduce pollutant volatilization and release into the atmosphere. Bioventing approach can be exploited to efficaciously eradicate pollutants from vadose zone. Moreover, this method could successfully remediate low molecular weight hydrocarbons, absorbed fuel residuals, spilled petroleum, and volatiles from the soil (Logeshwaran et al. 2018).

Bioslurping

Bioslurping combines a number of techniques, including vacuum-assisted pumping, bioventing, and soil vapour extraction, to remove contaminants from soil and groundwater utilizing an indirect oxygen source together with accelerating microbial biodegradation (Kayastha et al. 2022). The limited soil permeability affecting the oxygen transfer rate and inhibiting microbial activity are constraints to this approach. In general, these procedures are employed to remove volatile and semi-volatile organic pollutants from soil and liquid matrix.

Phytoremediation

To minimize the hazardous effects of pollutants, this strategy uses plant interactions (physical, biochemical, biological, chemical, and microbiological) in contaminated areas. Phytoremediation involves a variety of methods (accumulation or extraction, degradation, filtration, stability, and volatilization) depending on the pollutant type (elemental or organic) (Sharma 2020). Extraction, transformation, and sequestration are the most common methods for removing elemental contaminants (toxic heavy metals and radionuclides). Organic pollutants (hydrocarbons and chlorinated chemicals) are mostly eliminated by degradation, rhizoremediation, stabilization, and volatilization, with select plants like willow and alfalfa allowing for mineralization (Azubuike et al. 2016).

When selecting a plant as a phytoremediator, some important factors to consider include: root system, which may be fibrous or tap depending on the depth of the pollutant, above ground biomass, which should not be available for animal consumption, toxicity of the pollutant to the plant, plant survival and adaptability to prevailing environmental conditions, plant growth rate, site monitoring, and, most importantly, the time required to achieve the desired level of cleanliness. In addition, the plant should be disease and insect resistant. According to San Miguel et al. (2013), the process of contaminant removal by plants in some polluted settings includes: absorption, which is mostly passive, translocation from roots to shoots, which is carried out by xylem flow, and accumulation in shoot. Furthermore, transpiration and partitioning between xylem sap and neighbouring tissues affect translocation and accumulation.

Permeable Reactive Barrier (PRB)

Because of its design and pollutant removal process, this technology is commonly seen as a physical method for remediating polluted groundwater. Nonetheless, researchers found that biological response is one of the various pollutant removal processes in PRB approach (degradation, precipitation, and sorption). Although terminology like biological PRB, passive bioreactive barrier, and bio-enhanced PRB have been proposed to encompass the bioremediation or biotechnology part of the process, the involvement of microorganisms has been observed to be mostly augmentation rather than a separate biotechnology. Unless otherwise noted, PRB will be used to define all forms of this technology, including the permeable reactive barrier itself. PRB is an in situ method for remediating groundwater that has been polluted with a variety of pollutants, such as toxic metals and chlorine-based chemicals. A permanent or semi-permanent reactive barrier (medium) largely formed of zero-valent iron is immersed in the path of polluted groundwater in this technology (Maitra 2019). Pollutants become trapped and undergo a sequence of processes when filthy water passes through the barrier under its natural gradient, resulting in clean water in the flow across.

In an ideal world, the barriers would be reactive enough to catch pollutants, permeable enough to enable water to flow but not pollutants, passive (requiring little energy), affordable, and easily accessible (Azubuike et al. 2016). The efficiency of this technology is mostly determined by the medium utilized, which is impacted by the kind of pollutant, biogeochemical and hydrogeological conditions, environmental and health impacts, mechanical stability, and cost. Researchers have recently concentrated on combining PRB with other approaches such as electrokinetics for the treatment of various contaminants (Yao et al. 2012).

Saturated and unsaturated zone remediation is achievable using both in situ and ex situ techniques, but with limits based on the selected method. However, the effectiveness of any technique is affected by a number of factors. Ex situ methods provide more alternatives for regulating or designing clean-up situations. Although ex situ settings may be more advantageous in some locations, in situ treatment is more successful in others. For instance, many sites are located near industrial and commercial zones, which make excavation difficult. In addition, excavation will be quite difficult if the pollutant is situated deep below the surface. Moreover, if the contamination is buried deep inside the subsurface, excavation may present additional difficulties, making ex situ biotreatment problematic. There may be other elements that affect the therapy technique. At the majority of locations, contamination is largely sequestered under the surface. The excavation of pollutants may expose them to the outside environment, posing potential health and safety risks. Moreover, depending on the circumstances, the public may have an unfavourable view of contaminated excavation. Clearly, in situ biotreatment is preferable in all of these situations. Prior to implementing a plan, it is necessary to properly assess the factors associated with each site.

4 The Significance/Impact of Bioremediation

Following the removal of pollution from the soil or water, these bacteria encourage the growth of healthier microorganisms that devour the same pollutants for energy, ensuring long-term land preservation. This approach has the unusual benefit of being a naturally occurring phenomenon with a high success rate. When human effort and innovation are enabled and encouraged, the positive outcomes are amplified tenfold.

This comprehensive approach may appear far-fetched at first, yet comparable strategies have been employed by humans previously. When a person suffered an open wound and became infected in ancient and mediaeval times, maggots were introduced into the wound. The maggots would consume the dead and contaminated flesh, leaving only the healthy skin and tissue. This is precisely how bioremediation works: nature protects itself from illness or pollution by digesting it. Bioremediation may be divided into three categories (Raffa and Chiampo 2021). All three are designed to address a specific environmental requirement while without adding to the planet's technical or chemical pollution.

Microorganisms: Microorganisms are used by microbial to break down pollutants and use them as a source of energy. It is the most fundamental and well-known type of bioremediation.

Phytoremediation: Phytoremediation employs flora to bind, remove, and cleanse chemical pollutants often employed on agriculture, as well as the trace elements produced as a result of such pollutants. Pesticides, petroleum hydrocarbons, trace metals, and chlorinated solvents are all examples.

©Mycoremediation: Mycoremediation uses fungus and their digestive enzymes to break down the same contaminants, but to a higher extent in the case of wetlands and water purification.

5 Nano-Bioremidiation

Heavy metal, hydrocarbon, and radioactive waste poisoning of soil and water is a global problem since these variables have a cumulative effect on the environment and human health. One of the most difficult challenges in the twenty-first century is removing toxins from degraded soils. Nanobioremediation is a new technique that uses biosynthetic nanoparticles to clean up polluted areas. Nanoparticles have attracted the interest of experts from several sectors of environmental research due to their unique chemical and physical features. Nanobioremediation is a promising and quickly developing soil remediation technique that is an offshoot of nanotechnology. The synthesis of nanoparticles from yeast, fungi, bacteria, and plants, as well as their potential applications in the remediation of polluted soils and sludges, is summarized in this chapter. Because nanoparticles are less harmful to soil flora and increase microbial activity, nanobioremediation can be used in situations where other traditional remediation methods have failed. Even though several studies on the physical and chemical characteristics of nanoparticles have been undertaken, additional information on their interaction and adsorption with polluted soils is still needed. Further soil-bioremediation research should concentrate on combining the use of nanoparticles, genetically engineered bacteria, and plants to create environmentally acceptable, cost-effective, resilient, and long-term remediation solutions.

Nanoparticles are created in a manner that is very similar to that which occurs in nature—i.e. through biomimetics—but it is more "cleaner" and "greener". Several times, the biological systems involved in nanoparticle creation have been re-created. Plant-based nanoparticle creation is thought to be easier, more benign, faster, and more readily scaled up for large-scale production than microbe-based nanoparticle fabrication (Dinesh and Risal 2021). Because the latter rely on the maintenance of microbial culture and may potentially include harmful moieties, they pose a risk to both the environment and human health. The limitations associated with this synthesis approach reduce its utility even further, favouring the plant-based system for nanoparticle creation.

Beginning with the first report on metal-accumulating plants in 1855, numerous further studies on abnormally high metal ion accumulation in plant tissues occurred during the next five decades. Plant-based nanoparticles can be produced intracellularly or extracellularly. Richter and colleagues published the first paper on intracellular nanoparticle generation and characterization (2002). Sastry and co-workers, on the other hand, reported the first extracellular synthesis utilizing plant broth.

The majority of these nanoparticle production methods are complex and need stringent controls for shape and size management, such as pH, agitation, light, bioagent cum metal ion concentration, and so on (Dinesh and Risal 2021). By altering the mixing percentage as a process variable, the authors have developed new techniques for producing particles with desired shape.

6 Bioremediation Utilization of Nanomaterials and Nanoparticles

Nanomaterials have unique physical and chemical characteristics, and as a result, they have piqued the interest of scientists and researchers working in a variety of fields related to the environment, including bioremediation. Bioremediation is an effective waste clean-up approach for some types of waste, but it will not be suitable for all. Bioremediation, for example, may not be a viable option in locations with high levels of pollutants that are poisonous to most microorganisms. Heavy metals and salt are two examples. Furthermore, advances in science and technology have raised the level of life, which has resulted in a rise in waste and harmful materials, either directly or indirectly. As a result, using present technologies to remediate toxins is ineffective and inefficient in cleaning up the environment. So that, nanomaterials are used in bioremediation, which will not only have a lower harmful impact on microorganisms, but will also increase the microbial activity of the specific waste and hazardous substance, cutting down on overall time and expense (Alshabib and Onaizi 2019).

Different NMs are utilized in bioremediation for a variety of reasons; for example, when a substance is pushed to nanoscale, its surface area per unit mass rises; as a result, a higher amount of the material can come into contact with surrounding materials, affecting its reactivity. Because NMs have a quantum impact, they require less activation energy to make chemical reactions possible. Another phenomena displayed by NPs that can be exploited to identify harmful substances is surface plasmon resonance. In terms of form and size, a variety of metallic and nonmetallic NMs in various shapes and sizes may be employed to clean up the environment. Because NPs can diffuse or penetrate into a contaminated zone where microparticles cannot, and they have stronger reactivity to redox-amenable pollutants, it can employ various single metal NPs, bimetallic NPs, carbon base NMs, and so on. Oxide-coated Fe0 has been shown to form weak and outer-sphere complexes with pollutants such carbon tetrachloride (CT). In batch experiments and field assessments, oxide coating increases reactivity, allowing CT to be broken down into methane, carbon monoxide, or format via electron transfer, whereas benzoquinone, bytrichloroethene, and other chlorinated aliphatic hydrocarbons can be broken down into chemicals with lower toxicities via electron transfer.

TiO_2 nanotubes may be employed in a laboratory context to destroy pentachlorophenol (PCP) via a photoelectrocatalytic process, in addition to field applications (Daghrir et al. 2012). Single metal NPs can also be employed as reductive dechlorination biocatalysts. Palladium, Pd (0) NPs, may be deposited on the cell wall and inside the cytoplasm of Shewanella oneidensis, and they can be charged with radicals by using various substrates as electron donors in a bioreductive assay incorporating Pd (0) NPs (II). When chlorinated compounds are brought into contact with these charged Pd (0)-deposited S. oneidensis cells, the radical on the Pd (0) can catalytically combine with PCP, resulting in the elimination of the chlorine molecule from the chlorinated compounds (Ramezani et al. 2021).

Microbial cells that can breakdown or biorecover certain compounds can be immobilized using NPs. Rather of using micron-sized media or a fixed surface to immobilize cells, magnetic NPs (Fe_3O_4) were designed and synthesized with ammonium oleate and coated on the surface of Pseudomonas delafieldii (Hastak et al. 2022). These magnetic NP-coated microbial cells were concentrated at a specific point on the reactor wall, removed from the bulk solution, and recycled for the treatment of the same substrate by applying an external magnetic field to them. These microbial cells were put to a bioreactor at a high biomass concentration and shown to desulfurize organic sulphur from fossil fuel (dibenzothiophene) just as well as non-NP-coated cells. The individual NMs are focused on waste clean-up of various forms.

Solid waste, groundwater, and wastewater, petroleum and petroleum products (hydrocarbon), soil remediation, uranium remediation, and heavy metal pollution remediation are some of the uses. The capacity of NMs to reduce pollutant output is in the works, and it has the potential to trigger some of the most dramatic environmental reforms in the coming decade.

6.1 Heavy Metal Toxic Effects

For heavy metals, 6.0 g per cubic centimetre is the upper limit. HMs have a major biological influence on animals and plants, but only at levels below WHO-approved recommended consumption limits. Heavy metals improperly disposed of are a major cause of pollution in both developed and developing countries. In wastewater, industrial and commercial activities are the main producers of heavy metals. Forest fires and volcanic eruptions are frequently less devastating than those that infiltrate the environment through man-made sources such as mines, smelters, and foundries. Plants, the ecosystem, humans, and aquatic life all suffer when these pollutants contaminate wastewater. Heavy metal contamination is caused by human activities like mining, untreated industrial waste discharge, and the use of pesticides and fertilizers containing heavy metals in agriculture. Fora and fauna are harmed, and seed viability and pollen grains are reduced when heavy metals are present in higher concentrations. They are very toxic and non-biodegradable in nature. Many of the binding sites key metal ions use in various cell structures are well-suited to them. Destabilization occurs as a result of this, and mistakes in replication, cancer, and mutagenesis follow. Many physiological and biochemical activities may be affected by heavy metals. They not only increase the quantity of free radicals but also denature microorganisms. Their bioremediation abilities may also be hindered by them. The most common method of heavy metal poisoning is outlined here. When ingested by humans, these chemicals interact with biomolecules. Due to a lack of biomolecule antioxidants, oxidative stress may occur during interactions. This results in an increase in the formation of reactive oxygen species (ROS) such as H_2O_2, O_2, and hydroperoxides. Fat peroxidation, which may damage the plasma membrane, is caused by an increase in reactive oxygen species (ROS) (Stambulska and Bayliak 2020). Enzymes, nucleic acids, and lipids may be damaged by ROS, resulting in decreased cellular function and, in some cases, cell death. Enzyme structure and function are altered by heavy metals that interact with substrates. Toxic heavy metals are also known to cause cell adhesion and penetration via carriers or channels, leading to ion imbalance.

Ecosystems may be endangered if HM makes its way up the food chain from water to plants and humans. Humans may get very sick or die if they consume water that has been tainted with HM. Hg (0.003 ppm), Ni (0.05 ppm), Cu (1.3 ppm), Pb (0.016 ppm), Cd (0.006 ppm), and Cr (0.2 ppm) are in the higher contaminant category for HMs in drinking water. These heavy metals may affect aquatic life, people, and the fertility of the soil when concentrations surpass acceptable limits. Human health is often harmed by the widespread use of HMs. Many diseases and syndromes, including Parkinson's and Alzheimer's disease, may be induced by heavy metal exposure (Arun et al. 2021).

6.2 The Presence of Heavy Metals in Sewage

Mines, coal combustion, automobiles, agriculture, and extremely polluting industries such as smelting and petrochemicals, as well as the production of plastics, paper-pulp, textiles, and fabrics, as well as printing and ceramics, are all sources of HMs. They may also be found in sewage discharge. Acid mine tailings and drainage that contain HMs are released by metalliferous mining companies. Other large industries that release these pollutants include manure sewage, fertilizer production, slag reprocessing, paint and pigment manufacturing, and electroplating (Vardhan et al. 2019). These industries all produce and reprocess metals, as well as waste and tailings.

7 Application of Nanoparticles in Bioremediation

Apart from cost-effectiveness, nanoparticles (NPs) high surface area to mass ratio, exceptional electronic properties, sensitivity, and catalytic behaviour gained the interest of researchers for applying nanomaterials in environmental remediation. The primary mechanism for remediation by using NPs is Catalysis and Chemical reduction. NPs can remove heavy metals on the basis of adsorption as a result of wide distribution of active sites on surface and availability for coating modification (Corsi et al. 2018). Small size and innovative surface coatings of NPs increases their application in soil and water remediation as they can be able to penetrate to subsurface through small gaps at a faster rate and remain suspended in ground water. Factors that enhance the chemical reactivity of nanomaterials are composition of near surface regions and participation of interfacial ΔG. High surface to mass ratio also play a major role in remediation mechanisms such as adsorption for water and photocatalytic processes as well as exhaust gas treatment.

7.1 Nanoscale Zero-Valent Metals

The use of zero-valent metals came into light when its application towards reduction of environmental pollution was first traced out by swenny et al. in 1972. A few years later, further studies demonstrated the remediation of groundwater contaminated with volatile organic chlorides (VOCl) by the use of zero-valent metals, primarily zero-valent iron (ZVI). For example, a study conducted for degradation of chlorinated organic compound TCE using zero-valent metals resulted a tremendous effect. The biometallics formed by Zn0 or Fe0 in conjunction with Ni0 or Pd0

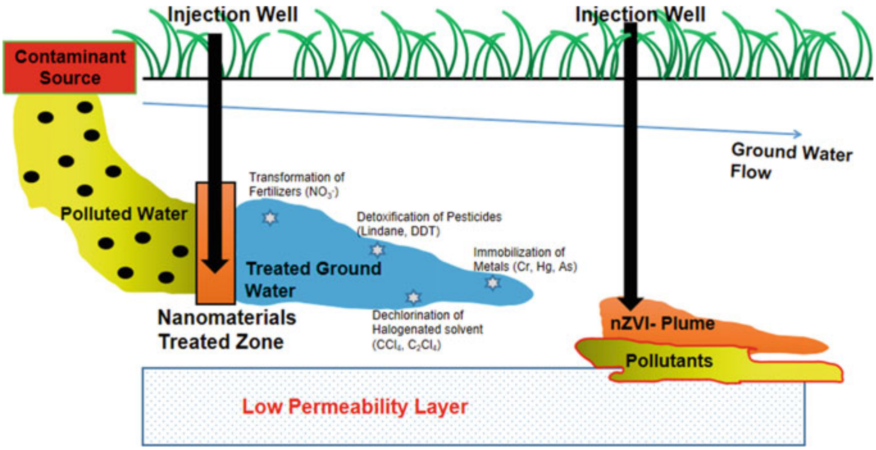

Fig. 1 Application of nZVI to reduce contamination in soil

have strong effect on the degradation reactions of TCE. Degradation of different halogenated aliphatic hydrocarbons, tetrachloroethylene (PCE), trichloroethylene (TCE), cis-dichloroethylene (cis-DCE), and trans-dichloroethylene (trans-DCE) with NZVI was reported (Arnold and Roberts 2000). Zero-valent metals such as Zn0, Ti0, Ni0, Pd0, Al0, Fe0, etc., with certain reduction capacity are known for low-cost and viable chemical medium for the removal of environmental contaminants.

In Europe and the United States, nZVI is the most often used nanomaterial for soil and groundwater clean-up. Nanoremediation with zero-valent iron is the most widely utilized approach for soil and groundwater clean-up. Because of their increased reactivity and mobility, nZVIs can be used to perform in situ therapies by injecting nanoparticles. These findings point to a highly favourable strategy for pollution treatment since it does not need preceding soil excavation or groundwater pumping. The introduction of highly concentrated nZVI slurries by injection at or near the polluted region is a frequent start to nanoremediation therapy. nZVI should be sprayed to contaminated soils or attached to them, where it will react with the target pollutants to produce less harmful or mobile compounds. Due to its small size, nZVI exhibits a higher reactivity and adaptability towards a wide range of pollutants, including halogenated mixes, nitrate, phosphate, and polycyclic hydrocarbons (Fig. 1). Because of its ability to produce reactive oxygen species (ROS) and its ability to consolidate in cells, nZVI could potentially cause mutagenicity (Fu et al. 2014). The toxicity of nZVI under toxic conditions is significantly lower as compared to anoxic conditions, implying that the formation of an iron oxide layer was caused by surface oxidation. Thus, nZVI is regarded as a promising remediation procedure applicable to a wide range of applications and widespread environmental issues, particularly in hydrophilic organic compounds (HOCs) and PAHs (polycyclic aromatic hydrocarbons) (Binh et al. 2016). The nZVI reduced the contaminants is given in table.

Metals	Cr (VI)		Chen et al. (2020)
	As (III), As (V)		Kanel et al. (2005)
	U (VI)		Li et al. (2013)
	Pb^{2+}, Zn^{2+}		Wang et al. (2016)
	Ni^{2+}, Zn^{2+}		Zhang (2003)
	Cd (II)		Boparai et al. (2013)
Non-Metal Inorganic Species	Nitrate		Liu and Wang (2019)
	Perchlorate (ClO_4^-)		Xie et al. (2016)
	Bromate		Lin and Lin (2017)
	Arsenic (AsO_4^{3-})		Bretzler et al. (2020)
Halogenated Aliphatics	Chlorinated Methane	Carbon tetrachloride (CCl_4) Chloroform ($CHCl_3$) Dichloromethane (CH_2Cl_2) Chloromethane (CH_3Cl)	Song and Carraway (2006)
	Chlorinated ethene	Vinyl chloride (C_2H_3Cl) 1,1-Dichloroethene ($C_2H_2Cl_2$) Trichloroethene (C_2HCl_3) Tetrachloroethene(C_2Cl_4)	Song and Carraway (2008)
		Cis-Dichloroethene ($C_2H_2Cl_2$) Trans-Dichloroethene ($C_2H_2Cl_2$)	Li et al. (2006)
	Brominated Hydrocarbon	Bromoform ($CHBr_3$) Dibromochloromethane ($CHBr_2Cl$) Dichlorobromomethane ($CHBrCl_2$)	Cohen et al. (2009)
Halogenated Aromatics	Chlorinated Benzene	Hexachlorobenzene (C_6Cl_6) Pentachlorobenzene (C_6HCl_5) Tetrachlorobenzenes ($C_6H_2Cl_4$) Trichlorobenzenes ($C_6H_3Cl_3$) Dichlorobenzenes ($C_6H_4Cl_2$) Chlorobenzene (C_6H_5Cl)	Xu and Zhang (2000)
	Chlorinated Phenols	Pentachlorophenol (C_6HCl_5O) p-Chloro Phenol	Reddy and Karri (2008)
Other Chlorinated Hydrocarbons	Polychlorinated Biphenyls		Wang and Zhang (1997)
	Polychlorinated Dibenzo p-dioxins, Polychlorinated dibenzo furans		Kim et al. (2008)
Other Organic Compounds	Dyes	Orange II ($C_{16}H_{11}N_2NaO_4S$)	Luo et al. (2013)

(continued)

(continued)

		Chrysoidine ($C_{12}H_{13}ClN_4$) Tropaeolin O ($C_{12}H_9N_2NaO_5S$)	Zhang (2003)
		Acid Red	Yari et al. (2015)
		Acid Violet Red B	Lin et al. (2014)
		Acid Green	Shojaei and Shojaei (2017)
	Explosive	2,4,6-trinitrotoluene (TNT)	Zhang et al. (2010)
		1,3,5-trinitroperhydro-1,3,5-triazine (RDX)	
		1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX)	
	Pesticide	DDT($C_{14}H_9Cl_5$)	Blundell and Owens (2021)
		Lindane ($C_6H_6Cl_6$)	Elliott et al. (2009)
	Herbicides	Alachlor	Thompson et al. (2010)
		Atrazine	Zhang et al. (2011)
	Other Organic Contaminants	N-nitrosodiumethylamine (NDMA) ($C_4H_{10}N_2O$)	Qin et al. (2019)

7.2 *Engineering Polymeric Nanoparticles for Bioremediation Purposes*

Polynuclear sweet-smelling hydrocarbons (PAH) are hydrophobic natural pollutants that cling to soil surfaces and are difficult to remove. Amphiphilic polyurethane (APU) NPs have been incorporated for application in soil remediation with PAHs. The particles are made up of polyurethane acrylates that have been anonymized or poly acrylates and urethane acrylate antecedent chains (PMUA). APU particles, like surfactant micelles, can increase PAH desorption and transportation, but unlike surface dynamic segments of micelles, they are not allowed to sorb individual cross-connected pre-runner chains on the dirt soil surface. As a result, regardless of their fluid fixation, the APU particles are stable. Exploratory results show that APU filters can be configured to have hydrophobic outdoor districts providing elevated phenanthrene (PHEN) and hydrophilic substrates that increase molecular portability in soil, the affinity of APU particles for pollutants can be limited, for example, by adjusting the size of the hydrophobic segment employed in the chain blend. The thickness of the load or the length of the swinging water-solvent chains on the surface of the molecule limits the mobility of colloidal soil APU suspensions. Different automated

NPs can modify the form of pollutant and soil conditions thanks to their ability to control molecular characteristics (Tungittiplakorn et al. 2005).

7.3 The Use of Nanoparticles as a Pollution Control Tool

According to pollution prevention act of 1990, the pollution control hierarchy is established at top of the list. Today, the main focus for technology development is remediation, rather than significance attached to pollution prevention in pollution control. Nanotechnology can play a major role in pollution control. A recent study of National Nanotechnology Initiative, USA, in 2001 stated that they had $100 billion savings occurred in a year by using nanotechnology-based home lighting. Therefore, the total energy consumption reduced by 10% and the C emission also decreased by 200 million tons per year.

Metal oxide nanocatalysts, particularly gold nanocatalyst, have shown enormous result in decreasing pollution at the site. It includes catalysts for pollution and emission control, chemical processing of a different chemicals, and sensors for detecting hazardous or flammable gases or compounds in solution. According to recent study, gold-based catalysts could be useful in hydrogen processing and related fuel cell systems. Advantage of gold nanocatalyst is it’s high reactivity under mild conditions and this brought down the running cost of chemical plants (Thompson 2007). Moreover, the main features of supported gold catalysts are, it activated by moisture and often very selective. Since 1992 in japan, Au nanopolymer is used for removal of odour in modern toilets. This is achieved by deposition of Au on Fe_2O_3, supported on zeolite wash coated honey comb. At low temperature Gold catalysts are more active, it can be employed for oxidative decomposition of many components in ambient air, particularly CO and nitrogen-containing odour compounds, such as trimethylamine.

In recent times, significant effort has been devoted to a particular method for removing hazardous contaminants from contaminated water. The growing demand for clean water, combined with the increasing use of nanotechnology, has prompted us to investigate several aspects of water treatment using nanomaterials. The nanofiltered membrane technique is a cost-effective way to purify huge amounts of polluted water into high-quality drinking water. Pesticides are rarely mineralized into carbon dioxide, water, or other inorganic minerals during degradation; instead, they are typically transformed into a variety of transformation products. The use of nanomembrane filters for the treatment of pesticide and pesticide transformation products contaminated groundwater. It has been observed that NF99HF membrane was able to remove the pesticides about 90%; however, it has a smaller size and so it is easy to employ low-pressure reverse osmosis (RO) membrane to achieve adequate pesticide removal (Madsen and Søgaard 2014).

Polynuclear sweet-smelling hydrocarbons (PAH) are hydrophobic natural pollutants that cling to soil surfaces and are difficult to remove. Amphiphilic polyurethane (APU) NPs have been incorporated for application in soil remediation with PAHs. The particles are made up of polyurethane acrylates that have been anonymized or poly acrylates and urethane acrylate antecedent chains (PMUA). APU particles, like surfactant micelles, can increase PAH desorption and transportation, but unlike surface dynamic segments of micelles, they are not allowed to sorb individual cross-connected pre-runner chains on the dirt soil surface. As a result, regardless of their fluid fixation, the APU particles are stable. Exploratory results show that APU filters can be configured to have hydrophobic outdoor districts providing elevated phenanthrene (PHEN) and hydrophilic substrates that increase molecular portability in soil, the affinity of APU particles for pollutants can be limited, for example, by adjusting the size of the hydrophobic segment employed in the chain blend. The thickness of the load or the length of the swinging water-solvent chains on the surface of the molecule limits the mobility of colloidal soil APU suspensions. Different automated NPs can modify the form of pollutant and soil conditions thanks to their ability to control molecular characteristics (Tungittiplakorn et al. 2005).

Nanotechnological Remediation Processes from Lab Scale to End User Applications for the Restoration of a Clean Environment (NANOREM) is a project that aims to harness the potential of nanoremediation and enable both in situ and off-site remediation. This work is being done in tandem with gaining a thorough understanding of the environmental risk-benefit of nanoparticle use, market demand, general sustainability, and stakeholder perceptions (Bardos et al. 2015).

7.4 Green Nanoremediation

Nanoremediation provided a number of solutions to remove the hazardous pollutants present in environment. It allows for deeper soil remediation, is compatible with other technologies such as bioremediation, and is a growing tool for contamination removal (Huang et al. 2016). To fully utilize the significant relevance of nanoremediation in PAH elimination, the invention and usage of nanofertilizers (bio stimulation and bioaugmentation), nanominerals (bio stimulation), or green synthesised nano-oxidizers (PAH oxidation) could be investigated further. Nanomaterials used for remediation have several advantages such as high adsorption capacity, more surface area, rapid and cost-effective. Nano-based approaches like nanocatalysis and adsorption have been used for the removal of harmful components. Nanofilters and nanomembranes are also employed for decontaminate the ground water. Nanomembranes have been used for the desalinization by reverse osmosis method. Nanomaterials can be prepared from either chemical or biological methods (microbes, plant, algae). However, chemical method may affect physiological processes of living system due to its toxicity. Moreover, mutation may cause by chemical-based nanomaterials (Kühnel et al. 2017).

To address the issues with chemical-based nanomaterial, now biological synthesis-mediated nanoparticles have gained more attention from researchers. In the following section, use of biological nanomaterials in the remediation of different environmental pollution is discussed. Uranium is a radioactive waste that comes from nuclear power plants and nuclear weapon production contaminates water. It can be remediated by cells and S layer proteins of Bacillus sphaericus JG A12. Generally, biological nanoparticles are of 2 types, i.e. microbial based and phytochemical based. Both have special ability to degrade the contaminants at the pollution site. Johnson et al. reported that C.pasteurianum BC1 cultures reduced Pd^{2+} to form Pd nanoparticles and it is present in cell wall. Different organic dyes such as azo, methyl orange and evans blue can be reduced with it. Removal of chromium and cadmium ions has reported using nZVI nanoparticles synthesized by reduction via P.aeruginosa. Fungi can also utilized for synthesis of NPs that can be used in bioremediation process. These fungal NPs may differ in size and composition but Fungi are considered as the vital source for preparation of metallic NPs. Thus, Fungi is called as "Nanofactories" (Dhillon et al. 2012).

Likewise plant synthesized nanomaterials can also help in green remediation in various ways. Iron-based NPs generally used for immobilization. Green tea derived nZVI could reduce Cr (VI) in the soil. Leaf extracted FeO_2 nanoparticles can stabilize Cd and As in the soil through precipitation. In the same way, 5–15 nm size NPs from M.azedarach and P.undulatum has the capability to remove Chromium level (Truskewycz et al. 2018). A study reported that dispersion of Colocasia esculenta plant's rhizome powder forms an Ag nanocomposite where this rhizome is act as supporting agent or stabilizer. It showed high catalytic reduction of nitroarenes such as Picric acid, 2-nitro phenols, 4-nitro phenols and 4-nitroaniline. The prepared catalyst also showed a high activity in the catalytic reduction of organic azo dyes such as Congo Red, Methyl Orange, Methyl Red, and Rhodamine B by sodium borohydride. 93% removal of Methylene blue was achieved by Silver nanoparticles (AgNPs) that were prepared from Kyllinga brevifolia extract. Green tea derived Au nanoparticles that decorated with TiO_2 (Au/TiO_2) showed degradation of Methylene blue via photocatalysis. AuNPs isolated from Salvia officials polyphenol exhibited size dependent catalytic activity displaying better catalytic reduction of 4-Nitro Phenol and methylene blue (Oueslati et al. 2020). Green synthesis can form different types of Zn-based catalysts such as Zinc oxide, Zinc hexacyanocobaltate, and Zinch hexacyanoferate which can induce the remediation of hazardous pollutants such as dyes, heavy metals, drugs, fertilizer, pesticides, etc. Zinc oxide nanoparticles by Eucalyptus globulus and jackfruit (Artocarpus heterophyllus) showed their photocatalytic activity against Methylene blue and methyl orange and rose bengal dye, respectively. Peganum harmala seed derived activated carbon when coated by green ZnO nanoparticles it shows a good result in removal of Cr (VI) removal. Spherical TiO_2 NPs of size 60–100 nm from Ageratina altissima removed Methylene blue, alizarin red, crystal violet, and methyl orange dye. Green synthesized CuONPs is also considered as good photocatalytic agent, it degrades 94% of RB dye on its Fifth cycle.

7.5 Dendrimers

(Dendri: Branch of tree; meros: Part of tree). Dendrimers is a highly branched and monodispersed polymeric compound which composed of many smaller subunits linked to it as a tree branches. Mainly, it consists of 3 components: a central core, interior branch cells (radial symmetry), and terminal branch cells (Peripheral group). Many composite structure can be developed using dendrimers as it has many void spaces for interaction of other compounds. It increases their catalytic property as well as reactivity. So this type of composite can be used in dye treatment water treatment (Rizwan 2014).

Polyamidoamine (PAMAM) is the most studied dendrimers among others due to its easy fabrication property (Fig. 2). Hybridization with other high performance adsorbents resulted this efficient dendrimeric polymer which is less toxic as well as cost-effective. The nature of terminal group decides the physicochemical property of dendrimer such as shape, solubility, stability rigidity, and viscosity. By repetition of reaction sequence, dendrimers up-to 10th generation to starting ammonium core were reported. In other words, number of end groups (radial branched layer) directly proportional to dendrimer generation. Although dendrimers remediates a wide variety of pollutants (metal ions, dyes, drugs, and pesticides) still requires various solid supports or adsorbents such as magnetite NPs, Graphene oxide, and carbon nanotubes for growing or immobilization because of difficulties to recover it from contamination site after remediation.

Different types of dendrimers are hyper-branched polymers, multi-arm star shaped polymers, dendronized or dendrigrifts polymer, hyper-grafted polymers, and dendritic linear block polymers. These can also help in adsorbent reclamation and reuse. Dendrimers adsorbed with either magnetic or non-magnetic supports like carbonaceous and silica nanoparticles or any other composite. Magnetic particles tethered dendrimers allow quick separation of used scavengers from the target solution by utilizing the magnetism power. PAMAM gained interest of researcher for water purification as adsorbents and can quantify, detect the toxic pollutants as sensors. PAMAM has been utilized in waste water samples contaminated with various metal ions such as Cu^{2+}. These dendritic polymers are able to encapsulate various cations in water (Cu^{2+}, Fe^{2+}, Fe^{3+}, Ag^{+}, Au^{+} Ni^{2+}, Zn^{2+}, and U^{6+}) by the help of attached functional groups such as primary amines, hydroxylates, and carboxylates. Advantage of using PAMAM as water purifier is it acts as chelating agent as well as antimicrobial agent to metal ions. Main feature of dendritic polymer is that it can't pass through the pores of ultrafiltration easily due to globular shape and monodispersity. For this reason, it is used to improve ultrafiltration and microfiltration processes for the recovery of dissolved ions from aqueous solutions. To recover clean water, contamination water can be transferred to filtration unit followed by treatment of functionized dendritic nanopolymer solution. In next step, by lowering the pH of the solution, dendrimers are separated and then concentrated solution of pollutants is collected for disposal or recycling.

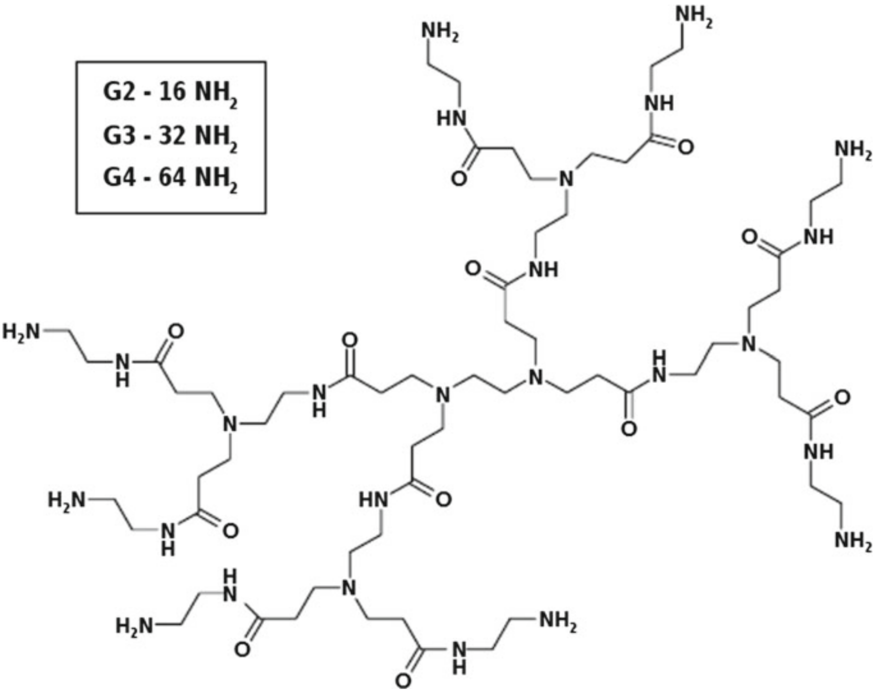

Fig. 2 Structure of PAMAM Generation 1 (Adopted from Borkowski et al. [2011])

Growth of generations increases the number of active sites that will act as scavenger and reduces the number of pollutants. PAMAM beads functionalized with halloysite nanotubes in alginate removes Methyl Green and Sunset yellow FCF (Kurczewska et al. 2019). A reusable chitosan-based magnetic-cored dendrimers reported for remediation of methylene blue. Chitosan-functionalized PAMAM is also used for removal of lanthanides and actinides from watsewater solution.

8 Sensing of Pollutants

A variety of factors influence wastewater treatment, including the use of tried-and true methods, costs, and environmental impact. Prior to the development of any technology, these concerns are considered. Immersion and isolation are required due to the difficulty of removing heavy metal contaminants using biological, physical, or chemical methods. Ion exchange chemistry, oxidation, reduction, and precipitation by ion exchange, as well as photocatalysis, are all commonly used processes nowadays. Heavy metals were removed from waste using a variety of methods, including conventional, microbial, plant-based, and nanomaterial-based methods.

One of the many advantages of these techniques is their controllability and resistance to high concentrations of heavy metals. The activated sludge method uses suspended bacteria to oxidize nitrogenous and carbon compounds, resulting in a process that meets regulatory requirements while having a low environmental impact. Whether by chemisorption or physisorption, adsorption in polymer adsorbents is based on two key factors: ease of use and electrostatic attraction. Traditional methods produce a lot of waste and consume a lot of energy, making it difficult to dispose of them.

These procedures may be harmful to the environment because they use nonrenewable resources. Physiochemical processes are out of reach for developing and underdeveloped countries. As a result, they reduce soil fertility, rendering them unsuitable for agricultural use. Traditional methods have several disadvantages, including high energy consumption, inadequate pollutant removal, and the generation of hazardous by-products. To remove HMs from wastewater, bioremediation employs both microbiological and physical methods.

Recently, analytical methods for water monitoring have branched out in a number of different areas. Techniques for measuring water quality include traditional instrumental analysis (laboratory-based analysis), sensor placement (sensing), spectroscopy, biosensors, microfluidic devices, and model-based detection. Choosing the right detection method depends on the objective of the analysis, whether it is quantitative, qualitative, or a combination of the two. The use of chemical and biological sensors in water monitoring technologies has been highly sought after, and it seems that device integration and commercialization are possible.

8.1 Biological Contaminants

In terms of microbiological characteristics in drinking water, the diagnosis of illness using indicator organisms takes precedence. Pathway of disease transmission occurs when faeces-borne germs enter the oral cavity of another host. According to the European Commission, concerns have been expressed concerning microbiological pollution and human health due to the growth of water monitoring devices. Traditional culture-based methodologies are still employed to determine microbial properties in water, despite technological developments (Sacher and Hambsch 2009).

8.1.1 Multiple Tube Fermentation (MTF) Method

Multiple tube fermentation (MTF) is a common technique for measuring microbial activity in water samples. The MTF technique consists of three phases: presumptive phase, confirmation phase, and complete test (Almasi and Sadeghi 2011). The presumptive stage entails tube incubation, which results in the production of gas, indicating a positive presumptive test. During the presumptive phase, each bacterial sample is counted using a broth medium that is suitable for that purpose. After gas generation, testing tube samples should be inoculated promptly. This is referred to as the confirmation phase.

It is the presence of gas and bacteria in culture colonies that indicate a successful test, according to EPA Standard Methods 9131 for Total Coliform: Multiple Tube Fermentation Technique (Zulkifli et al. 2018). In proportion to the microbial growth, turbidity will form, and the findings will be reported as a statistical estimate of the mean, also known as the most probable number (MPN). The presence of coliform bacteria, sometimes known as "indicator" organisms, is determined using the MTF method with an A-1 medium for an MPN test protocol outlined in Standard Methods for the Examination of Water and Wastewater. On the other hand, the MTF approach was proven to be successful for yeast isolation and identification of *Escherichia coli* and *Enterococcus* spp. The findings revealed a good correlation between yeast densities and conventional indicator counts, indicating that yeast may also be regarded as organism indicators of sewage pollution.

In addition to the membrane filter (MF) approach, the MTF method was used to identify *E. coli* in water samples, and a 100% detection rate was attained. Turbidity analyses corroborate the presence of faecal pollutants in water samples, which are represented by an increase in water temperature and a reduction in dissolved oxygen (DO) concentration.

8.1.2 Membrane Filtration (MF) Method

The USEPA and UNEP/WHO acknowledge the MF approach for identifying biological pollutants in drinkable water. Compared to MTF, this approach can isolate and eliminate discrete microbiological colonies in a larger sample volume. Both procedures were previously only used in laboratories, but with the advent of portable technology, they may now be used on-site. It is possible to count total coliform colonies by incubating MF on solid or liquid selective medium at the right temperature. Temperatures are determined by the bacterium indicator and the selective media. However, poor absorption and inactivation induced by elevated pH levels resulted in poor viral and phage detection inductively coupled plasma mass spectrometry. Microfiltration and ultrafiltration membranes are used for filtration because they have a wide range of surface pore sizes that may efficiently retain microorganisms. In vitro nitric oxide generation and binding response of Limulus amebocyte lysate (LAL) experiments have revealed that microfiltration may remove bacterial pollutants from water (Zídek et al. 2013). In recent years, it has been tried to employ both microfiltration and ultrafiltration membrane filters simultaneously. The integrated technique has also been employed to retain natural colour stability in red beet stalks. It reduced peroxidase activity and turbidity by 99.9%. Previously, the membrane bioreactor (MBR) technology was used for aerobic fermentation.

MBR combines membrane filtration with a biological reactor. Water sample monitoring effectiveness relies on bacterial adhesion to inert substance causing high biomass. MF, selected medium broth, and culture plate procedures are now standard (ISO 16654:2001) for water pathogen detection. These approaches are time-consuming and tedious and have limited sensitivity in identifying tiny amounts of pollutants (Zulkifli et al. 2018). The inconsistency of phenotypic differentiation in selective culture or immunological approaches may also impair detection accuracy. Snyder et al. described how MF may reduce the concentration of pollutants with certain qualities. The degree of contaminant removal was observed to be strongly dependent on membrane characteristics and analyte molecular properties. Microfiltration and ultrafiltration membranes removed the least pollutants, whereas reverse osmosis membranes removed practically all contaminants tested. An osmosis membrane might remove practically all pollutants with levels below the method reporting limits (MRL).

8.1.3 DNA/RNA Amplification

DNA amplification detects molecular biology by amplifying a single or few copies of targeted DNA molecules in vitro. Kary B. Mullis devised the first DNA amplification technology intended to examine specific DNA molecules. Traditionally, DNA replication takes days or weeks. However, PCR amplification of DNA sequence only takes a few hours. PCR can replicate small amounts of DNA sequences and is particularly valuable in commercial applications such as genetic identification, forensics, quality control industrial applications, and in vitro diagnostics. A thermostable DNA polymerase, a dNTP mix, and two oligonucleotide primers are the main components of a PCR amplification procedure. After the reaction, one cycle amplification amplifies the quantity of targeted DNA sequence (Foo et al. 2020). These are 1 min denaturation, 45 s annealing, and 1–2 min elongation. A multiplex PCR approach might be used to identify *E. coli*, *Campylobacter* spp., and *Salmonella* spp. in both drinking and surface water.

The detection of Actinobacillus actinomycetemcomitans by traditional hot-start PCR involves heating reaction components to DNA melting temperature before mixing with polymerase to decrease non-specific priming amplification (Zulkifli et al. 2018). Then, in succeeding cycles, the primer annealing temperature was progressively reduced to detect bacteria in aquatic samples. Various innovative technologies have emerged in recent decades to circumvent these restrictions. Examples include quantitative real-time PCR (qPCR), reverse transcription real-time PCR (RT-qPCR), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), ligase chain reaction (LCR), rolling circle amplification (RCA), helicase-dependent DNA amplification (HDA), and the newly developed random amplified polymorphic DNA analysis (RAPD). qPCR automates quantitative amplification and detection. Quantification cycles (Cqs) are measured by fluorescence threshold or maximal second derivative. The qPCR exponential phase may be seen for 30–50 Cqs and used to determine the initial number of targeted DNA. There are various commercial real-time PCR tests for pathogen identification (e.g. F. tularensis, B. anthracis, and Y. pestis) with excellent sensitivity and pathogen diversity. Melting curve analysis SYTO9 and qPCR TaqMan assays have been proven to be helpful for detecting Naegleria sp.

RT-qPCR has been used to identify pathogens such as mRNA in *E. coli* cells, Filoviridae viruses, Ebola viruses, cereulide generating Bacillus cereus, Salmonella, rotaviruses, and coronavirus in stool contaminations. Reverse transcription PCR (RT-PCR) is a very efficient method for amplifying DNA and RNA sequences. However, conventional PCR only amplifies DNA.

Isothermal amplification technologies are an alternative to PCR. Loop-mediated isothermal amplification (LAMP) uses two primer pairs (inner and outer) and relies on DNA polymerase strand displacement synthesis to construct loop amplifications. LAMP is commonly used for biological specimen diagnosis and is commercially accessible for environmental monitoring (Rubinfien et al. 2020). It has good sensitivity, quick detection, and can quantify more germs. It is also more sensitive and may be used for on-site water contamination monitoring. Using extension PCR with regular PCR might be quicker, fewer false positives, and more compatible for various pathogen identification.

Taking into mind each approach's premise and practice, no method is flawless. For example, disinfection may identify dangerous viruses, but not coliform bacteria owing to the low concentration of bacterium indicator. The development of DNA-based amplification methods has progressed to meet the need for more specific and quick detection. Then the probe-based real-time loop-mediated isothermal amplification (RT-LAMP) test for measuring Salmonella invasion gene (InVA) came.

8.1.4 Fluorescence In Situ Hybridization (FISH)

In situ hybridization is a method for detecting, identifying, localizing, and counting microorganisms. Using fluorescent in situ hybridization (FISH), cellular components, and specific analytes may be seen. The FISH method is widely used in cytogenetics, microbiology, and genetic diagnostics. Before doing FISH investigations, crucial issues including probe design and fluorophore selection must be considered. The probe is 15–30 nucleotides long and has a 3' or 5' fluorophore on either end (Huber et al. 2018).

Microbial ecology was one of the first applications of FISH. Similar to DNA amplification, 16S rRNA sequences are the most often employed DNA probe for detecting bacteria in live tissues and aquatic materials. FISH has recently been used in microbiological surveillance. However, FISH has modest fluorescence signals; therefore, it can only identify a limited microbial population. A multi-labelled FISH approach is proposed to improve the fluorescence signals and identify many microbial groups simultaneously. Previous research found Proteobacteria and Cytophaga-Flavobacterium clusters in an urban river 3–7 days after creation. A similar approach was utilized to identify several Cytophaga Falvobacterium cluster members, proteobacteria classes, and Planctomycetales members in an aquatic environment. A FISH probe utilizing Bacillus subtilis 16 s rRNA was also found to discriminate between 465 and 483 genes. However, FISH was unable to identify B.

altitudinis, B. cereus, B. gibsonii, B. pimulus, and B. megaterium. The use of FISH in determining MRNA and DNA molecules has risen in recent years. To increase fluorescent brightness, photochemical cohesion, and coherence emission spectra, fluorescent nanomaterials (QDs) have been developed. QD-FISH can identify particular target genes. For example, employing a dNTP combination to synthesize biotin-streptavidin deoxyuridine triphosphate (dUTP)-tagged DNA probes enabled the detection of Ectromelia virus (ECTV), a Poxviridae family member, with an 80% genome detection after 36 h post-infection. By 5S-1 and 5S-2 probes, U. polifera, Ulva linza, and Ulva flexuosa molecular genes were detected using FISH. Six green algae species were investigated, but only U. prolifera could be marked by both probes. Single-cell bacteria are challenging to identify and quantify with FISH due to their complicated structure. Thus, combining flax desegregation with quantitative FISH is advised.

8.2 Inorganic or Non-biological Contaminants

Non-biological water pollutants fall into two groups. Chemical pollutants include components and compounds such as volatile organic chemicals, disinfection by-products, and synthetic organic chemicals. Radioactive pollutants are unstable atoms that release radiation, such as plutonium and uranium. Engineered nanoparticles/nanomaterials are another water pollutant gaining attention. Metallic nanoparticles (Ag, Au, and Fe), oxides (CeO_2, TiO_2, and ZnO), and quantum dots are examples (e.g. ZnS). While nanoparticles are useful in many industrial applications, their discharge may inadvertently encourage harmful jobs for the environment and human health. Chemical Pollutants—CCL 4 newly drafted by the EPA finds non-biological contaminants in tap and drinking water. The bulk of organic water pollutants come from industrial operations, pollution, agricultural runoff, and natural elements. Inorganic water pollutants occur from erosion and discharge of natural minerals. Non-biological pollutants are always analysed using chemical parameters such as pH, hardness, temperature, dissolved organic nitrogen, total organic carbon (TOC) (Zulkifli et al. 2018), and chemical oxygen demand (COD). According to the WHO Guidelines for Drinking Water Quality, municipal drinking water should be derived from the tolerable daily intake (TDI).

8.2.1 Capillary Electrophoresis (CE)

Capillary electrophoresis studies molecule polarity and atomic radius using ions electrophoretic mobility. The mobility of analytes in electrolyte solutions is directly proportional to the applied voltage. CE may separate in mm capillaries and micro- to nano-fluidic channels. Other CE-based approaches include capillary gel electrophoresis, capillary isoelectric focusing, and micellar electrokinetic chromatography (MEKC) (Fung et al. 2012). Several studies have used the CE detection approach to determine NH^{4+}, Na^+, K^+, Mg^{2+}, and Ca^{2+} ions in environmental materials. The electrophoretic mobility of existing unions (nucleotides, metal ethylenediaminetetraacetic acid, haloacetics, etc.) in aquatic settings may be determined. To find existing unions, researchers suggest using 50 m straight capillaries with baseline noise reduction. This approach works well for screening anions in liquid samples. Anions were separated using a very alkaline pH to attract a negative charge, causing migration towards anode. Then, the anions in the aquatic environment may be evaluated using electrophoretic mobility within a certain wavelength, as well as peptide analysis.

Pan et al. devised a CE approach for detecting trace chloroanilines in water samples. The optimal enrichment factors were 51–239, with linear calibration across three orders of magnitude ($r > 0.998$). Herbicide-contaminated water is infectious and possibly harmful. An expanded CE approach with low voltage eigenmode expansions (EME) models may identify and quantify pesticides. In order to maximize extraction efficiency, a Box-Behnken design (BBD) and response surface methodology (RSM) were used to pre-concentrate and identify contaminants in water samples. Herbicides might therefore be identified utilizing a unique MEKC technique.

Online sweeping pre-concentration in MEKC technology was developed to identify five triazine herbicides in water samples. Water pollution with estrogenic chemicals is unavoidable due to the widespread use of animal-based fertilizers. Estrogenic chemicals were identified in worrying amounts in mineral and wastewater samples. Electrophoretic mobility near the intake capillary might increase analyte detection sensitivity. The sensitive capillary is necessary for the simultaneous detection of analytes that bind particularly to it. Its industrial deployment is still uncertain due to complicated equipment configurations. Gel electrophoresis or capillary electrophoresis is often employed to differentiate nanoparticles. Using polyacrylamide gel electrophoresis, engineered nanoparticles (ENPs) like bio-conjugated quantum dots may be detected (PAGE). Nevertheless, the tiny pore size of polyacrylamide gels (10 nm) makes separation difficult. Thus, Hanauer et al. established nanoparticle separation methods using agarose gel electrophoresis (AGE), with pore sizes ranging from 10 to 100 nm. Using polylethylene glycols as electrophoretic mobility controllers, silver and gold nanoparticles were synthesized. However, results for gold nanoparticle separation under typical CE settings were inferior to ICP-MS and UV detection approaches. Using CE, bio-conjugated quantum dots and protein nanoparticle interactions may identify environmental samples. Using several inorganic buffers as electrolytes enabled the identification of metal and metal oxide nanoparticles. Using CE sodium dodecyl sulphate, Au, AU, Pt, and Pd nanoparticles were identified with a 5 nm resolution.

The CE methodology is gaining popularity, and there are several papers on modifying and integrating CE-based detection systems. Unsymmetrical peak identification, poor mobility time repeatability, low separation precision, and injection efficiency of only 103 to 107 L are some of the shortcomings addressed by these improved approaches.

8.2.2 Liquid/Gas Chromatography-Mass Spectrometry (MS)

Mass spectrometry (MS) is an analytical technique that measures molecular mass. Environmental mass spectrometers are widely employed with separation methods like gas chromatography and liquid chromatography. Non-biological pollutants in water have recently been determined using combinational approaches (Rice et al. 2020). Albishri et al. employed UV-based reversed-phase liquid chromatography with liquid phase micro-extraction to determine five organophosphorus pesticides in tap, well, and lake water samples. Pesticides were identified in water samples employing a new ultrasound-assisted temperature-controlled ionic liquid-phase micro-extraction method coupled with reversed-phase liquid chromatography. Pesticides dimethoate, carbaryl, simazine, atrazine, ametryne, tebuthiuron, diuron, and linuron were completely separated using liquid chromatography. It has also been claimed that GC with a nitrogen-phosphorus detector (NPD) may identify trace amounts of eight pesticides in drinking water. Despite its excellent detection sensitivity, GC is not recommended for non-volatile, high-molar mass compounds like insecticides. The LOD of 2.6 ng/mL for nicotine quantification in tap water and wastewater was reported utilizing a new gas chromatography mass spectrometry (GC–MS) with liquid–liquid extraction technique (Sieira et al. 2020). Franendez et al. used magnetic solid-phase extraction (SPE) before GC–MS to identify benzene, toluene, ethylbenzene, and xylenes in water samples. The LOD for benzene was 0.3 g/L and for other chemicals was 3 g/L. DBPs (disinfection by-products) are highly likely to be detected in drinking water and are associated to cancer (Zhang et al. 2019). The availability of mass spectral library databases has led to widespread use of GC–MS for DBP determination.

GS-MS can only identify molecules with low molecular weight (800 g/mol). Richardson et al. developed a novel approach that incorporated SPE, dual-column LCMS, and PIE. Verstraeten et al. and Erickson et al. used the same approach to evaluate public water samples for hormones, PPCPs, polyfluoroalkyl compounds, and herbicides. Barnes et al. used LC–MS to verify the presence of pharmaceutical pollutants and found sulfamethoxazole and carbamazepine concentrations above 0.1 ng/L in 9 wells and 0.07 ng/L in 5 wells. Llorca et al. employed LC–MS to identify 33 analytes, including pharmaceutical pollutants (Zulkifli et al. 2018). There are several studies on sulfamethoxazole and carbamazepine in drinking water and groundwater.

The ability to minimize friction makes perfluorinated compounds (PFCs) widely employed in heavy sectors including aircraft, automotive, building, and construction. PFCs, unlike other chemicals, are routinely discharged into the aquatic environment owing to their widespread industrial and culinary use. As previously described, LC–MS might be utilized to assess perfluorooctanoic acid in water samples (PFOA).

Field-flow fractionation (FFF) is a method used to obtain extensive information on chemical composition, functionality, and molecular architecture. Calvin Giddings invented it to separate macromolecules and colloids. FFF works by applying an external field perpendicular to the direction of phase flow inside a capillary to separate analytes. Compared to classic chromatography, FFF offers excellent separation components, little shear degradation, ultra-high resolution, flexible separation ability, and gentle operating conditions that enable analysis of delicate analytes. The asymmetric FFF approach combined with high resolution inductively coupled plasma mass spectrometry (ICP-MS) and membrane filtration was used to measure colloidal phosphorus in natural waterways. This technology uses centrifugal force-based sedimentation (SdFFF) and perpendicular flow-based sedimentation (FlFFF) to separate engineered nanomaterials (ENMs) in aquatic (Montaño et al. 2019) settings. SdFFF recognized high density particles, such as metallic nanoparticles with a large size, because to their capacity to produce better separation resolution.

9 Pollution Prevention

The increase in the population of the world has resulted in a rise in the amount of contaminated land and water. As the world's population continues to expand, so does the demand on air, water, and land resources. Rapid growth of industries, food, health care, automobiles, and other services is required to meet people's needs. However, if effective management is not done, it will be very difficult to sustain the quality of life with all of these new innovations, which are detrimental to the environment in which we live. Various fungus, bacteria, and microbes are continually at work in nature to break down organic molecules, but when pollution happens, who will clean up? Because the total condition of the environment is intrinsically tied to the quality of life, the world's attention has been drawn to strategies to protect and preserve the environment. Biotechnology is used to make this attempt possible.

10 Conclusion and Future Prospective

Bioremediation is considered as one of the safe and the most sustainable technology as it depends on the action of microorganisms to remove toxins from waste, such as wastewater or soil. This may be carried out on-site without disrupting human activities or the environment. When compared to traditional treatment procedures, bioremediation is proven to be a cost-effective solution. With the help of microorganisms, it is performed anaerobically or aerobically. Although microorganisms such as bacteria, archaebacteria, yeasts, fungus, and algae aid in the process of bioremediation, biological treatment alone is insufficient for pollutant elimination. The effectiveness of bioremediation of home and industrial wastewater is influenced by a variety of environmental conditions. Some of these characteristics are said to be significant since they are connected to biological and metabolic activity, which is the backbone of bioremediation.

Future of bioremediation is that the microorganisms (mostly bacteria) may be employed to eliminate or change dangerous pollutants into less damaging forms. Almost every detoxifying response may be performed by microorganisms. Nonetheless, bioremediation as a commercial technique today is predominantly focused on the removal of petroleum hydrocarbons. Bioremediation's full potential to cure a wide range of chemicals will not be achieved as long as there is confusion about what it does and how effectively it works. The committee expects that by offering instructions on how to assess bioremediation, the study would dispel the mystique surrounding this highly interdisciplinary subject and pave the path for further scientific advancements.

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Treatment of Trace Organics and Emerging Contaminants Using Traditional and Advanced Technologies

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1 Introduction

Developments in laboratory instrumentation and analytical methods have made it possible to detect and monitor trace organic compounds (TOrCs) and emerging contaminants (ECs) in different water sources. For example, advanced chromatographic techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) have enabled the quantification of pharmaceuticals, hormones, personal care products, perfluoroalkyl and polyfluoroalkyl substances (PFASs), disinfection by-products, flame retardants, and plasticizers in μg/L and/or ng/L concentrations (Ademollo et al. 2021; Borrull et al. 2021; Grung et al. 2021; Liu et al. 2021; Quintana et al. 2019). These compounds are designed to prevent, cure, or treat diseases (pharmaceuticals); control weeds, insects, and plant diseases (pesticides); control physiology, and behavior (hormones); to enhance quality of materials such as softness and flexibility (plasticizers); flame retardants (polychlorinated biphenyls or PCBs) and synthesis of firefighting foams, paper, and cardboard coating materials (PFASs) (Sumpter and Johnson 2005; Appleman et al. 2014).

The presence of TOrCs and ECs in water is a major concern due to previous reports of associated negative effects in animal health. For instance, various ECs cause dermal lesions, weight loss in infants, respiratory disorders, ocular signs, neurological disorders, reproduction disorders, immunodeficiency damage to the endocrine system, and liver. Further, exposure to PCBs causes birth defects and infertility to fish, birds, and amphibians (Haarstad et al. 2012). Although TOrCs and ECs have

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been demonstrated to be toxic, their monitoring is still poor, thus more awareness is needed to ensure minimum pollution and risking or compromising human health.

2 Global Occurrence of Trace Organic Compounds and Emerging Contaminants

Common ways through which TOrCs and ECs enter into water include discharge from the factory, inappropriate discarding of surplus medication, sewer leaks due to old infrastructure, ejection by humans and animals, and sewage from fish farms (Fauzan et al. 2018). Although several classes of TOrCs and ECs exist, pharmaceuticals are the most investigated due to their prevalence in wastewater. Notably, pharmaceuticals water contamination is caused by marked increase and consumption in the last few decades. This is linked to the growing world population, expanding investment in the health-care sector, and ever-growing global market availability (Van Boeckel et al. 2014). For this reason, global occurrence of TOrCs and ECs will be restricted to pharmaceuticals, but not to undermine the occurrence and health concerns of other classes of TOrCs and ECs widely reported in the literature.

The TOrCs and ECs are not only detected in ng/L concentrations in water bodies but also in concentration levels up to μg/L in different continents including North America (Bernot et al. 2016), South America (Carolina et al. 2019), Europe (Kuczy et al. 2021), Asia (Minh et al. 2006), Africa (Otieno et al. 2020), and Australia (Xie et al. 2020). A study by aus der Beek et al. (2016) reviewed numerous databases reporting on the occurrence of pharmaceuticals (either in water or other sources) to understand their global occurrence (Beek et al. 2016). Reportedly, 47% of the database entries registered on surface water, 8% on groundwater and drinking water, 40% on wastewater (influent and effluent), 3% on manure, dung, or soil and the remaining 2% on particulate matter. Among 71 countries globally, at least one pharmaceutical substance was reported in the literature. Figure 1 presents a global detection of TOrCs together with their possible sources. Based on database entries, monitoring of pharmaceuticals is dominating in Europe and Asia. According to aus der Beek et al. (2016), environmental water samples were characterized for 713 various pharmaceuticals and related compounds, from which 631 were successfully quantified.

Numerous techniques are employed for the removal of TOrCs and ECs from water to ensure compliance to wastewater discharge limits or drinking water standards. Herein various techniques including biological treatment, adsorption, electrocoagulation, microbial fuel cell, sorption by wetlands, photocatalysis, activated sludge, ion exchange processes, and membrane processes are discussed. Attention is paid to the basic operating principles, advantages as well as challenges associated with each technique. Also, recent advancements in each technology are highlighted together with complementary techniques that have been coupled to enhance water treatment. Finally, knowledge gaps and prospects of each technology for further advancement are debated.

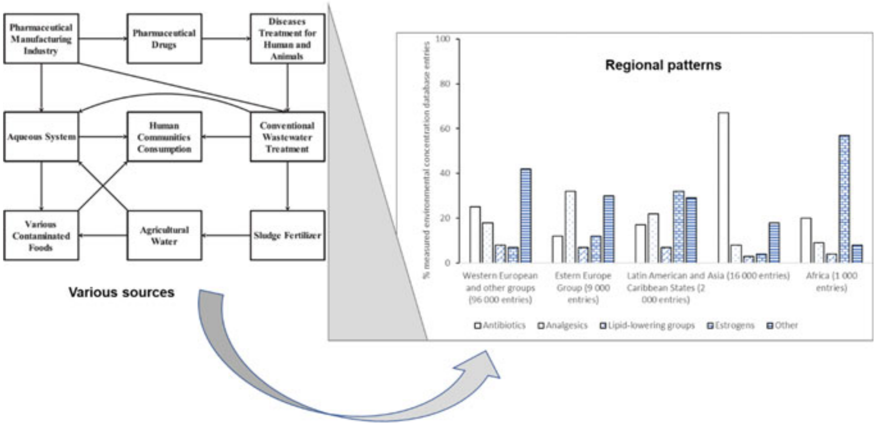

Fig. 1 Overview of sources and regional patterns of pharmaceuticals (adapted from Beek et al. [2016] and Zaied et al. [2020]). Copyright © 2015 John and Sons; Copyright © 2020 Elsevier

3 Removal of Trace Organic Compounds and Emerging Contaminants

ECs are primarily refractory compounds whose removal from water requires advanced treatment processes. Notably, TOrCs and ECs as well as their metabolites reacts with other existing contaminants, presenting toxic effects to animals and humans (Vasilachi et al. 2021). Although their direct impact on human health is not yet fully understood, some TOrCs and ECs bioaccumulate, thus compromising human health. Therefore, their removal from water sources is imperative. This section discusses some of the techniques evaluated for the removal of TOrCs and ECs from different water sources (Patiño et al. 2015; Feng et al. 2013; Mautner 2020) (Fig. 2).

3.1 Membrane Filtration Processes

Membrane processes have been extensively evaluated for the removal of emerging disinfection by-products, endocrine-disrupting compounds (EDCs), personal care products (PCPs), pharmaceutical residues, and other organic compounds (Kamaz et al. 2019; Urase et al. 2005; Wang et al. 2018). These processes include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), where full rejection mechanisms are well-established. The said processes involve the use of membranes characterized by different physicochemical properties including hydrophobicity, surface charge, functionality, surface roughness as well as surface and cross-sectional morphology. The membranes are often modified to achieve high rejection efficiency. Notably, appropriate modification approach depends on the targeted type of the feed or water chemistry. For instance, Narbaitz and

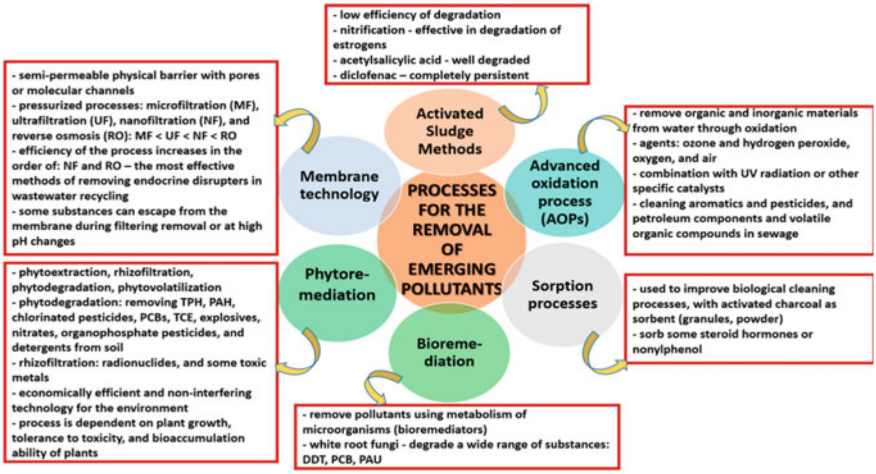

Fig. 2 Physicochemical, biological, and advanced processes for removal of organic and micropollutants from water sources (Vasilachi et al. 2021). Copyright © 2021, Creative Commons Attribution License (CC BY)

co-workers (Narbaitz et al. 2013), modified cellulose acetate (CA) NF membranes by incorporating charged surface modifying macromolecules (CSMM) to maximize the removal of charged pharmaceuticals and personal care products (PCPs). The modified CA membrane achieved excellent removal of ibuprofen and sulfamethoxazole. However, removal of carbamazepine. The major removal mechanisms are governed by membrane-solute interactions and include size exclusion, hydrophobic interactions, electrostatic interactions, and non-electrostatic interactions (Mahlangu et al. 2017). Thus, the membrane as well as solute properties are key in determining the overall rejection of TOrCs and ECs. The major challenge in applying membranes for wastewater treatment is fouling which deteriorates membrane performance and lifespan. Hence, membrane modification does not only improve performance but also resistance to fouling. Another problems associated with the application of membrane in water treatment are capital expenditure (CapEx) and operation expenditure (OpEx) (Nthunya et al. 2022). Although membrane filtration highly removes TOrCs and ECs, some pollutants are still detected in the permeates, showing incomplete removal of the contaminants. Therefore, membrane filtration has been coupled with other processes (such as biological processes) to further improve rejection.

3.2 Biological Treatment Processes

Several bacteria can degrade TOrCs and ECs even at higher concentrations to harmless products and sometimes to complete mineralization. To achieve highly efficient biological operations at industrial level, bioreactor systems ensuring scalable bioprocesses should be developed. Conventional biological systems used to remove TOrCs, and ECs include activated sludge process, trickling bed bioreactor and biofilm-based bioreactor. However, advanced biological processes namely membrane-based bioreactor (MBR), cell-immobilized bioreactor systems, and two-phase partitioning bioreactor are currently evaluated at laboratory scale with minimal industrial upscale (Feng et al. 2013; Deshpande et al. 2010). Compared to conventional processes, advanced biological processes can tolerate high concentrations of pollutants. Also, these processes can treat waste discharge containing insoluble pollutants. To improve treatment efficiency, a hybrid of biological systems is used. The evaluation of biological processes for their efficiency in removing TOrCs and ECs has been widely reported in the literature. Zhang and co-workers (2011) investigated the removal of pharmaceutical compounds, namely diclofenac, carbamazepine, naproxen, and ibuprofen from wastewater using constructed wetlands equipped with Typha angustifolia. Highest removal efficiencies for ibuprofen and naproxen were 80% and 91%, respectively. Recalcitrant carbamazepine and diclofenac showed low removal efficiencies due to their higher hydrophobicity. These variations in removal efficiencies are linked to disappearance/alterations of parent molecules, thus omitting the potency of intermediate structures. Among various biological treatment processes including bioremediation, phytoremediation, and mycoremediation, this chapter focused on biological processes restricted to sorption by wetlands (a phytoremediation process) and activated sludge (a bioremediation process). Membrane bioreactor (MBR) systems are discussed briefly under coupled processes.

3.2.1 Sorption by Wetlands

Among ecological wastewater treatment technologies, constructed wetlands (CWs) emerged as attractive method to remove TOrCs and ECs from polluted water sources. This technology was discovered in the 1950s and tested in Germany. Since then, the technology grew gradually and evaluated for the removal of micropollutants, thus ensuring strategic environmental remediation. The CWs are systems engineered to follow controlled natural wetland processes, such as biological, physical, and chemical interactions among plants and soil. The CWs remove pollutants through microbial processes (e.g., nitrification and denitrification) and physicochemical processes including fixation of phosphate to the soil filter. As a result, micropollutants are biotransformed to high-value products for the chemical and pharmaceutical industries. For instance, Anthracene is metabolized by a strain of *A. niger* to produce gentisic acid (Parshikov et al. 2015). The CWs are classified into three categories, namely (1) subsurface flow where the effluent moves vertically or horizontally to the planted layer; (2) surface flow where the effluent moves horizontally to the planted layer; and (3) floating treatment wetland where growing plants float as hydroponic mats on the water surfaces. Due to the self-sustainability of CWs, their operational costs are lower compared to other water treatment processes. Subsurface flow CWs were tested by Hijosa-Valsero and co-workers for their efficiency in removing pharmaceutical and PCPs from the wastewater. To ensure process efficiency, the influence of plants in the CWs and their feeding regime (continuous flow or batch flow) was also monitored. The removal efficiency was greatly depended on temperature where 98% of the micropollutants were removed in summer while remaining relatively low in winter (Hijosa-Valsero et al. 2011).

3.2.2 Activated Sludge Processes

Activated sludge is a form of aerobic treatment (a biological treatment process) applied in wastewater treatment for the elimination of emerging organic compounds from water. This process was developed over a century ago and used for the elimination of biodegradable organic compounds from water thus preventing oxygen depletion of receiving water if such water is discharged in the treatment plant effluent. During water treatment using activated sludge, organics are oxidized into carbon dioxide (CO_2) and water (H_2O) by live microorganisms which reside in the sludge. This oxidation is coupled to the growth of microbes and the microorganisms take up a portion of the organics. There are activated sludge processes based on non-oxidative mechanisms. These processes are used for the removal and valorization of the hydrophobic organic content in wastewater. In this case, the organic compounds are not oxidized to CO_2 and H_2O but they are removed from wastewater in a reduced form (Modin et al. 2016).

In wastewater treatment, the elimination of organic compounds through activated sludge processes is often incomplete (60–90%), and it is mainly polar compounds that are removed. Apolar compounds with low biodegradability and adsorption coefficients are poorly eliminated. In Brazil, the application of activated sludge for wastewater treatment showed 12–90% removal of polar pharmaceutical compounds and the major removal mechanisms was degradation and adsorption (Ternes et al. 1999). In the more famously used activated sludge treatment process, biotransformation has been tipped as the most important but inadequate step in eliminating hormones and antibiotics (Carballa et al. 2004). However, it must be noted that biodegradation rates for hormones and antibiotics can differ under aerobic and anoxic conditions existing in most activated sludge systems. Plósz et al. (2010) studied the fate of hormones and antibiotics in wastewater treatment using activated sludge (Plósz et al. 2010). The authors could not detect estriol in the aerobic effluent liquid due to the high affinity of the compound to sorb onto activated sludge (thus implying complete removal at the activated sludge treatment process). The underlying mechanisms of biotransformation of some compounds (such as trimethoprim) in activated sludge remains uncertain and this points out to the need for further research. However, Plósz et al. (2010) believe that the presence of growth substances is a pre-requisite of aerobic biodegradation and this condition can exist in aerobic reactors with high hydraulic loading. This would prevent growth substances from bleeding through to the aerobic reactor from the anoxic zone in remarkable quantities.

3.3 Adsorption Processes

Adsorption processes have shown potential to remove TOrCs and ECs from wastewater (Patiño et al. 2015; Álvarez et al. 2015). The key advantages of adsorption over other processes include simple process design as well as low CapEx and OpEx. However, recent studies are focused on development of low-cost adsorbents with high pollutant binding capacities. This involves the use of agricultural and industrial waste biomass. Also, activated carbon produced from various sources including waste has shown high pollutant removal efficiencies. Research advancements are establishing adsorption processes with effective removal of ECs. This involves exploitation of nanotechnology due to the requirements of large surface area of adsorbents. Nano-adsorbents include carbon nanotubes, metal oxide-based nanomaterials, silica-based nanoparticles, and chitosan-based nanoparticles (Álvarez et al. 2015; Delgado et al. 2019). Also, metal-organic frameworks adsorbents have been extensively evaluated for the removal of micropollutants from water sources.

3.3.1 Activated Carbon Processes

Over the years, activated carbon has been used more than the other adsorbents to remove greater than 90% of ECs due to the high surface area and porosity of the adsorbent. Activated carbon is one of the well-established adsorption techniques that is preferred for the treatment of water with extremely low pollutant concentrations. Activated carbon is highly selective, thus greatly removing target pollutants (including heavy metals) to levels below the detection limit. However, to achieve high removals, the process must be applied over a long time. The extraction efficiency of activated carbon depends on the source of the activated carbon. For example, Cabrita et al. (2010) found that activated carbon derived from wood sorbed greater than 90% acetaminophen, while 60–87% of the same pollutant was sorbed by activated carbon generated from other sources (Cabrita et al. 2010). The variation in adsorption capacity has been ascribed to the carbon composition of the raw resource where in some instances the resources may have lower pore diameters and the lack of additional activation prevents formation of new pores (Yunus et al. 2020).

In a study by Delgado et al. (2019), the removal of carbamazepine and sildenafil citrate by powdered activated carbon of vegetable origin was investigated (Delgado et al. 2019). Over 85% removal of the pollutants was achieved. In comparison with granular activated carbon, powder activated carbon presented more advantages such as higher adsorption capacity and rate of adsorption. Thus, the authors marked powdered activated carbon as a promising technology for the removal of carbamazepine and sildenafil from water.

Although activated carbon has proved effective in eliminating emerging organic compounds, there is little information known about the role of other parameters on sorption-based techniques and their competitiveness. Scaling up of research studies on activated carbon remain a challenge, thus hindering practical application of the technology (Khan et al. 2022). Further, adsorption using activate carbon is costly because activated carbons are hardly regenerated after adsorption. Although, regeneration has been successful using thermal regeneration, the regenerated activated carbon is not as effective as newly synthesized activated carbon (Marques et al. 2017). In addition, the regeneration of activated carbon is costly, making the process less economically feasible. When applied for the removal of per- and polyfluorinated substances (PFASs), granular activated carbon requires frequent replacement (Franke and Franke 2019). Due to the high cost of commercial activated carbon, recent trends have focused on feasibility studies utilizing activated carbon derived from agricultural waste and bio-materials. In this regard, more affordable but less effective adsorbents such as sepiolites and diatomites are used in many industrial applications (Cabrita et al. 2010). Alternatively, new synthetic routes may be explored to lower the production costs.

3.4 Electrocoagulation Processes

Electrocoagulation which involves coagulation, electrochemical processes, and flotation is a promising excellent technology used for the removal of TOrCs and ECs from water sources (Feng et al. 2013; Zhang et al. 2015). In this process, the electrodes are subjected to an electrical current, thus increasing the concentration of the cation which finally precipitates as oxides. During water treatment, the contaminants are coagulated and precipitated as sludge. Common anode materials used for electrocoagulation removal of micropollutants include aluminum, stainless steel, and iron acting as electro coagulants. Electrocoagulation neutralizes the charge of the pollutant, thus ensuring the formation of stable flocs. Electrocoagulation offers advantage of low secondary pollution and highly efficient removal of small colloidal substances. Normally, electrocoagulation is used in the event where micropollutant removal impedes the application of chemical coagulation. Also, electrocoagulation produces small amounts of settleable sludge that can easily be dewatered and discarded. Electrocoagulation has been actively studied for the treatment of pharmaceutical wastewaters with a potential 72% reduction of COD. Feng et al. (2013) evaluated various parameters for the treatment of wastewaters contaminated with micropollutant using a stirred batch reactor. Approximately, 97% of total organic carbon (TOC) associated with diclofenac, and paracetamol was removed. Also, the COD reduction was 33% after 120 min of operation. Electrocoagulation is often used as a pretreatment step in anaerobic fixed film bed reactor (AFFBR) to achieve high micropollutant removal from high-strength pharmaceutical wastewater. This hybrid system was reported by Deshpande et al. (2010) where COD and BOD were reduced by 80% and 85%, respectively.

3.5 Microbial Fuel Cell Processes

Microbial fuel cells (MFC) have gained momentum in management of wastewaters contaminated by TOrCs and ECs (Zhang et al. 2015). This was motivated by challenges faced by bioremediation, where control of microorganisms becomes difficult (thus lowering efficiency of a treatment process). A MFC is composed on an anode, cathode, and a cationic membrane. The system stimulates bioremediation for effective removal of micropollutants (Yang et al. 2019). Microbes in the anode chamber degrade the provided substrate to release electrons. On the other hand, the cathode chamber reduces the oxidized pollutants, such as chlorinated solvents. Therefore, MFC ensures both oxidation and reduction of micropollutants in one system, thus ensuring high removal efficiency from the environment. Several studies reported the application of MFC for removal of various recalcitrant TOrCs and ECs. The bioelectrochemical degradation of paracetamol using a dual chamber MFC reactor was reported by Zhang et al., where 70% degradation efficiency was achieved (Zhang et al. 2015).

3.6 Advanced Oxidation Processes

Compared to conventional wastewater treatment processes, advanced oxidation processes (AOPs) have emerged as an attractive technology due to their wide range of application, complete transformation of pollutants to less toxic products and timely conversion (Nagargoje et al. 2014). In AOPs, highly oxidizing hydroxyl and sulfate radicals (•OH and SO_4•−) are generated to effectively degrade TOrCs and ECs in the water sources. Advanced oxidation processes were first introduced in 1987 to decontaminate the environment. Its performance is enhanced by high oxidation potential of the radicals, fast reaction, and possible operation at room temperature (Glaze et al. 2008). Nonetheless, complete removal of TOrCs and ECs requires a combination of several AOPs for high production of radicals rather than a standalone process. Therefore, a combination of these processes can be chosen from a wide range including photolysis, ozonation, photocatalysis, Fenton process, sonolysis, anodic oxidation, and wet air oxidation (Nagargoje et al. 2014). Each of these technologies possess a certain level of weakness, hence, the need for coupling. For instance, the effectiveness of photocatalysis is altered by electron-hole recombination and lack of oxygen supply to generate radicals. Ozonation can only be operated at alkaline pH while Fenton process requires acidic pH to generate radicals (Ikehata and El-Din 2004). Also, ozonation promotes generation of toxic halogenated by-products in the presence of halide salts. Sonolysis is limited to degradation of volatile and hydrophobic TOrCs and ECs. Lastly, anodic oxidation suffers anode fouling induced by the organic deposition on its surface, thus reducing its performance and stability. To ensure high process performance, degradation of 40 different TOrCs was carried out using UV/H_2O_2 where the results were compared with the stand-alone application of UV

(Wols et al. 2013). Reportedly, a combination of UV/H_2O_2 improved the rate of degradation due to speedy formation of radicals achieving 90% removal of pharmaceuticals (Wols et al. 2013). However, H_2O_2 was required in excess to speed up the process through high generation of radicals (Wols et al. 2013).

Among the wide range of advanced oxidation processes, photocatalysis, and ozonation are widely used. These processes are discussed in the next sections.

3.6.1 Photocatalysis Processes

Recently, photocatalysis, as one form of AOPs, has become a distinctive, effective, and an interesting promising ecofriendly process for the removal of recalcitrant and persistent TOrCs and ECs (Neghi and Kumar 2017). Notably, photocatalysis is less toxic, stable over a longer time, and can operates at room temperature using solar lights for irradiation. The major drivers of this process are catalysts and UV light ensuring the acceleration of a catalysis-based photoreaction. Therefore, to be initiated, photocatalysis requires UV wavelengths (Aiin et al. 2016). Generally, organic compounds are degraded within 10–200 min, ensuring 80–99% removal efficiencies. Commonly used photocalysts are titanium dioxide (TiO_2), magnesium oxide (MgO), molybdenum trioxide (MoO_3), cadmium sulfide (CdS), tin(II) oxide (SnO), zirconium dioxide (ZrO_2), tungsten trioxide (WO_3), zinc oxide (ZnO), and graphite-based carbon nitrides (g-C_3N_4). Owing to its stability, high performance, and cost-effectiveness, TiO_2 is the most widely used catalyst in wastewater treatment (Lee et al. 2017). Also, TiO_2 possesses a suitable band gap for adsorption of electrons, thus promoting photoreaction. TiO_2 has three crystalline forms namely anatase and rutile brookite. Anatase exhibits high photocatalytic activity compared to other two and is mostly used in photocatalysis reactions. More research is still underway to improve photocatalytic process in terms of complete degradation of pollutants and effective reuse of the photocatalyst.

3.6.2 Ozonation Processes

Recently, ozonation processes have proved suitable to transform trace pharmaceuticals into smaller and less harmful products before discharge into the environment. To ensure process visibility, residual ozone either in liquid or gaseous phase is used as a control parameter. Ozonation is widely used to eliminate recalcitrant antibiotics detected in the wastewaters (Lange et al. 2006). In the process, the major reactive oxidants ensuring degradation of non-biodegradable organic compounds are ozone molecule (O_3) and hydroxyl radicals (•OH). In their study, Bahr and co-authors used ozone (0.5 mg O_3/mg DOC_0) to reduce carbamazepine and 17 α-ethinylestradiol to levels below their detection limits (Bahr et al. 2007). Also, ozonation disinfected the processed water while ensuring minimal formation of bromate. Furthermore, the study evaluated the relation between UVA reductions and removal of EDCs, pharmaceuticals, iodinated X-ray contrast media, microbial parameters, and bromate formation. The findings reported a possible use for guidelines controlling the oxidation performance at large scale ozonation.

3.7 Ion Exchange Processes

In ion exchange, one or multiple unwanted ionic pollutants are removed from water by exchanging with another undesirable ionic substance; ions are exchanged between solid (ion exchanger or ion exchange resin) and liquid phases. The ion exchanger is insoluble in the aqueous phase, carries exchangeable ions, and remains unchanged during the reaction. Ion exchange is used in water treatment for softening, purification of chemicals, and separation of substances. Ion exchange resins such as functionalized polymers, zeolites, clay, and soil humus are normally used as either cation or anion exchangers. Ion exchange processes are governed by Eqs. 1 and 2; where X is the structural unit of the ion exchanger (Levchuk et al. 2018).

$$2\mathrm{NaX} + \mathrm{CaCl_2(aq)} \leftrightarrow \mathrm{CaX_2} + 2\mathrm{NaCl\ (aq)} \tag{1}$$

$$2\mathrm{XCl} + \mathrm{Na_2SO_4(aq)} \leftrightarrow \mathrm{X_2SO_4} + 2\mathrm{NaCl\ (aq)} \tag{2}$$

Equation 1 defines processes taking place during water softening where hard water is pumped through an ion exchange column (NaX) and cations of calcium (as an example) are eliminated from water and replaced by an equal amount of sodium. An ideal ion exchanger must be hydrophilic, chemically and physically stable, have relatively high speed of ion exchange, have enough ion exchange capacity, and have adequate particle size and effective surface area efficient for the application and economically feasible (Levchuk et al. 2018).

Ion exchange processes have been applied for the treatment of ECs from water. In a study by Franke and co-workers (2019), anion exchange was coupled with nanofiltration (NF) for the removal of PFASs, where the ion exchange process treated concentrates from the NF process. Coupling the NF process with ion exchange improved the removal of PFASs by four-folds, thus making the ion exchange process a good technique to treat retentate from membrane filtration. In another study, Feng et al. formed a cation exchanger using particle electrodes filled in the combination of an electrolytic system with biological process and used it to remove refractory organics and heavy metals (Feng et al. 2019). The system removed chemical oxygen demand (COD), TOC, chromium, and cupper by over 80%, reaching 96% removal for cupper. Table 1 presents a summary of various processes used for water treatment together with their capacities.

Table 1 A summary of processes used for removal of organic micropollutants from water

Processes	Material	Water source or micropollutants	Initial conc. (mg/L)	Time (h)	Removal efficiency or capacity	References
Membrane filtration	CA NF membrane	Pharmaceuticals and PCPs	5.2	–	88.4%	Narbaitz et al. (2013)
	PA RO membrane	Natural water spiked with TOrCs	0.001	–	90%	Comerton et al. (2008)
	UF membrane	Secondary effluent			50%	Acero et al. (2010)
Adsorption	Activated carbon	Carbamazepine	10	10	220 mg/g	Delgado et al. (2019)
	Carbon xerogels	Caffeine	100	48	182.5 mg/g	Álvarez et al. (2015)
	Carbon nanotubes	1,8-dichlorooctane	80	72	2740 mg/g	Patiño et al. (2015)
Electrocoagulation	Al–Al; Fe–Fe electrodes	Distillery spent wash	–	2.0	98% COD reduction	Khandegar and Saroh (2014)
	Four Fe electrodes	Licorice processing wastewater	–	1.5	90.1% color, 89.4% COD, 82% turbidity, and 73.3% alkalinity reductions	Abbasi et al. (2020)
	Al and Fe electrode	Textile wastewater	–		42.5% TOC, 18.6% COD, 83.5% Turbidity, 94.9% color reductions	Bener et al. (2019)

(continued)

Table 1 (continued)

Processes	Material	Water source or micropollutants	Initial conc. (mg/L)	Time (h)	Removal efficiency or capacity	References
Membrane bioreactor (MBR)	Hollow-fiber microfiltration membrane bioreactor	Ketoprofen	0.1	–	90%	Urase et al. (2005)
	Reverse osmosis-nanofiltration membrane bioreactor	Amoxicillin		–	77%	Wang et al. (2018)
	Microfiltration membrane bioreactor	Atrazine	5400	–	25%	Kamaz et al. (2019)
Ozonation	UV-TiO2-O_3	Wastewater treatment plant	–	45	100%	Aguinaco et al. (2012)
	O_3	Wastewater treatment plant	–	45	50%	Aguinaco et al. (2012)
	O_3/ UV	Sewage treatment effluent	–		96%	Ternes et al. (2003)
	O_3/ H_2O_2	Diclofenac	1		98%	Zwiener and Frimmel (2000)
Microbial fuel cells	Dual chamber MFC reactor	Paracetamol	–	–	70%	Zhang et al. (2015)
Constructed wetlands (CW)	Pilot-scale horizontal subsurface flow CWs	Diclofenac	–	Winter	32–70%	Ávila et al. (2010)
	Mesocosm-scale CWs	Naproxen	–	Winter	0–41%	Hijosa-Valsero et al. (2010)
	Mesocosm-scale CWs	Naproxen	–	Summer	60–95%	Hijosa-Valsero et al. (2010)

(continued)

Table 1 (continued)

Processes	Material	Water source or micropollutants	Initial conc. (mg/L)	Time (h)	Removal efficiency or capacity	References
	Subsurface flow CWs	Salicylic acid	0.025	Batch CWs	93–94%	Zhang et al. (2012)
	Subsurface flow CWs	Salicylic acid	0.025	Continuous CWs	88–89%	Zhang et al. (2012)
Activated sludge processes	Activated sludge	Polar pharmaceuticals	–	–	12–90%	Ternes et al. (1999)
Photocatalysis processes	TiO_2, LED lamp	Sulfamethoxazole	0.4	20	90%	Cai and Hu (2016)
	In_2S_3	Tetracycline	20	–	100%	Ai et al. (2015)
	V_2O_5/TiO_2	Formaldehyde	500	–	100%	Akbarzadeh et al. (2010)
Ion exchange	Particle electrodes	Refractory organics and heavy metals	–	–	Up to 96%	Feng et al. (2019)

4 Coupled Treatment Processes

Biological processes are the most widely used in wastewater treatment for the removal of TOrCs and ECs. However, biological processes are ineffective in completely removing organic contaminants, thus they are often coupled to other alternative treatment processes to enhance removal. Membrane technology has been combined with other processes resulting in more advanced and energy-efficient hybrid systems with improved performance in removing TOrCs and ECs. In 2002, Molinari et al. combined a membrane and catalyst for the first time to form a hybrid process which was applied for the removal of organic pollutants (Molinari et al. 2002). The membrane served both as a sieve for separation and catalyst. In 2003, Sun et al. followed suit by developing a hybrid inorganic membrane and photocatalyst to treat *E. coli* in a single module (Sun et al. 2003). The combined process showed superior performance than the individual processes employed separately. AOPs have been coupled to biological processes for the treatment of recalcitrant contaminants and the compounds generated during chemical oxidation are mineralized by biological processes (Cai et al. 2020).

García-Gómez and co-workers combined MBR with electro-oxidation (EO) (an advanced oxidation process), and noted high removal of carbamazepine (García-Gómez et al. 2016). In this system, hydroxyl radicals (*OH) that are formed near the high voltage O_2-overvoltage anode oxidized the pharmaceutical, thus degrading it.

MBR has also been coupled with electrocoagulation processes to improve the removal of organic pollutants while reducing foulant deposition on the membrane surface. During electrocoagulation, metal ions and hydrogen gas that are generated at the anode and cathode, respectively, assist in the removal of flocculated particles by skimming from the surface. Using combined MBR and electrocoagulation, Mendes Predolin et al. (2021) observed enhanced removal of organic pollutants due to the introduction of an electric field. The mechanisms for pollutant removal were biological degradation, membrane filtration, and electrochemical action (Mendes Predolin et al. 2021).

Recently, bio-electrochemical systems have been incorporated into MBR resulting in the improvement in the removal of organic pollutants from wastewater (Song et al. 2020). Organic degradation in bio-electrochemical systems is catalyzed by electrogenic microorganisms where organic pollutants that are not utilized by microorganisms under the action of electrochemistry are changed into intermediate products that could be easily biodegraded (Song et al. 2020). Figure 3 presents some of the processes that have been coupled to improve the removal efficiency.

5 Knowledge Gaps and Prospects

Although numerous techniques have shown effectiveness in the removal of organic contaminants, their application at industrial scale has not been achieved. For example, majority of processes combining MBR and AOPs are still at laboratory or pilot scale.

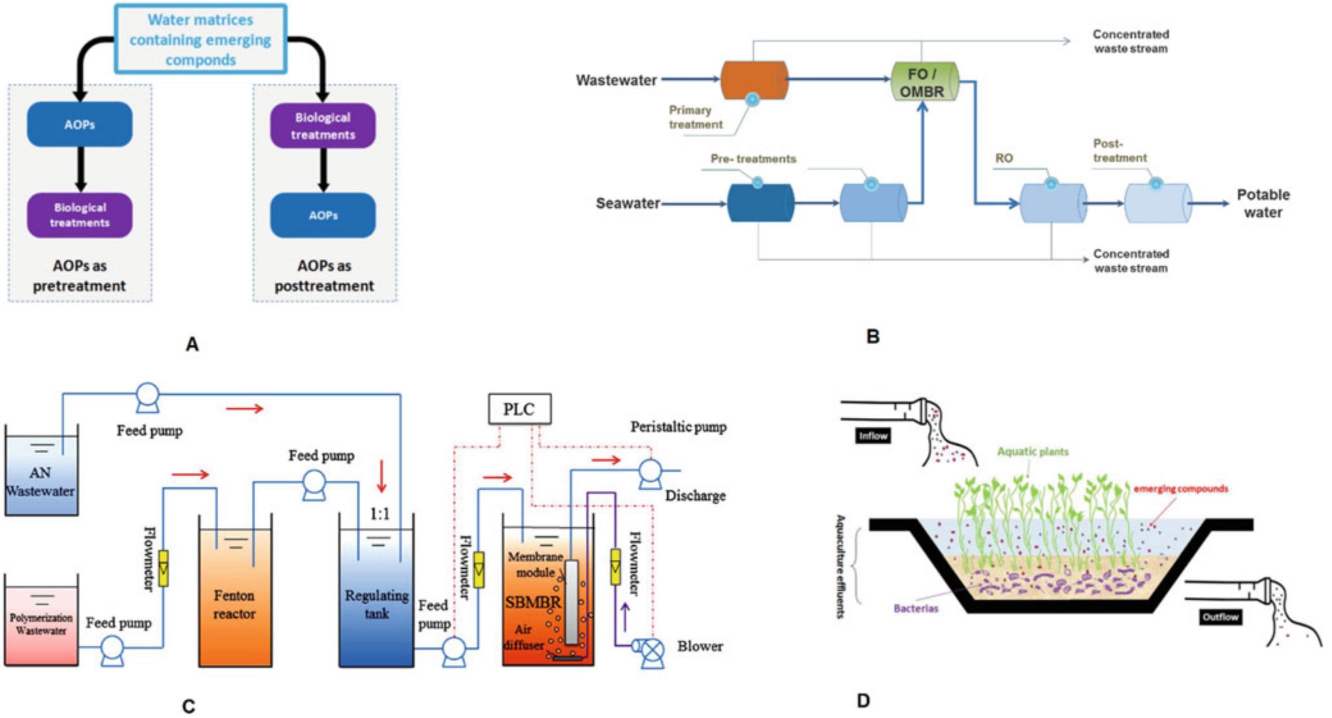

Fig. 3 Coupling different processes and removal mechanisms for treatment of emerging contaminants in water (Blandin et al. 2016; Taoufik et al. 2021; Rostam and Taghizadeh 2020): A—biological treatment coupled with advanced oxidation processes; B—biological treatment coupled with membrane filtration; C—combined Fenton (AOPs) and MBR process; D—wetland combining biological, physical, and chemical interactions for water purification. Copyright © 2016, Creative Commons Attribution; Copyright © 2020 and 2021 Elsevier

There is still incomplete removal of TOrCs and ECs by most of the applied processes. Therefore, further investigations are needed to ensure improved efficiencies of these processes. Some of the challenges especially for membrane-based processes include fouling control, pollutant removal, cost-effectiveness, and competitiveness in the field of application.

The toxicity of degradation by-products from AOPs is not fully known. These products may be more toxic than the parent compounds. Thus, their toxicity and removal need further investigation. Where possible, all by-products must be eliminated.

Membrane processes often produce waste containing high pollutants than the original feed. The recovery of precious minerals and/or nutrients remains ineffective while safe disposal of the concentrate remains a challenge. In MBR, describing fouling development using deterministic models and subsequent control is still a challenge due to the complexity of membrane fouling, especially combined fouling.

Recent studies are using nano-adsorbents for pollutant removal. However, the toxicity of the nanomaterials remains unknown. Future studies are encouraged to study the safety of the nanoparticles. Further, more research on long-term application and reusability of the material is needed to determine future integration of the technology by industry.

During wastewater treatment, the sludge may contain precious metals, nutrients as well as toxic compounds. More studies on efficient and cost-effective methods for nutrient recovery are required. Further, efficient methods for sludge treatment are required, otherwise the problem with TOrCs and ECs would be shifted from water treatment to sludge treatment; and this will influence sludge disposal. The use of some materials including carbon nanotubes (CNTs) at industrial scale is still hindered by the high costs of CNTs compared to other materials such as carbon black, clay, and carbon fiber (Ali et al. 2019). Several advanced water treatment technologies have been applied for the removal of perfluoroalkyl and polyfluoroalkyl substances (PFASs) (Banks et al. 2020). However, very little information is available on the effects of water quality conditions on the removal of PFASs and thus more research work is required on this regard.

6 Conclusions

The global detection of TOrCs and ECs in water has raised safety concerns because previous reports have shown that these compounds may cause dermal lesions, weight loss in infants, respiratory disorders, ocular signs, neurological disorders, reproduction disorders, immunodeficiency damage to the endocrine system, and liver. The traditional water treatment processes such as biological treatment have not been designed to remove xenobiotic organics, non-biodegradable compounds, and highly polar organics. Due to their inefficiency to completely remove pollutants of major concern, research focus has shifted to investigating modern/efficient alternative technologies. Thus, biological processes are often coupled to the more recent and effective approaches to target high removal of the pollutants. These coupled/hybrid processes often involve the combination of membranes with catalysis and MBR coupled with AOPs (electro-oxidation and electrocoagulation). Although modern techniques have shown promising results, their application at industrial scale has not been achieved partly due to associated costs with the treatment process and the production of degradation by-products whose safety remains unknown. Performance of other techniques under different conditions has not been fully uncovered. Finally, there is need for safe handling and disposal of sludge produced during wastewater treatment and where possible precious metals and nutrients be recovered prior to disposal.

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Declaration of Competing Interest

All authors declare no conflict of interest.

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