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Bioremediation of petroleum polluted soil (a review).

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ABSTRACT

In the last decade, the prevention and clean-up of polluted soil and water have become worldwide environmental priority. Remediation may be physical, chemical or biological. Bioremediation is slow and specific. It is cost effective, it is a natural process; although microbial seeding is inevitable, and it converts the hydrocarbons into harmless by-products. The extent of bioremediation can be measured by gravimetric method, CO_2 evolution method and gas-liquid chromatography method. Bioremediation strategies involve biostimulation, bioaugmentation and mineralization. Bacterial cultures degrade oil faster than fungi because bacteria have a shorter generation time than fungi. Petrophiles utilize pollutants as food by breaking them down using their intracellular oxygenase enzymes. The optimal strategies for any given site depend on the nature and concentration of the contaminants, pH, substrate toxicity, oxygen, moisture content, temperature, nutritional factor, salinity, sunlight, and the extent of contamination.

Keywords: Bioremediation, petroleum, soil.

INTRODUCTION

Worldwide, over 2 billion metric tons per annum (mta) of petroleum are produced and it has been estimated that 0.08-0.4% of the world production ends up polluting the ocean (National Academy of Science, 1985). Hydrocarbons vary in composition and the effect oil spill can create is often underestimated. A single gallon of oil can spread widely over areas of oceans as oil and water do not mix and tides and wave actions often compound this problem. Some parts of the oil evaporate. The oil that remains after evaporation undergoes emulsification that creates a highly viscous sticky material. After 3 months, only 15% of the original oil volume remains and it forms tarry coats that floats to shore (Atlas, 1981; Bartha and Bossert, 1984).

The short-term effects of oil spill include; contact toxicity on fine roots and root hairs of herbaceous vegetation, reduction in light transmission, reduction in the amount of dissolved oxygen, disruption of the marine ecosystems, adverse effects on marine birds. Oil covered birds can be drowned or become handicapped by reducing their ability to fly and float on water and their feathers no longer serve as insulation leaving them to die from exposure (Ijah, 1998).

In addition to short-term effects, there are also long lasting effects of oil spill. More stable oil components can be consumed by smaller species and passed unto animals higher up in the food chain, including humans resulting in growth retardation, spinal cord curvature and mortality (Pritchard, 1991).

To reduce these effects, clean-up strategies must be utilized. These include the use of skimmers, booms or barriers, chemical dispersants, detergents and solvents or burning off the oil. However, many of these treatment technologies do not actually destroy the hazardous components present; rather, the chemicals may simply be bound in a modified matrix or transferred from one location to another. Moreover, the procedures are expensive and time consuming (Bartha and Bosert, 1984).

Bioremediation involves the utilization of microorganisms to metabolically mediate desired physical and chemical processes .It is the newest and most effective method of oil spill clean-up. It makes use of indigenous oil consuming microorganisms called petrophiles by enhancing and fertilizing them in their natural habitats. Petrophiles are widespread in nature, they have been isolated from soil, water, and palm wine (Ijah, 1998). They include species of *Pseudomonas, Alcaligenes, Bacillus, Micrococcus, Flavobacterium, Corynebacterium*; yeasts such as *Hansenula, Candida albicans* and *Torula* species. They also include molds such as *Penicillium, Aspergillus, Mucor, Rhizopus.* Others are the actinomycetes: *Streptomyces* and *Norcardia* (Ijah and Okang, 1993).Moreover, bacterial cultures degrade oil faster than fungi because they reproduce at a faster rate asexually than fungi.

Bioremediation strategies include; biostimulation, which involves the addition of nutrients to increase the metabolic rate of indigenous microorganisms, bioaugmentation, the introduction of oil degrading microbes from natural population or from the test tube of genetically engineered microorganisms. Mineralization is the complete biodegradation of petroleum pollutants into inorganic molecule

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(Margesin and Schinner, 1999).

BIOREMEDIATION MECHANISMS

The process of bioremediation may be minor, involving modifications such as simple oxidation or reduction or even the loss of a functional group from a molecule. In the case of alkanes, the action of monooxygenase attack results in alcohol production. The alcohol product is oxidized to an aldehyde and finally to a fatty acid. Under anaerobic process, the fatty acid is converted to methane, carbon(iv)oxide and water by anaerobic genera such as *Methanospirillium*, *Methanococcus*, *Methanogenium* and *Methanobacterium* as illustrated below (Figs. 1 to 4).

The cycloalkanes are transferred by a not fully characterized system to a corresponding cyclic alcohol which is dehydrogenated to ketone. In the next step, a monooxygenase system distinctly different from the previously mentioned oxidase lactonizes the ring which is subsequently opened by a lactone hydrolase as illustrated below (Capone and Bauer, 1992).

Prokaryotic microorganisms convert aromatic hydrocarbons by an initial diooxygenase attack to trans-dihydrodiols that are further oxidized to dihydroxy product such as catechol in the case of benzene. Eucaryotic microorganisms use monooxygenases producing benzene 1,2-dioxide (cis-dihydrodiol). This is oxidized to form catechol. Catechol, the key intermediate in biodegradation of aromatics if opened by ortho or meta- cleavage yielding muconic acid or 2 hydroxy muconic acid semi-aldehyde. Both products are further metabolized to tricarboxylic acid cycle intermediates. Condensed polycyclic aromatics are degraded one ring at a time by a similar mechanism as shown below; (Capone and Bauer, 1992).

In the case of organochlorine compounds, they may undergo dechlorination resulting in a hydroxylated product. Once sufficient removal of chlorine atom has occurred, the remainder of the molecule can be metabolized by mechanisms similar to those of aerobic aliphatic and aromatic hydrocarbon degradation as earlier reported

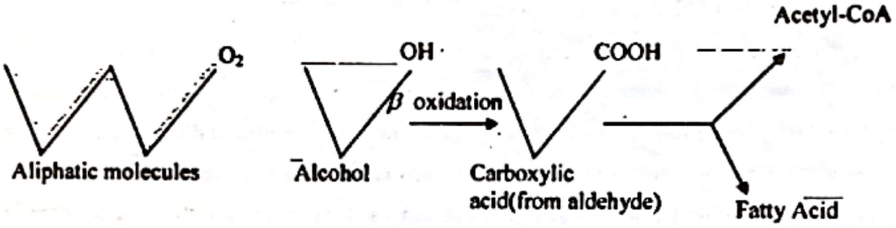

Fig. 1. Biodegradation of aliphatic molecules (Capone and Bauer, 1992).

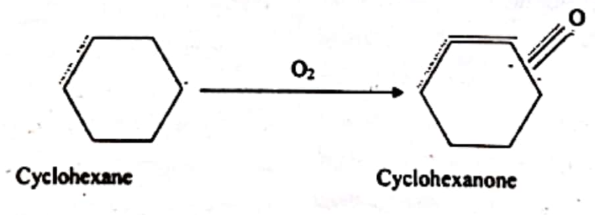

Fig. 2. Oxidation of cycloalkanes (Capone and Bauer, 1992)

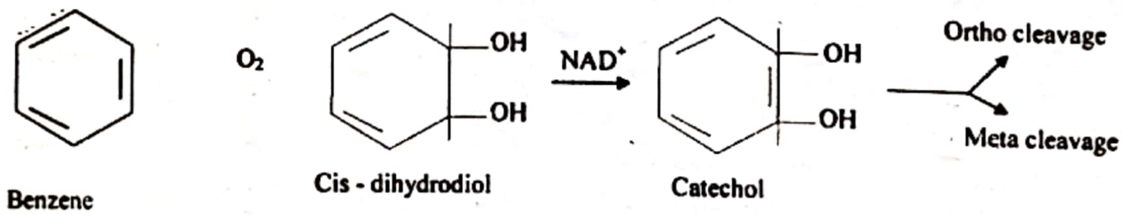

Fig. 3. Biodegradation of Benzene (Capone and Bauer, 1992).

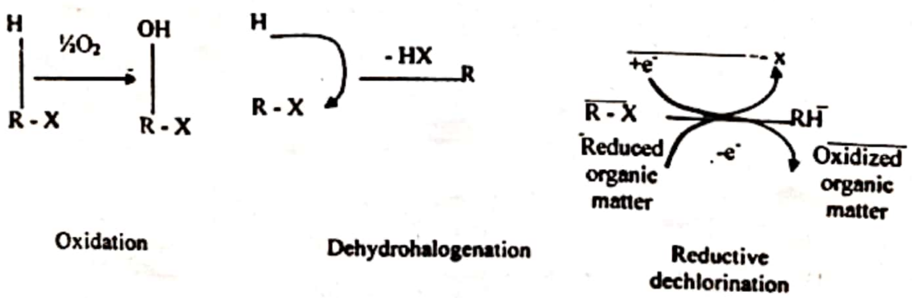

Fig. 4. Biodegradation of organochlorine (Capone and Bauer, 1992).

(Capone and Bauer, 1992).

Generally, extracellular enzymes secreted by petrophiles such as monooxygenase, dioxygenase, phenol oxidases and lignocellulases enhance these degradations (Ijah and Okang,1993).

The physical changes noticeable in oil degraded by microorganisms are that the oil appears darker, less slick, less transparent, it becomes separated into sections and it no longer covers the entire surface of the water. An increase in turbidity indicates the breakdown of the oil and the growth of microorganisms (Wardell, 1995).

Compost has an enormous potential for bioremediation as it is a nutrient substrate capable of sustaining diverse population of microorganisms such as *Pseudomonas, Penicilium, Alcaligenes, Aspergillus,* mesophilic; thermophilic actinomycetes and lignin-degrading fungi capable of degrading a wide variety of aromatic pollutants (Kaplan,1989). Soil composting has been used to destroy toxic explorer and propellants (Kaplan, 1989). At 45^{0}C, the compost was found to remove substantial amounts of the high molecular weight polychromatic hydrocarbons after 15 days through biodegradation (Civilini,1994).

Basidiomycetes such as *Gleophylum sepiratus* and *Pleurotus ostreatus* are often used in the biodegradation of petroleum effluents (Oyeleke,1999). *Phaenerochaete chrysosporium* has been used to degrade polychlorinated phenol, polycyclic aromatic hydrocarbon, DDT and polychlorinated di-benzo-p-dioxins into CO_2 and water due to the presence of cellulolytic enzymes (lignin and manganese peroxidases) (Civilini, 1994). Besides, *Lentinus edodes* could degrade pentachlorophenol from pentachlorophenol contaminated soil when supplemented with glucose, thiamine and mineral salts (Okeke *et al.*, 1993). However, successful biological treatment of contaminated soil requires the combined knowledge of microbial ecology and process engineering. Bioremediation technology could be ex-situ or in situ soil treatment.

Ex-situ treatment process includes landfarming and solidphase bioremediation. Landfarming uses the activity of soil microorganisms to degrade or immobilize the various components of hazardous waste. Solid phase bioremediation denotes landfarming operation that complies with various environmental regulations. It implies that soil piles be constructed as treatment cells with control over volatizations, leaching and run off in the absence of impermeable clay underlying the treatment zone.

In situ bioremediation involves subsurface bioremediation which involves the manipulation of aqueous constituents and the stimulation of air movement (bioventing). In each case; both passive and dynamic in situ strategies are employed. Passive in situ treatment consists of adding supplemental nutrients through infilteration wells or galleries without supplemental engineering to encourage activity while dynamic in situ treatment employs active pumping of an aquiver to better control the infiltration and distribution of nutrients (Bartha and Bossert, 1984).

Factors affecting bioremediation

Most of the factors affecting bioremediation are the factors affecting the microbial populations involve in the degradation processes. These include;

Temperature

Bacteria readily metabolize certain hydrocarbons at varying temperature range. Thermophiles are active at higher temperature, mesophiles at medium temperature range while psychrophiles at low temperature range (Ijah, 1998).

Nutritional factor

The presence of nitrogen and phosphorus increases the rate of bioremediation for example during the study on biological treatment of polluted effluent water from Escravos tank farm, samples with nutritional supplement showed a more enhanced biodegradation (Okeke *et al.*, 1993).

Oxygen

Oxygen is very essential for bioremediation of oil because, most hydrocarbon degraders are aerobes. In some instances, where the microbes have used up the available oxygen, oxygen must be delivered to the contaminated region (Bartha and Bossert, 1984).

Moisture content

If the moisture content is below 40%, degradation will be slow and if it is above 60%, there will be insufficient air space to sustain aerobic decomposition hence 50-60% of moisture is somewhat the best range of moisture content for rapid aerobic thermophilic degradation (Ijah and Okang, 1993).

Concentration of contaminants

The more the concentration of the contaminants, the more the time taken for the degradation to take place. Thus biodegradability tends to decline with the number of rings and degree of condensation and contaminant concentration (Bartha and Bossert, 1984)

Physical and chemical state of the pollutant

The lower concentration of pollutants depending on their chemical compositions have more chances of degradation. Thus the degree of spreading determines in part the surface area of oil available for microbial colonization by hydrocarbon degrading microorganism (Kaplan, 1989).

pH

The optimum pH for rapid aerobic degradation is between 6.0 and 7.5. However, there is a wide range over which sewage sludge can be decomposed with little apparent rate of decomposition (Okeke *et al.*,1993).

Salinity and sunlight

The rate of biodegradation in hypersensitive environment reduces as salinity increases. Besides, photooxidation has been shown to have a substantial effect on the chemical, physical and toxicological properties of petroleum which subsequently influences the rate of biodegradation (Wardell, 1995).

MEASUREMENT OF THE RATE OF BIOREMEDIATION

There are several methods used in measuring the rate of bioremediation. These include gravimetric method, gas liquid chromatographic technique and CO_2 evolution method. The gravimetric method involves the determination of the difference in the weight of control oil sample and that of the residual oil after treatment with the test organism. In the gas liquid chromatographic method, the residual oil is injected into an injection medium and the extent of bioremediation is determined from the various peaks indicated on the screen., Although this method is tedious and involves the use of sophisticated equipment, it is faster, more reliable and more accurate than the other methods include, the progressive degradation and the persistent components are also indicated. In CO_2 evolution method, a hundred gram of the soil sample from the contaminated site is mixed with 10mls of water in 250mls Erlenmeyer flasks and then sterilized. The organism under test is introduced into the flask with 5mls of the effluent sample. A control (with no organism is equally set up). 0.5g of barium peroxide is taken in a vial and to this barium peroxide was added 5mls of distilled water. Each of the vials is then lowered at 20 degrees into each of the Erlenmeyer flasks containing soil and each flask covered with cotton wool. The flask is then agitated gently two or three times daily to break up the scum of $BaCO_3$ formed on the surface of the liquid vial. At 5 days intervals, the vials are removed and new ones replaced inside the Erlenmeyer flasks for a period of 21 days. The amount of CO_2 absorbed by $Ba(OH)_2$ can be determined by titrating $BaCO_3$ in the vial with 1N hydrochloride acid at room temperature using phenolphthalein as an indicator, which corresponds with the volume of the acid used. The amount of CO_2 produced by the organism utilizing the substrate can be estimated using Stolky's formular; (B-V)BE, where B is the volume (ml) of the acid for the control, V is the volume (ml) of acid treated, N the normality of the acid and E, the equivalent weight data ($E;CO_2=22$) (Oyeleke,1999).

ADVANTAGES AND LIMITATIONS OF BIOREMEDIATION

Bioremediation is a natural process. It is cheaper than all other remediation techniques. It is theoretically useful for the complete destruction of a wide variety of contaminants. Thus, many compounds that are legally considered to be hazardous can be transformed into innocuous products thereby eliminating the chances of future liabilities associated with treatment or disposal of contaminated materials (Bartha and Bossert, 1984).

Bioremediation is a slow process often highly specific. Moreover, not all xenobiotics are susceptible to rapid and complete biodegradation. Besides, products of biodegradation may be more hazardous than the initial parent compounds. For example, biotransformation of chlorinated aliphatic trichloroethylene can result in the formation of vinyl chloride; a suspected carcinogen (Ensley,1991).In some cases, severe negative impacts from newly introduced microbial populations are known, although, the effects is generally described as low probability of high-consequence risks (Bartha and Bossert, 1984).

CONCLUSION AND RECOMMENDATION

In order to maintain a reasonable level of water quality and minimize land pollution, petroleum spills should be prevented by strict compliance to government regulations and national standards. Besides, bioremediation is the most effective method for oil spill clean up hence, to realize its full potentials, innovative research and technology in the field should be encouraged.

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