THE EFFECTS OF TEMPERATURE AND PH ON DECOLOURIZATION OF DYE CONTAMINATED SOILBY *PSEUDOMONAS AERUGINOSA* DM1

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ABSTRACT
This study investigated the effects of pH and temperature on the decolourization of textile effluent contaminated soilby *Pseudomonas aeruginosa* DM1. The physicochemical parameters of the textile effluents were temperature (33.5 ^{o}C), pH (6.10), Total Dissolved Solids (465.5 mg/L), Total Soluble Solids (310 mg/L), Dissolved Oxygen (0.162 mg/L), Biological Oxygen Demand (0.13 mg/L), Chemical Oxygen Demand (123.5 mg/L). The *Pseudomonas aeruginosa* DM1 was isolated from textile effluent contaminated soil samples in Challawa, Kano State, Nigeria. The 3 mL culture of the isolate was inoculated into medium comprising 2 g of yeast extract, 2 g of glucose, 2.8 g of lactose broth in 50 ml of different concentrations of the effluent (0%, 5%, 10%, 30%, 50%, 70%, 90% and 100 %) and incubated for 30 days at 37 ^{o}C. The effects of temperature (30 °C, 35 ^{o}C, 40 ^{o}C, 45 ^{o}C) and pH (4, 6, 8, and 10) on the decolourisation potential of the isolates were determined. The rate of biodecolourisation was determined using spectrophotometry at 600 nm. The optimum biodecolorization temperature was 35 ^{o}C (60.7%) after 30 days while the optimum pH was 6 with biodecolorization rate of 85.1%. The results obtained in this study revealed that 35 ^{o}C and pH 6 were optimum for decolourization by *Pseudomonas aeruginosa* DM1 of textile effluent contaminated soil.

KEYWORDS: *Pseudomonas Aeruginosa* Dm1, Textile Effluents, Biodecolourisation, Spectrophotometry.

INTRODUCTION

Nature has blessed man with abundant gifts like water, air, land, forest, minerals, and fossil fuel. The essences of these gifts were to improve his standard of living (Tamil *et al*., 2012). Within the last few decades, uncontrolled urbanization has caused serious pollution problem due to the disposal of sewage and textile effluents to water bodies (Indu, 2011). Most of these industries generate large volume of waste water which is generally channelled into water bodies either untreated or inadequately treated thus causing water pollution (Sarala, 2009). Textile industry is one of the important industries causing water pollution. The effluent generated from these industries constitute large amounts of organic substances as well as varying high concentrations ammonium salts, chromium, chlorides, sulphides which have been used during the process (Ayisa*et al*., 2017).

The removal of recalcitrant dye stuffs using physical and chemical methods (adsorption, chemical transformation, incineration, photo-catalysis, ozonationetc) are not suitable because of high cost, low efficiency and in-applicability to a wide variety of dyes (Andleeb*et al*., 2010).

Generally, the wastewater from a typical textile industry is characterized mainly by high values of biological oxygen demand (BOD), chemical oxygen demand (COD), colour and pH (Tufekci*et al*., 2007). The untreated textile wastewater if discharged directly into the surface water sources can cause rapid depletion of dissolved oxygen due to its high BOD value. The effluents containing high levels of BOD and COD values are highly toxic to biological life (Olukani*et al*., 2006). The high alkalinity and traces of chromium which is employed in dyes adversely affect the biological treatment processes and also interferes with aquatic life (Melgoza*et al*., 2004). It acts by inducing persistent colour coupled with organic load leading to disruption of the total symbiotic/ecological balance of the receiving water stream (Puvaneswari*et al*., 2006).

Dyes with striking visibility in recipient waters reduce light penetration in aquatic environment which in turn significantly affects the photosynthetic activity. The high concentration of nitrogen in the textile industrial effluents can also cause the eutrophication of closed water bodies. In addition, colored materials are objectionable as it can spoil the beauty and aesthetics of water environments (Ashutosh*et al*., 2010). In view of the earlier mentioned adverse effects of dyes, there is a

need for textile industry effluents to be discharged after proper treatment.

Current research has been focused on the biodegradation of the industrial effluents (Andleeb*et al*., 2010). It goes to show interest towards the pollution control making use of bacteria or fungi in combination with physicochemical methods such as pH, electrical conductivity, organic carbon, available nitrogen (McMullan *et al*., 2012). The attractive features of biological treatment include low cost, renewable and regenerative activity with little or no secondary hazard (Sharifi*et al*., 2013; Morias and Zamora, 2015). The conventional biological processes are not effective because the dye content in the textile effluent is toxic to the microorganisms used (Koch *et al*., 2002).

Microorganisms belonging to different taxonomic groups of algae, bacteria, fungi, and actinomycetes have been reported for their ability to decompose azo dyes (Olukami*et et al*.,2006) The bacteria used include *Escherichia coli, Bacillus sp, Clostridium sp*, and *Pseudomonas sp*. (McMullan, 2012)**.**

The northern part of Nigeria is where most of these textile industries are cited and the environmental pollution caused by these textile industries is increasing at an alarming rate. This necessitatedthis study on the biological control of dye pollutants by isolating the dye degrading microbes from contaminated soil. Within the past two decades there has been an upsurge in the search for cost effective and environmentally friendly alternative to the conventional method for dealing with waste water (Ezeronye*et et al*., 2014). The major methods for treatment of textile waste water involve physical and chemical processes (Saraswalthi and Balakumar, 2014) and these methods are less effective, expensive and have limited application (Chen *et al*., 2013). Biological decolourization has been investigated as a method to transform, degrade or mineralize dyes. Moreover, such decolourization and degradation is an environmental friendly and cost-competitive alternative to chemical decomposition processes (Verma, 2013). Therefore, the aim of this study was to utilize *Pseudomonas aeruginosa* DM1 to decolorize dye contaminated soil and investigate the effect of pH and temperature on the decolourization potential of *Pseudomonas aeruginosa* DM1.

MATERIALS AND METHODS

Sample collection

The effluent samples were collected from the textile manufacturing industry in Challawa, Kano State Nigeria in a clean 25-liter polyethylene container and transported to the laboratory using ice pack chest stored in the refrigerator at about 4 $^{\circ}$C prior to analysis. Contaminated soil samples (approximately 20 g) from the three locations were also collected using some clean, dry and sterile polythene bags along with sterile spatula.

Isolation and identification of *Pseudomonas aeruginosa* DM1

Soil samples were collected using a hand trowel from the field to a depth of 30 cm combined and mixed thoroughly. The 50 g of soil sample was dissolved in 100 mL of sterile distilled water and centrifuged for one hour at 3000 rpm. The 0.5 ml of the soil suspension was then added to 4.5 ml of broth consisting of centrimide and salt. Ten fold dilution of the soil sample was made and after 48 hours of incubation at 37 ^{0}C, 0.1 ml of each dilution was plated on King B medium with 0.03% centrimide added and plates incubated at 37 °C.

After incubation for 24 - 48 hours, cultures grown in centrimide broth and King B agar which fluoresced with an ultra violet lamp was suspected to be *P. aeruginosa*. In addition, representative yellow or blue-green fluorescent colonies from the soil samples isolated from King B medium was confirmed as *P. aeruginosa*.

The DNA of the organisms was extracted using Polymerase Chain Reaction for molecular characterizations using 16S rRNA gene from the bacteria, chromosomal DNA was extracted with QIAamp DNA Mini Kit (Qiagen Inc, Germany). The 16S rRNA gene was amplified using polymerase chain reaction (PCR) with the primer 27 F (5^{1}- AGA GTT TGA TCM TGG CTC AG-3$^{1)}$ and reverse primer 1492R (5^{1}- ACC TTG TTA CGA CTT-3^{1}) from Macrogen, Korea. The PCR mixture will include 0.2 µg of template, 20 pmole of each primer, 10 mM of dNTP mixture, 5 µl of 10x pfu DNA polymerase buffer (Mg included) and 3 units of pfu DNA polymerase (Bioneer Co., Korea) in a 50 µl reaction volume. After which the phylogenetic analysis was performed to determine the evolutionary relationship of the strain with other validly published strain (Sriram and Reetha, 2015).

Biodecolorization experiment

The biodecolorization test was carried out in accordance with a modification of the method reported by Walter *et al*. (2013) thus: 100 ml flask containing 50 mL of the effluent with 2 g of yeast extract and 2 g of glucose added as a co-substrate to augment the activity of the bacterial isolates that is serving as a booster for the organisms to perform optimally. The flask was sterilized by autoclaving at 121 $^{\circ}$C for 15 minutes. The sterilized flask containing the effluent sample was inoculated with 3 mL inoculum of *Pseudomonas aeruginosa* DM1 and compared with the corresponding Mcfarland standard to determine the size of inoculum and the test was done on 100% of effluent and other concentrations that included 90%, 70%, 50%, 30%, 10%, 5% to 0%. The rate of biodegradation was measured spectrophotometry at 600 nm.

Determination of the effects of temperature and pH for biodecolorization

In order to determine the effects of temperature on the biodecolorization potential of the isolates, the procedure reported by Walter *et al*. (2013) was repeated with the

following temperature ranges 30 ^{o}C, 35 ^{o}C, 40 ^{o}C and 45 ^{o}C in water bath while pH was adjusted to 4, 6, 8, and 10 using dilute HCl and NaOH. All other parameters such as available nutrient, oxygen were kept constant.

RESULTS

The molecular characterisation of the isolate gave the following sequence: This gave a99% identity to *Pseudomonas aeruginosa* DM1 16S ribosomal RNA gene, partial sequence

AAAAAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGT
GGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGG
ACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACG
ATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTAGGGGAG
GCAGCAGTGGGGAATATTGGACAATGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCT
TCGGATGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAA
CAGATAAGCACGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATT
ACTGGCGTAAAGCGCCCGTAGGTGGTTCAGCAAGTTGATGTGAAATCCCCGGGCTCAACCTGGGAACTGC
ATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGAT
ATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGACTGATACTGACACTGAGGTGCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGA
GACTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAA
TGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGC
ATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGT
TACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
CAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGC
CGGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGT
CGGAATCGCTAGTAATCGGAATCAGAAGACTGAT

The results of PCR reaction was visualized using gel electrophoresis (Figure 1). The result indicated that the DNA fragments form a band on the gel at location corresponding to 1500 base pair of the DNA ladder.

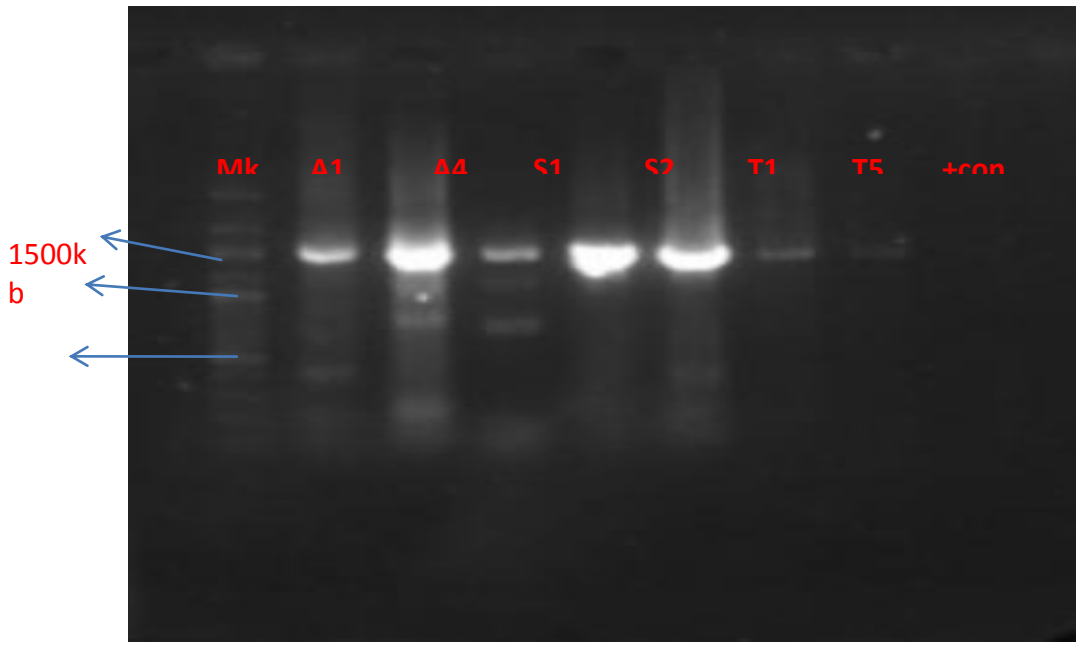

Figure 1: Gel image of *Pseudomonas aeruginosa* DM1

Biodecolorization Abilities of the Isolates on the Textile effluent

Pseudomonas aeruginosa DM1 possessed the ability to biodecolourize textile effluent as they grew on the minimal basal medium which contained the textile effluent. *Pseudomonas aeruginosa* DM1 possessed the ability as they grew on minimal basal medium containing 5% textile effluent. Their increased performance on higher concentration of textile effluent necessitated their selection.

Effects of temperature on biodecolorization of textile effluent

The results in Table 1 showed the effects of temperature on biodecolorization of the textile effluent. The biodecolorization activities increased with increase in temperature from 30 °C to 35 °C but later reduced at 40 °C to 45 °C. Table 1 also recorded the highest biodecolorization of 60.7% at 35 °C after 25 days.

Effects of pH on biodecolorization of textile effluent

The result of the effects of different pH values on biodecolorization activity are illustrated in Figure 2. The activity of the isolate decreased with increase in pH values except in some instances. The *Pseudomonas aeruginosa* DM1 showed highest biodecolorization of 85.1% at pH 6.

		% biodecolorization/ volume of effluent					
Days	T (°C)	100	90	70	50	30	10
5	30	2.00^{b}	3.80^{a}	5.20^{b}	5.40^{c}	6.22^{c}	7.40^{c}
	35	4.00^{a}	3.40^{b}	5.00^{c}	10.00^{a}	10.40^{a}	13.20^{a}
	40	1.01^{c}	1.09^{c}	2.60^{d}	5.20d	7.00^{d}	10.30^{b}
	45	0.10^{d}	0.70^{d}	7.00^{a}	7.80^{b}	10.40^{a}	10.60^{a}
10	30	4.90^{b}	6.30^{a}	11.50^{b}	16.80^{b}	18.30^{b}	19.00^{c}
	35	5.20^{a}	4.39^{b}	11.00^{a}	17.00^{a}	21.00^{a}	20.90^{a}
	40	1.01^{d}	1.20^{d}	4.20^{d}	6.00^{d}	6.20^{d}	7.02^{d}
	45	1.20^{c}	2.00^{c}	7.10^{c}	9.00^{c}	12.00^{c}	11.30^{c}
15	30	8.00^{a}	9.30^{a}	17.10^{a}	21.20^{b}	22.00^{b}	22.40^{b}
	35	5.80^{b}	4.82^{b}	14.00^{b}	26.00^{a}	26.00a	28.00^{a}
	40	2.00^{d}	2.30^{c}	5.90^{d}	7.50^{d}	8.10^{d}	8.60^{d}
	45	2.70^{c}	2.20^{d}	9.80^{c}	10.70^{c}	13.70^{c}	14.90^{c}
20	30	11.40^{a}	13.10^{a}	18.00^{b}	23.30^{b}	23.70^{b}	24.90^{c}
	35	6.40^{b}	6.12^{b}	25.00^{a}	41.00^{a}	41.00^{a}	44.50^{a}
	40	4.10^{c}	4.00^{c}	5.60^{d}	7.00^{d}	7.30^{d}	8.70^{d}
	45	3.20^{d}	4.00^{d}	10.30^{c}	19.70^{c}	21.20^{c}	27.10^{b}
25	30	13.30^{a}	15.70^{a}	22.40^{b}	25.70^{b}	27.50^{c}	30.20^{c}
	35	7.20^{b}	6.90^{b}	44.00^{a}	48.00^{a}	48.00^{a}	60.70^{a}
	40	4.20^{c}	5.70^{c}	7.20^{d}	8.24^{d}	9.40^{d}	10.00^{d}
	45	3.60^{d}	5.10^{d}	14.40^{c}	22.60^{c}	35.00^{b}	38.00^{b}
30	30	14.90^{a}	17.50^{a}	30.60^{b}	34.50^{b}	37.90^{c}	41.20^{d}
	35	1.00^{c}	1.80^{c}	3.01^{d}	3.20d	5.12^{d}	6.20^{c}
	40	1.01^{c}	1.09^{c}	2.6^{d}	5.2^{d}	7.0^{d}	10.0^{c}
	45	0.10^{d}	0.70^{d}	7.0^{a}	7.80^{a}	10.40^{a}	10.60^{a}

Table 1: Effect of varying temperature on *Pseudomonas aeruginosa* DM1 after a period of 30 days

Value are means ± standard deviation, value with the same alphabet in the same column are not significantly different from each other at $P \leq 0.05$.

Table 2: Effect of varying pH on *Pseudomonas aeruginosa* DM1 after a period of 30 days

Days	pH	100%	90%	70%	50%	30%	10%
5 days	4	4.00^{d}	5.50^{d}	6.00^{d}	9.00^{d}	11.30^{d}	15.60^{c}
	6	5.10^{b}	7.40^{c}	8.00^{b}	11.10^{b}	11.50^{c}	15.10^{d}
	8	5.00^{c}	7.60^{b}	7.80^{c}	11.00^{c}	12.10^{b}	16.50^{b}
	10	6.10^{a}	9.10^{a}	9.90^{a}	12.30^{a}	13.40^{a}	17.60^{a}
10 days	4	5.20^{d}	6.41^{d}	12.00^{d}	16.00^{d}	22.30^{c}	22.80^{c}
	6	6.20^{c}	8.30^{c}	14.10^{c}	18.30^{c}	22.10^{d}	21.50^{d}
	8	6.30^{b}	8.50^{b}	14.00^{b}	18.10^{b}	23.40^{b}	23.60^{b}
	10	7.30^{a}	10.80^{a}	15.30^{a}	19.20^{a}	24.10^{a}	24.90^{a}
15 days	4	6.10^{d}	6.73^{d}	15.00^{d}	25.00^{d}	27.10^{d}	30.00^{c}
	6	7.40^{b}	8.60^{b}	17.40^{b}	27.10^{b}	27.40^{c}	29.20^{d}
	8	7.20^{c}	8.10^{c}	17.00^{c}	27.00^{c}	28.20^{b}	31.40^{a}
	10	8.40^{a}	10.50^{a}	18.10^{a}	28.10^{a}	29.50^{a}	31.30^{b}
20 days	4	7.30^{d}	8.60^{d}	27.00^{d}	35.00^{d}	43.10^{c}	45.30^{c}
	6	9.00^{b}	10.10^{c}	29.60^{b}	37.20^{b}	43.00^{d}	45.20^{d}
	8	8.00^{c}	10.20^{b}	29.00^{c}	37.00^{c}	44.30^{b}	46.20^{b}
	10	9.60^{a}	12.40^{a}	30.40^{a}	38.30^{a}	45.10^{a}	47.40^{a}
25 days	4	7.90^{d}	9.22^{d}	45.50^{d}	53.10^{a}	49.30^{d}	62.20^{a}
	6	8.50^{c}	11.20^{c}	47.50^{b}	49.30^{d}	55.10^{b}	60.40^{c}
	8	9.10^{b}	11.50^{b}	47.00^{c}	50.40^{c}	55.00^{c}	62.10^{b}
	10	10.30^{a}	13.60^{a}	48.20^{a}	51.70^{b}	56.40^{a}	62.10^{b}
30 days	4	8.10^{d}	9.41^{d}	67.90^{d}	71.40^{d}	78.20^{d}	83.10^{d}
	6	10.10^{b}	12.70^{b}	70.40^{c}	74.50^{a}	79.20^{c}	85.10^{a}
	8	9.50^{c}	11.60^{c}	69.60^{b}	73.10^{c}	79.30^{b}	84.30^{c}
	10	10.70^{a}	13.70^{a}	70.80^{a}	74.20^{b}	80.10^{a}	84.60^{b}

DISCUSSION

The role played by some bacterial species in the decolourization of textile effluents has been reported (Kalyanee*et al*., 2010; Olayinka*et al*., 2011; Walter *et al*., 2013; Karthikeyan and Anbusaravanan, 2013; Sriram and Reetha, 2015; Ayisa*et al*., 2017). Bacteria capable of decolourizing dyes from industrial effluent samples collected from waste water treatment sites contaminated by dyes have been isolated, screened and reported by Ndasi*et al*. (2011). Idisi*et al*. (2014) isolated *Pseudomonas* and *Bacillusspecies* capable of degrading crude oil and textile effluent. Temperature has a significant effect on the biodecolorization of textile effluent by micro-organisms (McMullan *et al*., 2012). The maximum biodecolorization potential of *Pseudomonas aeruginosa* DM1 72.2% at 35 °C. Similarly, Ayisa*et al*., (2017) reported the highest biodecolorization activities of 60.7% at 35 °C.

Pseudomonas aeruginosa DM1 achieved highest biodecolorization of 85.1% at pH 6. The results agreed with work carried out by Walter *et al*. (2013). The ability of microorganisms to degrade textile effluent is as a result their constant metabolic activities. The isolates probably may have acquired the natural adaptive ability to survive in the presence of the textile effluent. The nitrogen, sulphate, and carbon found in the effluent medium are utilized by the microorganisms for their nutrition (Sriram and Reetha (2015).

Similarly, Karthikeyan, and Anbusaravanan (2013) and supported the concept of Pandev*et al*. (2006) that the optimum growth of bacteria usually occurs at pH7. However, the rate of biodecolorization of *Pseudomonas aeruginosa* DM1 at pH 7 was slightly lower than at higher pH. It is possible that alkaline pH was detrimental to the bacteria and caused the release of enzyme or redox mediators to cause dye reduction and the dye could also be reduced by alkaline hydrolysis (Kalyanee*et al*., 2010). Thus, the activities of the *Pseudomonas aeruginosa* DM1 resulted in the biodecolorization of textile effluents within a period of 30 days. The pH 6 and temperature of 35 °C are optimum for the biodecolorization of the isolate.

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