ISOLATION AND IDENTIFICATION OF EFFECTIVE DYE DEGRADING BACTERIA FROM TEXTILE EFFLUENT

Laju, S[1], Susheela Gomathi [2], Antony S[2] and Bibin K.Mathew[3]
1 Research Scholar Reg. No. 8537, Department of Microbiology Malankara Catholic College, Mariagiri Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627 012, Tamil Nadu, India
2. Assistant Professor, Malankara Catholic College, Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627 012, Tamil Nadu, India
3. Researcher, Malankara Catholic College, Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627 012, Tamil Nadu, India

Abstract

Degradation and de-colouration of textile dye using microorganisms has been one of the most promising technologies for dye removal from aqueous environment. The present research work highlights the isolation and identification of indigenous bacteria from textile dye effluent and evaluation of their ability to decolorize dye. The decolorizing activity was quantified using standard methods. Among the different microbial isolates, for the present study, three bacterial strains were selected from the mother plate of effluent source as all isolates have exhibited decolorizing activity after one to three days of incubation. Among them *Micrococcus luteus* are the bacteria able to decolorize the textile dye efficiently. Decolouration studies to be carried out with different concentration of dye at varying pH and temperature. The 16s rRNA sequence matching the potential isolate was identified as *Micrococcus luteus* strain JW-22.

Key Words: Decoloration, Degradation, Dye, Bacterial isolates

I.INTRODUCTION

India's dye industry produces every type of dyes and pigments. Production of dye stuff and pigments in India is close to 80,000 tones. India is the second largest exporter of dyestuffsand intermediates (developing countries) after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80% (Lorimar, 2001). Industrialization is vital to a nation ‘economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment. Many of these products are problematic because of persistence and toxicity.

The discharge of dye effluents containing recalcitrant residue into rivers and lakes (Manikandan et al., 2012). Especially in textile industries produced more than 70% of the total quantity of waste in India (Rajeswari et al., 2013). Dyes are chemicals which bind to one of the material then imparts colour due to presence of chromophore group. Industrially the following azo dyes are commonly used and they are acid dye, basic dye, direct dye, disperse dye, mordant dye, reactive dye and solvent dyes. The acid, basic, direct and reactive dyes are ionic (Sudha et al., 2014). Azo dyes are one of the largest and most versatile classes of synthetic dye. Azodyes consist of a diazotized amine coupled with an amine or phenol, and contain one ormoreazo linkages .Specifically reactive azo dye has complex aromatic structures. Mainly, those dyes are stable because they are very difficult to degrade (Aftab et al., 2011). These are mainly used in the textile, rubber products, paper printing, colourphotography, pharmaceuticals, cosmetics, foods, and other industries.

The presence of very small amounts of dyes in water (less than1.0 ppm for some dyes) is visible and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies giving displeasing appearance. Treatment of such wastewaters is therefore, essential but difficult. The discharge of dye wastewater into the environment, impedes light penetration, damages the quality of the receiving streams and may be toxic to food chain organisms and to aquatic life. Azo dyes and their metabolites are to be mutagenic and carcinogenic, that cause bladder cancer in humans and

hepatocarcinoma (Srinivasan et al., 2014). Through mineralisation, these dyes can be broken down into an aromatic amine, an arylamine to be carcinogenic and mutagenic. They reduce the efficiency of seed germination and plant growth, inhibits the elongation of shoot and roots.

Several physico-chemical techniques have been proposed for treatment of coloured textile effluents, includes anion exchange resins, flotation, electro flotation, electrochemical destruction, irradiation, ozonation, adsorption and the use of activated carbon etc. All these physical or chemical methods are very expensive and result in the production of large amounts of sludge, which creates the secondary level of land pollution. Therefore, economic and safe removal of the polluting dyes is still an important issue. Some of physical and chemical treatment techniques are effective for colour removal but use more energy and chemicals than biological processes. In recent years, a number of studies have focused on some microorganisms which are to biodegrade and bio absorbs dye in wastewaters. A wide variety of micro - organisms capable of decolorizing a wide range of dyes include some bacteria, fungi, yeast .Bioremediation is a pollution control technology that uses natural biological species to catalyse the degradation or transformation of various toxic chemicals to other forms. This process completed is called as ‘’mineralization’’. Compared with physicochemical methods, biological process has more interest because of their cost effectiveness, lower sludge formation and is environment friendly (Gurulakshmi, 2008). These organic materials can be degraded both aerobic and anaerobically. Over the past decades, biological degradation has been investigated as a method to transform, degrade or mineralize dye effluents.

II. MATERIALS AND METHODS

2.1. Sample collection

The dye containing waste water as sample was collected from a textile dying industry located in Nemam, Thiruvananthapuram. The sample was collected in sterile polyethylene bottle. The collected sample was shifted to the laboratory within 24 hours and stored under 4^0C in refrigerator for further analysis.

2.2. Selection of dyes

Three different types of dyes were selected for the present study such as direct black, direct blue and direct orange.

2.3. Microbial analysis of sample

2.3. a.Enumeration of total microbes in the dye effluent sample

Total microbial counts in the dye effluent sample was enumerated by serial dilution from 10^{-2} to 10^{-7}. One ml from each of the dilution were plated on to the respective Nutrient Agar plates, using spread plate method and incubated at 37ºC for 24 hrs for bacterial counts. Colonies on the plates were counted by using colony counter.

The bacterial loads in the sample were calculated by using the formula

$$\text{Bacterial load/ml} = \frac{\textbf{Total number of colonies obserbed}}{\text{volume of sample taken}} \times \text{Dilution factor}$$

2.3.b. Isolation of bacterial strains

Based on their predominant and distinct colony morphology such as size, shape, colour, elevation, transparency etc. three bacterial Isolates were selected from the mother plate. They were named as D1, D2 and D3.The Bacterial cultures were isolated and identified by pure culture and Biochemical methods. These isolated strains were maintained on Nutrient agar slants and stored in refrigerator at 4^0C.

2.3.c.Characterization of bacterial isolates

The characterization of selected bacterial strains was carried out by Macroscopic, Microscopic, biochemical test and molecular methods. The comparison of the results obtained with Bergey,s manual of determinative bacteriology 9th edition was carried out.

2.4. Assay of decolourization activity

The commercial available dyes were used for decolourization study. They are direct orange, direct blue and direct black. 100 ml of nutrient broth was taken in 250 ml sterilized conical flask. 0.1 gm of dye was added and mixed well. After that flasks were inoculated with 1 ml of isolated test organism individually and incubated at 37^0C for 1-3 days. The decolourization percentage was read after incubation. Two replications were carried out.

After incubation aliquot was centrifuged at 8000rpm for 10 minutes to separate the bacterial cell mass and clear supernatant was used to measure the decolourization at the absorbance maxima of 540nm by using calorimeter. Controls were carried out in the same conditions without inoculum. The percentage of decolourization effluent was determined by using the formula

Percentage of decolourization (D) = $\frac{Ao-A1}{Ao} \times 100$

Where,

D= decolourization in %; A0 = initial absorbance; A1= Final absorbance.

2.4. Effect of dye concentration on decolourization

Textile dyes of direct black, direct blue and direct orange were chosen. Each dye were set at different concentration such as 100mg/L, 200mg/L, 300mg/L, and 400mg/L in the test tubes. After that tubes were inoculated with 1 ml of isolated test organism individually on each set of dye. Before incubation, the optical density measured was taken as the initial value. After 24 hours of incubation, the sample was centrifuged at 5000 rpm 15 minutes. The supernatant was collected and absorbance was measured using calorimetrically and the rate of decolourisation was determined.

2.5. Effect of pH on dye decolourization

Textile dyes of direct black, direct blue and direct orange were chosen. Each dye was set at different pH such as 5, 6, 7, 8, and 9 in the test tubes. After that tubes were inoculated with 1 ml of isolated test organisms individually on each set of dye. Before incubation optical density was taken as initial. After 24 hours of incubation sample was centrifuged at 5000 rpm 15 minutes. The supernatant was collected and absorbance was measured using calorimetrically and the rate of decolourisation was determined.

2.6. Antimicrobial activity of D1, D2 and D3

The antibacterial activity was determined by the diffusion method of Kirby Bauyer described by (Dupuid et al., 1989). The antibacterial activity of selected isolated strain (D1, D2 and D3) against clinical pathogens (*Salmonella*, *Staphylococcus, Streptococcus* and *Shigella*) was determined.

2.7. The PCR primer which target the 16s rRNA sequences analysis

Isolated bacterial DNA B14 (D2) was prepared for each sample as follows. Two microliters of lytic enzyme solution from the Pure gene DNA Purification Kit (Flowgen) was added to each sample, which was then briefly mixed and incubated at 37°C for 30 to 45 min. Samples were pelleted and re-suspended in 100 µl of sterile distilled water, boiled for 10 min, and then stored at −20°C until required. Ten microliters of sample was used as a template in each PCR.

2.8 PCR amplification

PCR amplification was performed in a volume of 50 µl consisting of 5 µl of concentrated lysate or 10 µl of 1:10 and 1:100 dilutions of the lysate in sterile MilliQ-grade water (Millipore, Boston, Mass.). The remainder of the reaction mixture contained 1× PCR buffer (50 mMKCl, 10 mMTris-HCl [pH 9.0], 0.1% Triton X-100, 1.5mM MgCl2), 0.4mM each of the four deoxynucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), 1.0 U of Taq DNA polymerase (Promega UK Ltd., Southampton, United Kingdom) and 0.2 µM (each) PCR primer. Thirty-five microliters of DyNAwax was used to separate the primers and lysate from

the rest of the reaction mixture to reduce the incidence of nonspecific PCR products and also improve the yield of the desired DNA fragments. The PCR was performed in an Omni-Gene thermal cycler (Hybaid, Teddington, United Kingdom). The cycling conditions were as follows: (i) an initial denaturation step at 94°C for 5 min; (ii) 35 cycles, with 1 cycle consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min; and (iii) a final extension step at 72°C for 10 min. Positive and negative controls were included in every set of PCRs performed. The positive control was a standard reaction mixture containing 10 ng of bacterial DNA instead of sample, whereas the negative control contained sterile water instead of sample. Reaction products were either analyzed immediately or stored at −20°C until required. The mixed 16S rRNA gene products were ligated into the pCR2.1-TOPO vector (Invitrogen BV, Groningen) and transformed into TOP10 cells (Invitrogen) according to the manufacturer's instructions

2.9 Sequencing

Plasmid minipreps were prepared from recombinants using the Promega Wizard Plus purification system (Promega UK Ltd.) according to the manufacturer's instructions. Sequencing was performed using the Thermo Sequenase sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech, Amersham, United Kingdom). The sequencing reactions were set up with 5 µl of plasmid DNA, 1 µl of sequencing primer (M13 Universal [5′-Signature sequence forward primer-3′] or M13 Reverse [5′- Signature sequence reverse primer-3′], both labeled with IRD800 dye), 0.7 µl of dimethyl sulfoxide, and 14.3 µl of sterile molecular biology-grade MilliQ-grade water (Millipore). For each clone, 4.5 µl of the sequencing reaction was added to 1.5 µl (each) of A, C, G, and T reagent (primer termination mixes for each dideoxynucleotide). A commercial master mix kit (HotStarTaq Plus Master Mix; Qiagen, Hilden; with added SYBR green I during cycle number optimization) was used for PCR amplification under the following cycling conditions: 1x 15 min at 95°C, 32x (for the 8f-534r set) or 35x (for the 968f-1401r set) 15 sec at 95°C, 30 sec at 55°C, 30 sec at 72°C, and 1x 1 min 20°C. Optimal amplification conditions were defined for each primer combination by the cycle number before the real time PCR amplification curves entered a plateau with no further increase of total fluorescence. Cycling was performed on a Stratagene MX3000p (Stratagene, Amsterdam, The Netherlands). PCR products were removed immediately after the last cycle and stored at −20°C until further analysis. The PCR products were purified with a commercial kit (Qiaquick nucleotide removal kit, Qiagen, Hilden, Germany) and the amount of DNA was determined as described above. Equimolar dilutions of all samples were then combined into one master sample per extraction procedure.

2.10 Sequence analysis

Sequences obtained from the LI-COR image analysis program were converted to FASTA format and analyzed for chimeric forms using the Chimera-CHECK 2.7 program from the Ribosomal Database Project II. After elimination of chimeric sequences, the partial 16S sequences were then compared with 16S rRNA gene sequences from the public signature sequences.

III. **RESULTS**

3.1. Physical analysis of sample

The collected waste water sample from textile industry was dark black in colour with pungent smell. The pH of tested sample was 8, above the neutral level.

3.2. Microbial analysis of sample

3.2.a.Enumeration of total microbes in the dye effluent sample

High bacterial load were observed on Nutrient agar plates. The colonies formed on the plate were enumerated. The microbial load of the sample was observed and the observation was tabulated (Table 1)

3.2.b. Selection and characterization of bacterial isolates

i Macroscopic observation

Based on their predominant and distinctive colony microbial characters such as shape, size, colour, elevation, transparency. Three bacterial isolates were selected from the mother plate. They were named as D1, D2, and D3(Table 2).

ii Characterization of bacterial isolates

Based on their microscopic, biochemical, physiological and selective plating. Culture properties upon comparing with Bergey's manual of determinative Bacteriology 9th edition. The three bacterial isolates were characterized (Table 3)

3.3.Decolourization of dye by the isolate

In the present study three bacterial isolates were observed. They were *Bacillus* sp, *Micrococcus luteus* and *Psuedomanas* sp. These bacteria were tested for their ability to decolourize three different commonly available textile dyes (direct blue, direct black and direct orange) at dye concentration about 1000 ppm. The flasks were incubated under shaking conditions. After 24 hours of incubation colour change was observed. (Plate 1 and 2).

3.4.Effect of concentration on dye decolourization

Decolourization percentage of three different textile dyes at a concentration (100mg/L. 200mg/L, 300mg/L, 400mg/L and 500mg/L) by using the isolated bacterial strains were tabulated. *Bacillus sp* shows maximum average decolourization on 200mg/L, *Micrococcus luteus* shows maximum average decolourization on 400 mg/L and *Psuedomonas* sp shows maximum average decolourization on 100mg/l (Table 4 and Plate 3)

3.5. Effect of pH on dye decolourization

The effect of pH on decolourization activity of selected bacterial isolates with different pH 5, 6, 7, 8, 9.*Bacillus* sp shows maximum decolourization on pH 8, *Micrococcus luteus* shows maximum decolourization on pH 7 and *Pseudomonas* sp shows maximum decolourization on pH 7. After 24 hrs of incubation (Figure 1and Plate 4)

3.6. Effect of temperature on dye decolourization

The effect of temperature on decolourization activity of selected bacterial isolates with different temperatures 28^0C, 37^0C and 40^0C.*Bacillus* sp shows maximum decolourization 37^0C, *Micrococcus luteus* shows maximum decolourization at 40^0C and *Pseudomonas* sp shows maximum decolourization at 40^0C (Figure 2and Plate 5).

3.7. Antimicrobial activity of selected bacterial isolates

From the data, it was revealed that the clinical pathogen (*Staphylococcus*) was sensitive to the isolated organism. *Bacillus sp* shows the maximum inhibition of 14mm against *Streptococcus* and minimum inhibition of 8mm against *Shigella* followed by *Micrococcus luteus* also shows a high antibacterial activity of 20mm against *Streptococcus* and minimum inhibition of 15mm against *Salmonella. Pseudomanas sp* shows maximum inhibition of 14mm against *salmonella* and minimum inhibition of 9mm against *Shigella* (Table 5)

3.8. Species identification

The PCR primer which target the 16srRNA sequences analysis

Name of the identified organism D2 was" *Micrococcus lutes* strain JW-22*"*

Type: species-specific signature sequences

The PCR primers which target the 16S rRNA Sequence Analysis

OPG -- 5'-TGTACACACCGCCCGTC-3′ 3'-CTCTGTGTGCCTAGGTATCC-5'.

Table 1 Bacterial load of the textile waste water

Sl No	Sample	Dilution	Bacterial Load(CFU/ml)
	Textile waste water sample	10^{-4}	196
		10^{-5}	70
		10^{-6}	35
		10^{-7}	Too low to count

Table 2 The morphological characters of the bacterial isolates

Sl. No	Bacterial isolates	Growth on agar plates				
		Colour	Elevation	Transparency	Shape	Size
1.	D1	white	Flat	Opaque	irregular	Large
2.	D2	cream	convex	transparent	circular	Small
3.	D3	Dissusible green	umbonate	Transparent	oval	medium

Table 3 showing microscopic characterization of bacterial isolates

Sl. No	Biochemical, physiological and selective plating	Bacterial isolates		
		D1	D2	D3
1.	Gram's staining	G+ve rod	G –ve, cocci	G-ve rod
2.	Motility	+ve	-ve	+ve
3.	Spore staining	+ve	-ve	-ve
4.	Indole	+ve	-ve	-ve
5.	Methyl red	-ve	-ve	-ve
6.	Vogesproskaur	-ve	-ve	-ve
7.	Citrate utilization	+ve	+ve	+ve
8.	TSI test	A/AL	A/AL, gas	A/AL
9.	Catalase test	+ve	+ve	+ve
10.	Oxidase test	+ve	+ve	+ve
11.	Urease	+ve	+ve	+ve
12.	Nitrate	+ve	-ve	+ve
13.	Casein	+ve	+ve	+ve
14.	Gelatine	+ve	-ve	+ve
15.	starch	+ve	-ve	-ve
16.	Lipid	+ve	-ve	+ve
17.	Carbohydrate	+ve	+ve	+ve
18.	Growth on selective medium	*Bacillus* cereus agar	Tripticase soy agar	Cetrimide agar
19.	Isolates	*Bacillus* sp	*Micrococcus* sp	*Pseudomanas* sp

+ve- Positive A- Acid production

-ve- Negative A – Alkali production

Table 4 showing effect of concentration on dye decolourization

Organisms	Colour of dye	Percentage of decolourization (%)				
		100mg/L	200mg/L	300mg/L	400mg/L	500mg/L
D1	Blue	81	86	51	39	36
	Black	11	14	21	28	28
	Orange	82	84	85	85	87
D2	Blue	33	37	57	63	60
	Black	96	95	88	85	71
	Orange	88	88	89	93	97
D3	Blue	16	17	25	16	19
	Black	70	80	53	64	58
	Orange	66	49	57	22	20

Table 5 showing antimicrobial activity of selected bacterial isolates

Sl. No	**Bacterial Isolates**	**Zone of inhibition in Diameter(mm)**			
		Streptococcus	*Staphylococcus*	*Shigella*	*Salmonella*
1	*Bacillus sp*	14	12	8	No
2	*Micrococcus luteus*	20	17	18	15
3	*Psuedomanas sp*	No	No	9	14

Plate 1 Decolouration of textile dye by isolated bacterial strains

Plate 2 Decolouration of direct orange by isolated bacterial strains


Plate 3 Decolouration of textile dye at different concentration using isolated bacterial Strains


Plate 4 Decolouration of textile dye at various pH by isolated bacterial strains


Plate 5 Decoloration of textile dye at different temperature by isolated bacterial strains

Plate 6 PCR product of the sample *Micrococcus lutes*

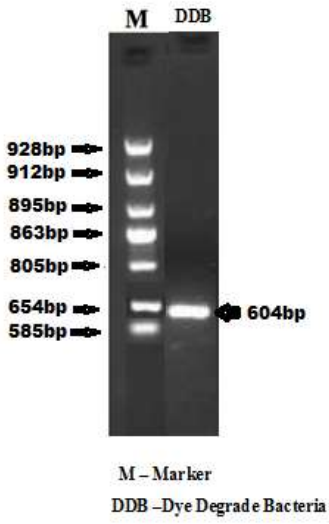


Figure 1 Effect of pH on dye decolourization

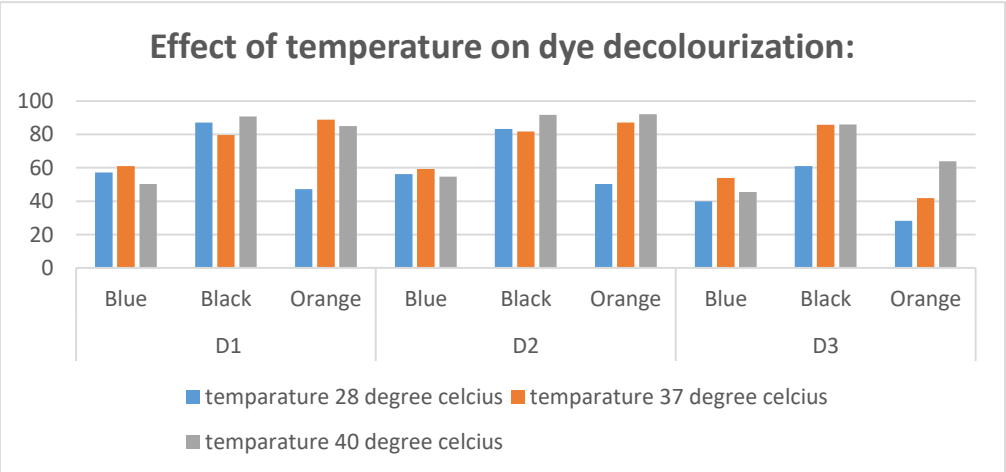


Figure2 Effect of temperature on dye decolourization

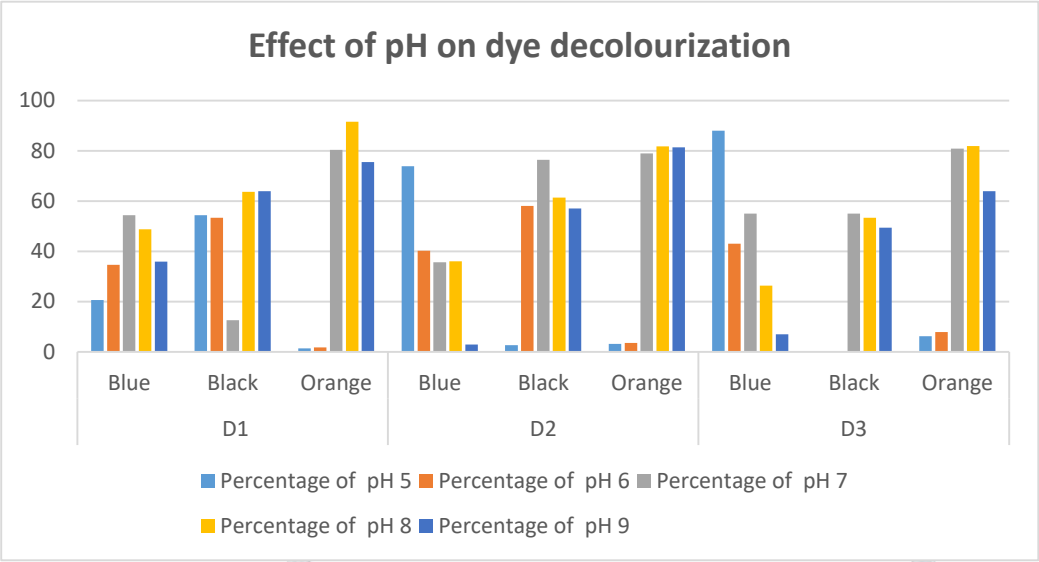
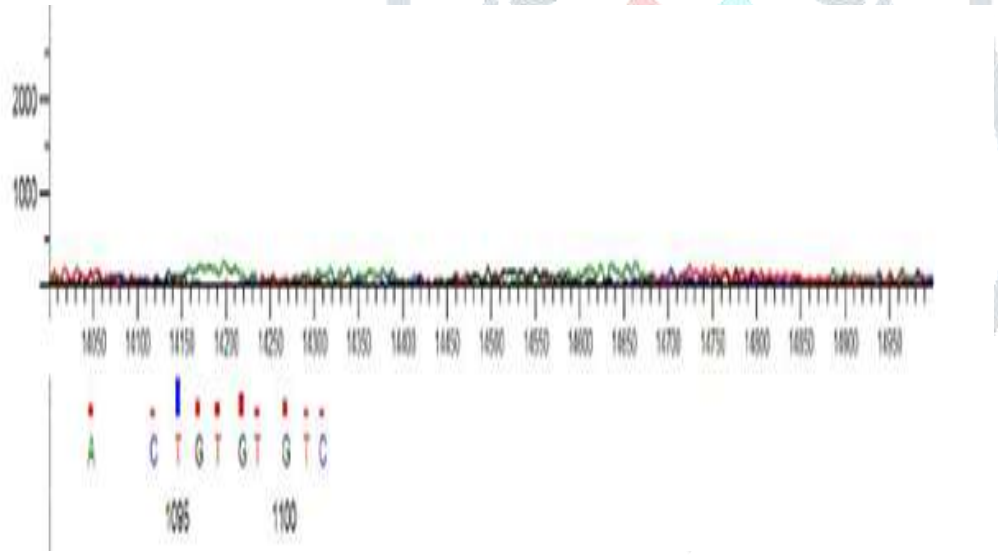


Figure3 showing Electrophotogram of the sample *Micrococcus luteus*



Forward Cycle

TCCAGAGTTTGATCATGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCAGC
TTGCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCTTAACTCTGGGATAAGCCTGGGAA
ACTGGGTCTAATACCGGATAGGAGCGTCCACCGCATGGTGGGTGTTGGAAAGATTTATCGGTTTTGGATGGACTCG
CGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCC
TGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAAGAAGCGAAAGTG
ACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTATCC
GGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGTCGTGAAAGTCCGGGGCTTATCCCCGGGATC
TGCGGTGGGTACGGGCAGACTAGAGTGCAGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATA
TCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCTGTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCG
AACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGACCATTCCACGGTTTCCGC
GCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGTTCTCGA
TCGCCGTAGAGATACGGTTTCCCCTTTGGGGCGGGTTCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAG

ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCACGTAGTGGTGGGGACTCATGGGAG
ACTGCCGGGGTCAACTCGGAGGAAGGTGAGGACGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCAT
GCTACAATGGCCGGTACAATGGGTTGCGATACTGTGAGGTGGAGCTAATCCCAAAAAGCCGGTCTCAGTTCGGATT
GGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCC
CGGGCCTTGTACA

Reverse primer

ATGAGACACCCGATCCCCGACTACCTGGCGAGCCTGGTCACCGAGCTCGGCGCCGTGAACCCGGGCGAGACGGCC
CAGTACATCCCCGTGCTCGCGGAGGCGGACCCGGACCGCTTCGGCATCGCGCTGGCCACCCCCACCGGGCGGCTGC
ACTGCGCGGGCGACGCGGACGTCGAGTTCACCATCCAGTCCGCGTCCAAGCCGTTCACGTACGCGGCCGCGCTCGT
GGACCGGGGCTTCGCCGCCGTGGACCGGCAGGTGGGGTTGAACCCCTCGGGCGAGGCCTTCAACGAGCTCTCCCTC
GAGGCGGAGTCGCACCGCCCGGACAACGCCATGATCAACGCCGGCGCGCTCGCGGTGCACCAGCTGCTCGTGGGA
CCGGAGGCGAGCCGGAAGGAGCGGCTGGACCGGGCCGTGGAGATCATGTCCCTGCTCGCCGGGCGTCGGCTCTCG
GTGGACTGGGAGACGTACGAGTCCGAGATGGCGGTCTCGGACCGCAACCTCTCCCTGGCGCACATGCTCCGCAGCT
ACGGGGTGCTGCAGGACTCCGCCGAGGAGAT

CGTGGCCGGGTATGTGGCGCAGTGCGCCGTGCTCGTCACCGTCAAGGACCTCGCTGTGATGGGCGCGTGTCTGGCG
ACCGGCGGCATCCACCCGATGACGGGAGAGCGGATGCTGCCGTCCATCGTGGCGCGGCGCGTGGTGTCCGTGATG
ACCTCCTCCGGCATGTACGACGCCGCCGGACAGTGGCTCGCCGACGTCGGGATCCCGGCCAAGTCCGGGGTGGCCG
GCGGTGTGCTCGGCGCGCTGCCGGGCCGGGTGGGGATCGGCGTGTTCTCGCCGCGCCTGGACGAGGTGGGCAACTC
TGCGCGCGGCGTGCTGGCGTGCCGCCGCCTCTCCGAGGACTTCCGGCTGCACCTCATGGACGGGGACAGCCTGGGA
GGCACCGCCGTGCGCTTCGTGGAGCGCGAGGGTGACCGCGTGTTCCTGCACCTGCAGGGCGTGATCCGGTTCGGCG
GCGCCGAGGCGGTGCTGGACGCGCTCACGGACCTGCGCACCGGTGCGGAGAAGCCCGGCACCGGGTGGGACGCCG
CCGTCTACCCGCGGTGGCAGGAGGCCGCGGCGGACAGGGCGGCGCTCTCGGCCGCGACCGGCGGCGGGGCGGTGC
ACGAGGCCGCCGCCGCGGCCGCCCGAGACGAGAACGACGGGCCCATCCGCACCGTGGTGCTCAACCTCGCGCGGG
TGGACCGGATCGACGACGTCGGCCGACGGCTGATCGCCGAGGGCGTGCGCCGGCTGCAGGCCGACGGCGTGCGCG
TGGAGGTCGAGGATCCGGAGCGCATCCTGCCGCTGGAGGAGGCCGGCGCCCACTGA.

IV. DISCUSSION

The textile industry plays an important role in the world economy as well as in our daily life, but at the same time, it consumes large quantities of water and generates large amount of waste waters. The chemical reagents used in the textile sector are diverse in chemical composition ranging from inorganic to organic molecules (Subhatra et al., 2013).

The release of a wide range of compounds from industries is creating disturbance to the ecosystem. Bioremediation has been identified as safe technique in which using microbes as biological agent is effective. Sufficient indigenous microbial population with effective degradation capacities and the environmental conditions are crucial in the in situ bioremediation process (Bento et al., 2005). In the present study, the observation of physical characters of the collected textile water samples had revealed a high bacterial load. The Colour of the sample was dark black colour with pungent smell and $P^{H}8$ shows alkaline. The textile dye colors taken for the study were direct black, direct blue and direct orange. Chikkara and Rana had observed the colour and smell of textile effluent sample which was black and pungent respectively at pH 9.4 (Chhikara and Rana, 2013) whereas Verma and Sarma tested textile waste-water which was brownish-black in color with unpleasant odor at pH 8.3 (Varma and Sharma, 2011) Mohan et al. (2013) reported textile dye effluent in Thirupur of Tamil Nadu shows alkaline pH.

Based on their colony morphology bacterial colonies were examined by microscopically and macroscopically three colony character was selected. On the basis of decolourising capacity and colony character these three isolates were named as D1, D2, and D3. These bacterial colonies were confirmed based on their morphological, Biochemical characteristics and comparing with Bergy's manual of determinative bacteriology the isolated colonies were *Bacillus* sp, *Micrococcus* sp and *Psudomonas* sp. After 24 hours of incubation D2 show 76% of dye decolouration. While the other two organisms exhibited 58% and 32% after 72 hours of incubation. Thus D2 was selected for identification based on16S rRNA gene sequencing analysis. The potential isolate was identified as *Micrococcus luteus* strain JW-22.Dipankaret al., (2018) studied the biodegradation of crystal violet dye by Enterobacter isolated from textile industry effluent showed the maximum decolouration of 81.25% after 72 hours of incubation. Marimuthu et al., (*2013)* isolated the strains of *Bacillus sp*., *Acinetobacter* and *Staphylococcus* and reported the

decolourization ability of the isolated organism. Also in the present study antibacterial activity of *Micrococcus luteus* was observed and it show maximum zone of inhibition against *streptococcus sp*. Mohan et al., (2013*)* isolated *Planococcus sp.* and *Bacillus sp* from textile dye effluent and found that these organism exhibited maximum decolourization which was about 80%. *Eisangela* et al., (2009) have reported that *Staphylococcus* can decolourizeazo dye in microaerophilic or aerated process in the presence of yeast extract. *Micrococcus* shows maximum decolouration at pH 7 and temperature at 40^{o}Cby Singh et al.,(2014) observed the biodegradation of azo dye Direct Orange16 was done by *Micrococcus luteus* .Also supported by Ramdan et al.,*(*2015) observed decolorization of reactive black 5 by the isolated organisms *Micrococcus luteus* from the textile effluent.

V.CONCLUSION

The study concluded that, bacterial analysis of textile waste water sample showed the bacterial isolates such as *Micrococcus luteus, Bacillus sp and Pseudomonas sp.* These isolates have the ability to decolorize commonly available chemical textile dyes. In that *Micrococcus luteus* showed maximum decolourization ability. In future these bacterial isolates can be used as a good tool for bioremediation of various textile effluent treatment by converting toxic dye into colourless, harmless product., the treated effluents can be reused for textile industry.

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BIBILOGRAPHY

[1] Aftab, U., Khan, M.R., Mahfooz, M., Ali, M., Aslam, S.H and Rehman, A. 2011. Decolouration and degradation of textile azo dye by *Corynebacterium* sp. isolated from industrial effluent, Paskistan Journal of Zoology, 43(1):1-8

[2] Bento, F.M., Camargo, F.A.O., Okeke, B. C., Frankenberger, I.F. 2005. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresource Technology. 96. 1049 – 1055.

[3] Chhikara and Rana 2013. Chhikara S, Rana L. Physico-chemical characterization of textile mill effluent: A case study of Haryana, India. Environment and Waste Management. 8:19–23.

[4] Cripps C., Bumpus, J., Dust S., 1990. Applied Environmental Microbiology, 56:1114-1118.

[5] Dipankar, C.R., Sudhangshu, K.B., Ananda, K.S., Biswanath, S., Mizanur, R., Apurba, K.R., Zakaria, H.P., and Swee, S.T. 2018. Biodegradation of crystal violet dye by bacteria isolated from textile industry effluent. *Peer J* 6: e5015.

[6] Elisangela, F., Andrea, Z., Fabio, DG, Cristianao, R.M., Regina, D.L and artur, C.P 2009.Biodegadation of textile azo dyes a faculatative *Staphylococcus arlettaestarin* VN-11 using sequential Microaerophilic and aerobic process. International Biodeterioration and Biodegradation.63: 280-288.

[7] Guru Prasad Srinivasan 2014. Biodegradation of carcinogenic textile Azo dyes using bacterial isolates .Journal of coastal life medicine, volume 2(2):154-162.

[8] Gurulakshm, M., Sudarmani, D.N.P and Venba. R., 2008. Biodegradation of Leather Acid dye by *Bacillus subtilis*. Journal of advanced biotechnology.

[9] Lorimer 2001. School of science and Environment, Coventry University, Coventry CVI 5FB,UK.[3]N. Pure Appl,chem.,73: 12.

[10] Manikandan, S. Surumbar Kuzhali and R. Kumuthakalavalli 2012. J. Microbiol. Biotech. Res. 2 (1): 57-62.

[11] Marimuthu, T., Rajendran.S, Manivannan. M 2013. Decolourisation of reactive textile dye effluent by using Bacterial strain, *international journal of science and Engineering Research* .**4**:1646-1656

[12] Mohan,V., Mathumitha, M., Sangeetha Menon and saranya, D.K. 2013. Isolation and screening of potential dye decolouizing bacteria from Textile dye effluents in Tamil Nadu,India. Journal of Academia and Industrial Research **2:**63-69

[13] Rajeswari, K., Subashkumar, R., and Vijayaraman, K.2013. J. Microbiol. Biotech. *Res.* 3 (5): 37-41.

[14] Ramdan, A.M., Hashem, M.A., Shareef and, A.A., Essam, T. 2014. Decolorization of Reactive Black 5 by *Micrococcus luteus* and *Candida albicans* in waste waters. World Applied science Journal 32(2):153-163.

[15] Saranaik S, Kanekar P. 1995. Bioremediation of colour of methyl violet and phenol from a dye industry waste effluent using Pseudomonas sp. Isolated from factory soil. Journal of Applied Bacteriology **79:** 459–469.

[16] Singh, S., Chatterji. S., Nandini. P.T., Prasad. A.S.A., Rao., K.V.B. 2015. Biodegradation of azo dye Direct Orange 16 by *Micrococcus luteus* strain SSN2.International Journal of Environmental Science and Technoogy, .**12**:2161-2168

[17] Subhathra, M., Prabakaran,V., Kuberan,T and Balamurugan, I. 2013. Biodegradation of azodye from textile effluent by *Lysinibacillus sphaericus*. Sky Journal of soil science and Environment Management 2(1):1-11

[18] Sudha, M., Saranya.A, Selvakumar G and Sivakumar. N. 2014. Microbial degradation of Azo Dyes: A review, International Journal of Current Microbiology and Applied Sciences .**3:** 670-690

[19] Varma and Sharma J. 2011. Analysis of physical and chemical parameters of textile waste water. Journal of International Academy of Physical Sciences, 15(2):269–276.