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Isolation and characterization of dye degrading bacteria from textile dye effluents

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ABSTRACT

Textile dye industry waste is one of the most serious problems in the environment. The dye wastes are severely deleterious to surface water bodies. The present study was an attempt for the assessment of different physico-chemical parameters such as pH, temperature, Electrical Conductivity (EC), Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total alkalinity, Total hardness and Chloride content. Therefore, the textile dye effluent degradation was herculean task because the textile effluents contain complex chemicals, highly toxic compounds and heavy metals. The experiment was carried out to degrade the dye effluents by using bacterial isolates from textile dye effluents. Five different bacterial isolates such as Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Pseudomonas fluorescens and Escherichia coli, were screened and they have capability to degrade and decolorize the textile dye effluents.

Key words: Textile dye, Effluent, Decolourization, Bioremediation and Bacteria.

INTRODUCTION

In modern life, rapid industrialization and urbanization resulted in the discharge of large amount of waste in to the environment, which in turn creates pollution. Water is essential for survival and existence of life on planet earth. The waste water and sewage are released from the industries, that wastes are entering into the water bodies, it is one of major source of environment toxicity [1], it also affect the soil micro flora and aquatic ecosystem [2]. The most environmental problem faced due to the textile dyeing industry is that the industry produces large volumes of high strength of aqueous waste effluents.

The discharge of dye effluents containing recalcitrant residue into rivers and lakes [3]. The residual dyes from different source such as textile industries, cosmetics, paper mills, pulp industries, dyeing and dye intermediates and bleaching industries, more than 80,000 tones of dyes and pigments are produced in these industries. Especially in textile industries produced more than 70% of the total quantity of waste in India [4]. India is the second largest exporter of dyestuffs and intermediates after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80%. Industrialization is vital to a nation's 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 (low biodegradability) and toxicity.

The effluent which is untreated is one of the major sources of consumed metal dyes, phenol, aromatic amines [4, 5, 6], several aromatic amines are known mutagens and carcinogens to human beings. Dyes also affect internal organ like kidney, liver, gastrointestinal tract.

The recycling of these effluents using several techniques such as chemical degradation and some physical methods. Physico-chemical methods such as adsorption, irradiation, ion exchange, oxidative process, ozonation, coagulation on have been used to decolorize textile effluent but these methods are costly, inefficient and sometimes produce hazardous by-products [7, 8], it also affects the environment during the degradation process. Extensive research in the field of biological dye effluent decolourization and degradation has shown promising results, but much of this work has been done with single model compounds [9]. However, industrial textile wastewater presents the additional complexity of dealing with unknown quantities and varieties of many kinds of dyes [10], as well as low BOD/COD ratios, and present the high amount of auxiliary chemicals and heavy metals. Therefore, the effluents produced are markedly variable in chemical composition, including organics, nutrients, sulphur compounds, salts and different toxic substances.

In biological treatment processes, various physicochemical operational parameters, such as the level of agitation, oxygen, temperature, pH, dye structure, dye concentration, supplementation of different carbon and nitrogen sources, electron donor and redox mediator, directly influence the bacterial decolorization performance of dye effluents. Thus, to make the process more efficient, faster and practically applicable, prior determination of the effect of each factor on the bacterial decolorization of dye effluents is essential. This may affect the efficiency of the biological decolourization [11].Over the past decades, biological degradation has been investigated as method to transform, degrade or mineralization dye effluents. Moreover, such decolorization and degradation is an eco-friendly method and cost comparative alternative to chemical degradation process [3]. Isolated bacteria are able to degrade dye either single (or) consortia methods. The objective of the present study is to analyze the physico-chemical characterization of textile dye effluents and isolate and characterization of dye degrading bacteria from dye effluents.

MATERIALS AND METHODS

2.1. Sample collection

The dye effluents were collected from three different dying industries in Chennai, Tirupur and Coimbatore, Tamil Nadu, India. The samples were named as, S1, S2 and S3 respectively, base on their place name. The effluent samples were collected in plastic cans. Before the sample collected the cans was rinsed tap water and distilled water.

1.1. Physico-chemical property analysis

The collected effluent samples have been analyzed to determine its physico-chemical parameters. The various parameters *viz.*, Temperature, pH, Electrical Conductivity (EC), Colour, Odour, Total dissolved solid (TDS), Total suspended solids (TSS), Chemical oxygen demand (COD), Biological oxygen demand (BOD), Dissolved Oxygen (DO), Total Hardness, Chloride, Ca Hardness and Mg Hardness were analysed in the laboratory by the standard protocol.

1.2. Isolation of dye degrading bacterial isolates from dye effluent:

The bacterial isolates present in the textile dye effluent were isolated by Serial dilution **(**Pour plate) technique. In this method, 1 ml of sample was thoroughly mixed with 9 ml of sterile distilled water, and then it was serially diluted by following standard procedure upto concentration of 10^{-6}. Then, 1 ml of serially diluted samples from each concentration of samples were transferred to sterile petriplates and evenly distributed throughout the plates and sterile unsolidified Nutrient agar was poured and it was allowed to solidify. The Nutrient agar plates were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were isolated and purified from the plates. The well grown bacterial cultures used for further screening technique and stored at 4°C.

1.3. Screening of dye degrading bacterial isolates from effluents:

Fifteen morphologically distinct bacterial isolates were tested for their ability to degrade the textile dyes. The isolated bacterial strains were screened out by incubating them on 100 ml of nutrient agar medium with 10 ml of dye effluent. The nutrient agar medium incubated at 37°C for 24 hrs. After the incubation, plates were observed for clear zone. The screened culture was transfer to agar slant and store 4°C for further study. Five morphologically distinct bacterial isolates showing more than 70% degradation of the added dye effluents. These efficient bacterial strains were selected for further studies.

1.4. Identification of selected isolates

The five selected dye degrading bacterial strains were named as DDB 1, DDB 2, DDB 3, DDB 4 and DDB 5 based on their dye degrading ability, and they were identified using morphological and biochemical properties for the standard protocol of Bergey's Manual [16].

1.5. Dye decolourization experiments

Dye decolorization experiments were carried out in three 250 ml Erlenmeyer flasks for three effluent samples. The each flask containing 100 ml of Nutrient Broth with 15 ml of dye effluents. The pH was adjusted to 7± 0.2. Then, the flasks were autoclaved at 121°C at 15 lbs pressure for 15 minutes. The autoclaved flasks were inoculated with 5 ml of bacterial inoculum of each isolates and bacterial consortium. The flasks were kept in mechanical shaker and incubated at 37 ºC for 4 days. Samples were drawn at every 24 hours intervals for observation. About 10 ml of the dye solution was filtered and centrifuged at 5000 rpm for 20 minutes. Decolourization was assessed by measuring absorbance at 510 nm of the supernatant with the help of spectrophotometer at wavelength maxima (λm) of respective dye.

1.6. Decolourization assay

Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated from the following formula,

$$\% \text{ Decolourization} = \frac{\text{Initial OD - Final OD}}{\text{Initial OD}} \times 100$$

2.8. Development of bacterial consortia

The isolates for the consortium development were selected based on three criteria; ability to degrade the dyes efficiently (> 70%), rapidly (within 3 days) and also ability to degrade a wide variety of dyes. A total of 13 consortia were developed using combinations of three to five isolates. A loopful of the selected isolates was individually inoculated into NB for 24 hrs to form a consortium. A 10% (v/v) aliquots of the culture mix were then transferred into a 250 ml Erlenmeyer flask containing 100 ml of Nutrient broth with pH adjusted to pH 7 with 15 ml of dye effluent samples and allowed to react in agitated and static conditions. The decolourization of the dye was determined as per the procedure proposed by Khadijah *et al.* [12].

RESULTS AND DISCUSSION

Table -1: Physico - chemical characterization of textile dye effluent samples

S. No	Name of the Parameters	Name of the dye effluent samples			NEQS*
		S1	S2	S3	
1	Temperature (°C)	35°C	45°C	38°C	40 °C
2	pH	7.9	9.2	8.6	6-9
3	Electrical conductivity (µS/cm)	223	248	237	80 - 450
4	Colour	Blackish blue	Blackish blue	Blackish blue	Colourless
5	Odour	Unpleasant	Unpleasant	Unpleasant	Odourless
6	Total dissolved solid (mg/l)	2187	2398	2211	3500
7	Total suspended solids (mg/l)	128	179	154	-
8	Chemical oxygen demand (mg/l)	763	856	837	156 - 400
9	Biological oxygen demand (mg/l)	178	213	210	80 - 250
10	Dissolved oxygen (mg/l)	115	157	146	-
11	Total Hardness (mg/l)	295	320	304	-
12	Chloride (mg/l)	1124	1257	1177	-
13	Ca Hardness (mg/l)	185	220	197	-
14	Mg hardness (mg/l)	56.96	70.12	65.83	-

**National Environment Quality* Standards

1.7. Physico –chemical analysis of textile dye effluents

Textile dye industrial effluents are one of major sources of environmental toxicity. It not only affects the quality of drinking water but also has deleterious impact on the soil microflora and aquatic ecosystems. Soil is the most favourable habitat for a wide range of microorganisms that includes bacteria, fungi, algae, viruses and protozoa. Industries keeps on releasing effluents which is quite toxic whether its sugar mill or fertilizer industries, or chemical treatment given to the fields also cause problems for the survival of the soil micro flora [2]. The dye effluents were collected from three dying industries in Chennai, Coimbatore and Tirupur in Tamil Nadu, India. These industries discharge the Blackish blue coloured effluents with dyes and toxic compounds into the open environment. It was found that all the dyeing industry is among those which contribute to water and soil pollution [8]. Therefore, the collected sample have been analyzed to determine their physico – chemical characteristics of the dye effluents and recommended level of NEQS was showed Table – 1. Temperature range recorded the three effluents between 35°C to 45°C. The temperature high in recommended level [3]. The maximum pH range was recorded S2 (8.2) followed by S1 (7.5) and S3 (7.9). The pH was alkaline in nature and samples have pH within the permissible limit also reported (Thoker Farook Ahmed *et al.* [2]; Mir Tariq Ahmad *et al.*[13]; Sofia Nosheen *et al.* [14]; Manikandan *et al.*

[3]. Electrical conductivity is commonly used as a measure of salinity of waste water also reported Thoker Farook Ahmed *et al.*, [2]; Mir Tariq Ahmad *et al.*, [13]; Sofia Nosheen *et al.* [14]. During the present study, the maximum electrical conductivity were recorded at S2 (248 µS/cm) followed by S1 (223 µS/cm) and S3 (237 µS/cm) which was also recommended of NEQS level. In the present study, the dye effluents have different dark colours and unpleasant odour [3].

The maximum values of total dissolved solid were obtained from S2 (2398 mg/l) followed by S1 (2187 mg/l) and S3 (2211 mg/l) respectively. High concentration of dissolved solids affects the density of water and influences solubility of gases in water (like oxygen) and osmoregulation of freshwater organisms [2,4,14]. Thoker Farook Ahmed *et al.* [2]; Arminder Kaur *et al.*, [1] also reported the TSS in textile dye effluent. The minimum total suspended solids were recorded at S1 (128 mg/l) while the maximum at S2 (179 mg/l) followed by S3 (154 mg/l). The maximum chemical oxygen demand were observed from S2 (856 mg/l) followed by S1 (763 mg/l) and S3 (837mg/l) which was much higher than maximum recommended limit of FEQS, it's impacted the receiving water-body to some extent and its effects on the quality of freshwater and subsequently cause harm to aquatic life [1,2,3,4,14]. The maximum biological oxygen demand were observed from S2 (213 mg/l) followed by S1 (178 mg/l) and S3 (210 mg/l) was recorded and its shows high level of BOD present in effluents, which above the recommended level [1,4].

Dissolved oxygen is a fundamental requirement for aquatic life [14]. The maximum dissolved oxygen were recorded at S2 (157 mg/l) followed by S1 (115 mg/l) and S3 (146 mg/l). The effluent waste discharge to surface water source is largely determined by oxygen balance of the system also recorded (Thoker Farook Ahmed *et al.* [2]; Manikandan *et al.* [3]). Total Hardness is the property of water which prevents the lather formation with soap and increases the boiling point of water. Hardness of water mainly depends upon the amount of calcium and magnesium salts and chloride [2]. The maximum hardness were recorded from S2 (320 mg/l) followed by S1 (295 mg/l) and S3 (304 mg/l). The maximum Chloride concentration was observed in S2 (1257 mg/l) as compared to other parameters like Ca (220 mg/l) and Mg (70.12 mg/l) in S2. The minimum values of chloride, Ca and Mg recorded in other two samples like S1 (1124 mg/l), (185 mg/l) and (56.96 mg/l) and S3 (1177 mg/l), (197 mg/l) and (65.83 mg/l) respectively, the Chloride occurs in all natural waters in widely varying concentrations. Excessive chloride in potable water is not particularly harmful microflora of aquatic life [4].

1.8. Identification and characterization of bacteria isolated from textile dye effluent

The degradation of dye effluents used several physical, chemical and biological methods. The physico-chemical methods are economic limited [9]. On the other hands, the biological methods are more effective and low expensive of treatment and amenability to scale up easily are the merits of biological methods. The present study was focused on biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. Therefore, fifteen different bacterial isolates were isolate from three dye effluent samples. Among the fifteen bacteria, the five bacteria are more effective against three effluents samples. Five different dye degrading bacteria of DDB1, DDB2, DDB3, DDB4 and DDB5 identified as *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Escherichia coli* respectively. The characteristics of the identified bacterial isolates were furnished in Table – 2.

Table – 2: Morphological and Biochemical Characterization of Screened Isolated Bacteria

(Positive +, Negative -)

S.No.	Characters	DDB 1	DDB 2	DDB3	DDB 4	DDB 5
1	Gram straining	-	+	+	-	-
2	Morphology	Rod	Rod	Rod	Rod	Rod
3	Motility	+	+	+	+	+
4	Spore	-	+	+	-	-
5	Indole	+	-	-	-	+
6	Methyl Red	-	-	-	+	+
7	Voges-Proskauer	+	-	+	+	-
8	Citrate test	+	+	+	-	-
9	Catalase test	+	+	-	+	+
10	Oxidase test	+	-	-	+	-
11	Nitrate Reduction test	+	-	-	-	+
12	Urease	+	-	-	-	-
13	Fermentation (Glucose)	+	+	+	-	+
14	Fermentation (Lactose)	-	+	-	-	+
15	Fermentation (Sucrose)	-	+	+	-	+
16	H_2S Production	-	-	+	+	+

Saranraj *et al.* [15] isolated five different bacterial isolates from the textile dye effluent sample and identified as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Escherichia coli.* Recently, Sriram *et al.* [8] isolated three different bacteria *viz.*, *Bacillus* sp., *Escherichia coli* and *Pseudomonas*

fluorescens were isolated from the textile dye effluent were investigated for the potential of textile effluent adapted bacteria in decolourizing it. *Bacillus* and *Pseudomonas fluorescens* were found to have use in effluent treatment.

Table – 3: Screening of bacterial isolates for dye degradation by plate assay

S.No.	Bacterial isolates	Zone formation (in mm)		
		S1	S2	S3
1	*Pseudomonas aeruginosa*	36	35	35
2	*Bacillus cereus*	35	33	32
3	*Bacillus subtilis*	34	32	31
4	*Pseudomonas fluorescens*	33	30	29
5	*Escherichia coli*	30	28	26

1.9. Screening of bacterial isolates for the decolourization of textile dye effluents by Plate assay

The bacterial strains exhibiting strong decolourizing activity was also investigated Hassan *et al.* [16]. The bacterial isolates were screened for the decolourization of dye effluents by Plate assay and the results were tabulated in Table – 3. The identified bacterial isolates *viz., Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Pseudomonas fluorescens,* and *Escherichia coli* were used for Plate decolourization assay. Maximum decolourization was recorded by *Pseudomonas aeruginosa* in the plate containing each three dye effluent samples, followed by *Bacillus cereus, Bacillus subtilis, Pseudomonas fluorescens* and *Escherichia coli* zone inhibition were recorded in the each three dye effluent plat. Rashid Mahmood *et al.* [18]; Karthikeyan and Anbusaravanan [17] reported the isolation and screening of microorganisms capable of decolourizing various azo dyes from sludge samples collected from wastewater treatment sites contaminated with dyes.

Table- 4: Decolourization of textile dye effluents by bacterial isolates

S.No	Bacterial isolates	% decolourization		
		S1	S 2	S3
1	*Pseudomonas aeruginosa*	72.12	70.33	69.45
2	*Bacillus cereus*	70.45	68.76	67.87
3	*Bacillus subtilis*	69.10	67.11	66.34
4	*Pseudomonas fluorescens*	68.00	66.98	65.09
5	*Escherichia coli*	66.12	65.33	64.65

1.10. Decolourization of textile dye effluents by bacterial isolates

The decolourization of textile dye effluents by five bacterial isolates *viz., Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Pseudomonas fluorescens* and *Escherichia coli* and bacterial consortium was studied and the results were showed in Table – 4. Maximum decolourization percentage was observed in the medium inoculated with bacterial consortium against the each three dye effluents of S1, S2 and S3, followed by *Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Pseudomonas fluorescens,* and *Escherichia coli* degradation percentage was recorded in three samples. Similar study was also carried out by Saranraj *et al.* [15]. They investigated the decolourization and degradation of direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. They isolated five different bacterial species from the textile dye effluent sample and the isolates were identified as *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli.*

Table – 5: Dye decolourization efficiency of bacterial consortia after 72 hours inoculation

S. No.	Consortium Number	Combination of isolates	% dye effluents decolourization (After 72 hrs)		
			S1	S2	S3
1	BC1	All Five Isolates	98.34	97.56	98.08
2	BC2	DDB1+DDB2+DDB3+DDB5	89.02	87	88.56
3	BC3	DDB4+DDB2+DDB3+DDB5	88.88	86.76	89.32
4	BC4	DDB2+DDB1+DDB4+DDB5	87.78	85.12	86.34
5	BC5	DDB3+DDB1+DDB4+DDB5	86.02	85.96	86.07
6	BC6	DDB1+DDB4+DDB5	78.12	75.43	76.34
7	BC7	DDB2+DDB3+DDB5	77.45	74.07	75.67
8	BC8	DDB1+DDB4+DDB2	87.44	86.34	87.23
9	BC9	DDB1+DDB4+DDB3	88.27	87	87.04
10	BC10	DDB2+DDB3+DDB1	87.09	86	87.78
11	BC11	DDB2+DDB3+DDB4	88.67	86.98	87.66

1.11. Dye degradation efficiency of bacterial consortia

Rashid Mahmood *et al.* [18] developed bacterial consortia for the decolorization of effluent, sludge and affected soil. They worked together and gave better results. The consortia were developed from bacteria. They are four consortia were developed using combinations of bacterial isolates. In this study, a total of 11 consortia were developed using

combinations of three to five isolates. A consortium based on the five isolates named BC1 was first tested for its dye degrading ability to three dyes effluent samples namely, S1, S2 and S3 were degraded recorded at 98.34%, 97.56% and 98.08% respectively; it's higher than other consortium. The minimum degradation percentages were recorded in consortia BC7 in three samples at 78.12%, 74.07% and 75.67% respectively. The results are showed in Table - 5. The development of consortium for dye degradation also recorded Khadijah *et al.* [12].

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