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BIOREMEDIATION OF TEXTILE DYE EFFLUENT BY *BACILLUS* AND *PSEUDOMONAS* SPP.

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Abstract: Textile effluent is one of the known sources of pollution, which usually contain several chemicals, which contaminate the receiving water. This study was conducted to understand the ability of *Bacillus* sp., and *Pseudomonas* sp., on decolourization and normalization of other physicochemical parameters, such as odour, temperature, pH, total solids, total dissolved solids, total suspended solids, total hardness, BOD, COD, calcium, magnesium and chlorine of textile dye effluent. The textile dye discharge was collected from a mill effluent outlet, Uppilipalayam, Erode, India, and subjected to biological characterization for the presence of total heterotrophic bacteria by serial dilution, and cultured on nutrient agar plates. The presence of *Bacillus* and *Pseudomonas* spp., were identified biochemically. The results indicated that *Bacillus* sp., have higher colour removing capacity and the ability to bring back normalcy in the physicochemical parameters of effluent than that of *Pseudomonas* sp.
Keywords: Textile dye effluent, *Bacillus* sp., *Pseudomonas* sp., Decolourization.

1. Introduction

Synthetic dyes are widely used in a number of industries, such as textile dyeing or printing, paper printing, cosmetics, pharmaceuticals and leather industries. These industries consume large amounts of energy and water, and they release a substantial amount of waste water with many contaminants [1]. With the increased demand for textile products, the waste waters generated from this industry have also increased proportionally and polluting the receiving water worldwide [2]. In India, an average mill discharge is about 1.5 million litre of contaminated effluent per day, which contains several types of toxic chemicals, such as dispersants, levelling agents, acids, alkalis, carriers and various dyes. The organic dye stuffs, chrome dyes and other chemicals during various operations and produce a large quantity of solid and liquid waste containing hexavalent chromium [chromium (VI)], salts of zinc,

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sulphates, copper, sodium and potassium etc., which causes severe toxicity to aquatic organisms [3, 4].

Concentration of chromium (VI), zinc, lead and cadmium should not exceed 0.05, 3.0, 0.015, 0.005 mg/l respectively in drinking water [5-7]. In Indian context the discharge concentration of chromium should not exceed from 0.1 mg/l as per waste water discharge standard of Central Pollution Control Board [8]. Currently, chromium, zinc, and copper pollution in the ground waters due to the environmental impacts of industrial effluent irrigation from a tanning industrial cluster at Bangalore, India has been reported [9]. This problem alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colourations [10-12]. The use of these water resources is limited and the ecosystem is also affected by microbial populations and can be toxic, mutagenic and/or carcinogenic to animals [13].

Several methods are used to treat textile effluents, which include physico-chemical methods, such as filtration, coagulation, activated carbon and chemical flocculation [14]. These methods are expensive and create a secondary disposal problem, whereas microbial degradation is an environmental friendly and cost competitive alternative to chemical treatment [15-17]. Some bacterial strains, such as *Bacillus cereus, Pseudomonas putida* and *Pseudomonas fluorescence* [18], *Pseudomonas desmolyticum* [19] and *Bacillus* sp. [20] have been used in the biodegradation of azo dyes. These microbial consortia were recommended for environmental remediation to degrade variety of pollutants [17-21]. Therefore, this study was aimed to evaluate the ability of microbial isolates (*Bacillus* sp. and *Pseudomonas* sp.) in decolourization of dye-effluent generated from local textile industry.

2. Materials and Methods

2.1. Textile dye effluent sample

The dye effluent was collected in screw capped sterilized bottles from a textile mill discharge point, Uppilipalayam, Erode, Tamil Nadu, India. The samples were brought to the laboratory with ice pack in a cooler box and stored at 4^{o}C.

2.2. Isolation and identification of bacteria from the textile dye effluent

Nutrient agar medium (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was used for the isolation of bacteria from the dye effluent. Serial dilutions from 10^{-1} to 10^{-7} were prepared by pipette out appropriate amount of distilled water suspension in 1 ml of dye effluent sample. The nutrient agar plates were prepared and labelled. Then 0.1 ml of aliquot from 10^{-6} and 10^{-7} dilutions was pipette out into the corresponding nutrient agar plate. The sample was spread

on the agar plate using the L-Rod (spread plate technique) and incubated at 37°C for 24 h. After incubation, bacterial colonies were observed. All colonies were isolated, sub-cultured and identified by several morphological and biochemical (Gram's Staining, Motility Test, Indole Test, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test, Starch Hydrolases, Gelatin Hydrolases, Casein Test, Urea Test) methods [22].

2.3. Determination of effluent decolourization

Different aliquots of textile effluent were taken in Erlenmeyer flasks (20, 40, 60 and 80 ml) in triplicates. 2.5 ml of 12 h old *Bacillus* sp. culture was added in each Erlenmeyer flask (cotton plugged and labelled). Similarly in another set of Erlenmeyer flasks containing similar quantity of effluent, 2.5 ml of *Pseudomonas* sp. culture was added. All the Erlenmeyer flasks were mixed thoroughly and incubated at room temperature for decolourization for 24 to 48 h. During the incubation period, samples were drawn at different time intervals. After 48 h, samples were withdrawn, centrifuged at 5000 rpm at 4^{o}C for 10 minutes using cooling centrifuge (REMI C-24BL). The decolourization potential of the isolate was determined by taking the absorbance reading of the cell free supernatant using Spectrophotometer (ELCO SL150) at 700 nm. The extent of decolourization was expressed as percent (%) decolourization and estimated as (Ai - At) / Ai × 100, where initial absorbance of the dye solution and absorbance at cultivation time denoted by Ai and At respectively.

2.4. Analysis of physicochemical parameters of the dye effluent

The physicochemical characterization of the effluent was made (before and after treatment with *Bacillus* sp. and *Pseudomonas* sp.) by analyzing various parameters, viz., Colour, Odour, Temperature, pH, Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Hardness (TH), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Calcium, Magnesium and Chloride. Water testing kits from HiMedia Laboratories Pvt. Ltd., Mumbai, India were used for the analysis of the physicochemical parameters of the effluent. The pH was determined by electronic digital pH meter (Model-Century CP901).

3. Results and Discussion

3.1. Bacterial species isolation from the textile dye effluent

In the present study, metal resistant bacterial strains were isolated from textile dye effluent on the basis of morphological and biochemical characteristics, it was tentatively identified as *Bacillus* sp. and *Pseudomonas* sp. (Table 1; Plates 1 and 2). Bioremediation is widely used to clean up both soil and waste water containing organic and inorganic contaminants [23-27].

Bacteria are omnipresent in nature with high resistance cell walls that are anionic which can fix metal and provide sites for nucleation and growth of minerals [28]. It has been reported that five different bacterial species, *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae and Escherichia coli* were isolated from the textile dye effluent [29]. It has also been reported that three different bacterial species, *Bacillus* sp.*, E. coli* and *P. fluorescens* were isolated from textile dye effluent contaminated soil, and used for the degradation of dye [30].

3.2. Decolourization

The bacterial isolates, *Bacillus* sp. and *Pseudomonas* sp. were found to have significant potential to decolorize the dye effluent, and their ability measured were given in table 4. In the present study, *Bacillus* sp., decolourization ability was 92-97%, whereas, *Pseudomonas* sp., produced 87-95%. In this study, *Bacillus* sp., has comparatively higher decoloration ability than *Pseudomonas* sp., The reason for effective and faster decolourization of the effluent by bacteria might be associated with the metabolic activities and interactions of these strains [31-36]. The bacteria, *Lysinibacillus* sp. require 96 h [37] and *Phanerochaete sordia* require 48 h [38] for optimum decolourization of azo dyes.

3.3. Physicochemical characteristics

In the present study, physicochemical analysis, pH, temperature, TS, TDS, TSS, TH, BOD, COD, calcium, magnesium and chloride were decreased when treated with *Bacillus* sp. and *Pseudomonas* sp. (Table 2). The reduction in concentrations of TDS and TSS were reported in the textile effluent due to degradation of these solids by the bacteria present in the consortium (*Providencia* sp. SDS and *P. aeuroginosa* BCH strains) [36]. The reduction in COD and BOD after treatment with consortium BMP1 was consequent of the removal of organic load from effluent and ultimately the toxicity [39]. The high levels of COD in the textile effluent indicated the toxicity level of pollution [40], which is very harmful for the whole ecology of aquatic ecosystem of the receiving water bodies. The concentration of the solids in textile effluent was another matter of concern and the carcinogenic effect of the dyes adds to it. In this study, the reduction of pollution parameters indicates the fact that the process of bioremediation carried out by *Bacillus* sp.*,* and *Pseudomonas* sp.

4. Conclusion

The results of the present study indicated that *Bacillus* sp.*,* and *Pseudomonas* sp., can be used for the treatment of effluent contaminated waters.

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Table 1. Biochemical characterization of bacterial isolates from textile dye effluent.

Characteristics	*Bacillus* sp.	*Pseudomonas* sp.
Source	Dye industrial waste	Dye industrial waste
Colony Morphology	Enumerated	Strikingly mucoid colonies
Cell Morphology	Straight rods	Slightly curved
Gram's Staining	+	-
Motility test	+	+
Indole Test	-	-
Methyl Red Test	-	+
Voges-Proskauer Test	-	+
Citrate Utilization Test	-	-
Starch Hydrolases	+	-
Gelatin Hydrolases	-	+
Casein Test	-	+
Urea Test	-	+

+, Positive; -, Negative.

Table 2. Decolourization of textile dye effluent after treatment with *Bacillus* sp. and *Pseudomonas* sp.

Concentration of dye effluent	Bacterial spp.	Hours		
		12 h	24 h	48 h
20%	*Bacillus* sp.	78.42±0.46	83.29±0.68	97.22±0.34
	Pseudomonas sp.	68.98±0.59	75.21±0.78	95.13±0.68
40%	*Bacillus* sp.	73.28±0.73	76.07±0.63	96.96±0.45
	Pseudomonas sp.	64.35±0.42	73.83±0.27	93.32±0.45
60%	*Bacillus* sp.	69.93±0.38	73.26±0.22	94.36±0.28
	Pseudomonas sp.	62.55±0.49	69.64±0.26	88.90±0.24
80%	*Bacillus* sp.	65.01±0.43	71.36±0.30	91.64±0.51
	Pseudomonas sp.	57.58±0.21	68.87±0.32	86.74±0.38

All values are mean ± SD (n=3).

Table 3. Physicochemical characterization of textile dye effluent before and after treatment with *Bacillus* sp. and *Pseudomonas* sp.

Characteristics	Textile effluent before treatment	Textile effluent after treatment with *Bacillus* sp.	Textile effluent after treatment with *Pseudomonas* sp.
Colour	Black	Colourless	Colourless
Odour	Disagreeable	Odourless	Odourless
Temperature (ºC)	30 ºC	29 ºC	29 ºC
pH	8.90±0.17	7.86±0.05	8.18±0.12
TS (g/L)	29.823±1.215	19.276±1.096	19.695±1.160
TDS (g/L)	28.845±1.106	18.756±0.923	18.990±1.050
TSS (g/L)	1.000±0.071	0.519±0.063	0.704±0.067
Total hardness	1.125±0.036	0.705±0.032	0.726 ±0.029
BOD (g/L)	0.472 ±0.009	0.273±0.005	0.280±0.008
COD (g/L)	2.360±0.016	1.435±0.012	1.443±0.015
Calcium (mg/L)	810.87±7.04	520.41±5.67	536.73±6.18
Magnesium (mg/L)	763.06±6.74	490.41±5.49	503.82±5.91
Chloride (mg/L)	710.47±6.59	460.75±4.97	469.92±5.78

All values are mean ± SD (n=3). TS, Total Solids; TDS, Total Dissolved Solids; TSS, Total Suspended Solids; BOD, Biochemical Oxygen Demand; COD, Chemical Oxygen Demand.

Plate – I. Colony morphology of *Bacillus* sp., and *Pseudomonas* sp., on agar plate

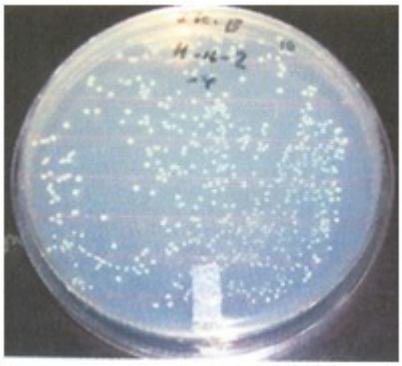

Fig.1. *Bacillus* sp.

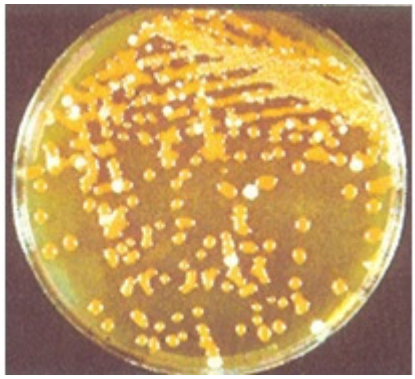

Fig.2. *Pseudomonas* sp.

Plate – II. Gram stained cell morphology of *Bacillus* sp., and *Pseudomonas* sp.,

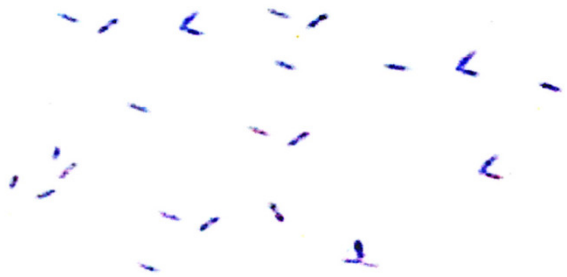

Fig.1. Gram positive and straight rod shaped *Bacillus* sp.

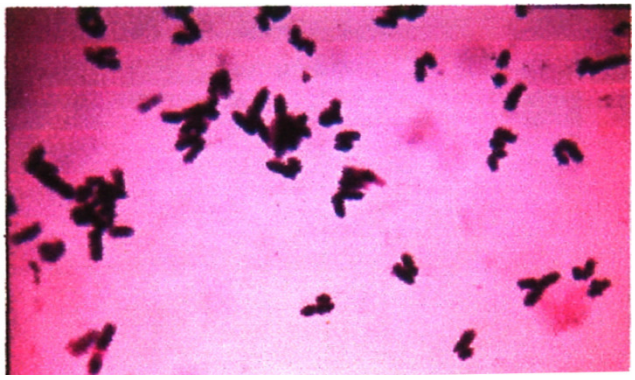

Fig.2. Gram negative and slightly curved *Pseudomonas* sp.