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Physico Chemical Characterization of Textile Effluent and Screening for Dye Decolorizing Bacteria

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Abstract: The physicochemical characterization of the textile industry effluent collected from Tiruppur in Tamil Nadu, India and the surrounding soil sediments were been carried out and the results for the textile effluent showed the high rates of temperature (41°C), pH (8.9) and EC (3.65 μs/m), BOD (485.5 mgl^{-1}), COD (876 mgl^{-1}), TSS (15398.5 mgl^{-1}), TDS (3672.5 mg l^{-1}), heavy metal ions, Total hardness (Ca^{2+}, Mg^{2+}, Cl^{-} & SO_4^{2-}) and colour over the prescribed fresh water limits. The soil sediments collected from the surrounding area of textile industry effluent was been analysed. The soil pH (8.3) was found to be moderately alkaline. Out of the 30 bacterial strains isolated from the effluent sample, 3 isolates showed the potential for decolorizing textile dye Reactive RED 5B employed in the industry. Based on the morphological and biochemical characteristics the 3 strains designated as VITEF1, VITEF2 and VITEF3 were identified as to belong to the genus Bacillus *sp.* Accounting to the ability of VITEF1, VITEF2 and VITEF3 to decolorize the textile dye, these strains could be used to decolorize and degrade different dyes of textile industry.

Key words: Textile Industry Effluent · Bacteria · Decolourization · Dyes

INTRODUCTION

Increasing urbanization and industrialization have led to a dramatic increase in the intensity of wastewater produced around the world. With the increased demand for textile products, the textile industry and its wastewaters have been increasing proportionally, making it one of the main sources of severe pollution problems worldwide [1, 2]. The chemical reagents used in textile industries are very diverse in chemical composition, ranging from inorganic compounds to polymers and organic products [3]. Waste water generated by different production steps of a textile mill have high pH, temperature, detergents, oil, suspended and dissolved solids, dispersants, leveling agents, toxic and non-biodegradable matter, colour and alkalinity. Wastewater from fabric and yarn printing and dyeing pose serious environmental problems both because of their colour and high COD and BOD [4]. Important pollutants in textile effluent are mainly recalcitrant organics, color, toxicants and surfactants, chlorinated compounds (AOX). The textile industry generally has difficulty in meeting wastewater discharge limits, particularly with regard to dissolved solids, ionic salts, pH, COD, color and sometimes heavy metals [5]. The most common textile-processing set up consists of desizing, scouring, bleaching, mercerising and dyeing processes. Dyeing is the process of adding colour to the fibres, which normally requires large volumes of water not only in the dyebath, but also during the rinsing step. Depending on the dyeing process, many chemicals like metals, salts, surfactants, organic processing assistants, sulphide and formaldehyde, may be added to improve dye adsorption onto the fibres. There are more than 8000 chemical products associated with the dyeing process named in the colour index while over 100,000 commercially available dyes exist with over 7×10^5 metric tons of dyestuff produced annually [6].

Dyes are classified according to their application and chemical structure. They are composed of a group of atoms responsible for the dye colour, called chromophores, as well as an electron withdrawing or donating substituents that cause or intensify the colour of the chromophores, called auxophores[7]. The most important chromophores are azo (-N=N-), carbonyl (-C=O), methane (-CH=),nitro ($-NO_2$) and quinoid groups.

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The most important auxochromes are amine (-NH_3), carboxyl (-COOH), sulfonate (-SO_3H) and hydroxyl (-OH). The auxochromes can belong to the classes of reactive, acid, direct, basic, mordant, disperse, pigment, vat, anionic and ingrain, sulphur, solvent and disperse dye[8]. To obtain the target colour, normally a mixture of red, yellow and blue dyes were applied in the dyebaths. These three dyes do not necessarily have the same chemical structure. They might contain many different chromophores, in which azo, anthraquinone and phthalocyanine dyes are the most important groups [9]. The current state of the art for the treatment of wastewaters containing dyes is physicochemical techniques, such as adsorption, precipitation, chemical oxidation, photodegradation, or membrane filtration [10, 11]. All of these have serious restrictions as economically feasible methods for decolorizing textile wastewaters such as high cost, formation of hazardous by-products or intensive energy requirements [12]. This has resulted in considerable interest in the use of biological systems for the treatment of wastewaters. Extensive studies have been carried out to determine the role of the diverse groups of bacteria in the decolourization of different textile dyes. Pure bacterial strains, such as *Pseudomonas luteola*, *Aeromonas hydrophila*, *Bacillus subtilis, Pseudomonas sp.* and *Proteus mirabilis* decolorized dyes under anoxic conditions [13]. Several bacterial strains that can aerobically decolorize dyes have been isolated during the past few years. Many of these strains require organic carbon sources, as they cannot utilize dye as the growth substrate [14]. The Objectives of the present study were: 1) Physico-chemical characterization of the textile effluent and the contaminated soil sediments for and 2) to screen for bacteria with the potential to decolorize various textile dyes.

MATERIALS AND METHODS

Sampling and Analysis of Effluent

Sampling: Tiruppur, the leading cotton knitwear industrial cluster in Tamil Nadu, South India (Lat.11°6' 0" N, Long. 77°. 21' 0" E) was chosen for effluent sample collection. The Effluent sample was collected from the middle point of the area. Standard procedures (Spot and Grab) were followed during sampling. The pH and Temperature were determined at the sampling site. The pH was determined by using pH meter (Hanna digital pH meter, model-671-p) and temperature with laboratory thermometer. The sample was transported to laboratory at 4°C as in accordance with the standard methods [15].

Analysis of the Effluent: The remaining physicochemical parameters (Colour, Electrical Conductivity (EC), Chemical Oxygen Demand (COD), Biological Oxidation Demand (BOD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), heavy metal ions) were determined as soon as the sample was brought to the laboratory. Sample colour was analysed by U-3010 spectrophotometer (Hitachi, Japan) while Electrical Conductivity (EC) was determined by conductivity meter (Jenvway EC meter, model-4070).BOD was determined by employing evaporation method by DO meter while COD was measured by COD instrument directly. Chloride and Sulphate contents were assessed by titrimetric and turbidity method, respectively [16]. While phenolic compounds were determined by photometric method. Analysis of different metal ions in the effluent sample was determined by Atomic Absorption Spectrophotometer (AAS) as per the standard methods.

Chemicals: The textile dye Reactive Red 5B was obtained from the Winner Dyeing Industry in Tiruppur, Tamil Nadu. A stock solution of the dye (1000 mg L^{-1}) was prepared in de-ionized water and used for all studies.

Isolation, Screening and Identification for Dye Decolorizing Bacteria from Effluent: Effluent sample collected from the textile mill was screened for the isolation of potential dye decolorizing bacterial strains. Sample was serially diluted in sterile distilled water and plated onto Luria Bertani Agar (g L^{-1} Casein enzymic hydrolysate-10, yeast extract-5, NaCl-10, Agar-15) and then incubated for 48h at 30°C [17]. Discrete bacterial colonies that developed on agar plates were initially grouped on the basis of pigmentation, colony morphology followed by gram staining and motility. Selected bacterial isolates were further purified and sub-cultured. The pure cultures were identified based on their biochemical activity and by Bergey's Manual of determinative Bacteriology.

Screening for Dye Decolorizers: Of the thirty morphologically distinct strains isolated from the textile effluent, only 3 isolates (VITEF1, VITEF2 and VITEF3) were found to possess the ability to decolorize the dye Reactive Red 5B. The Screening for the bacterial isolates was carried out on the Screening medium(SM) with the following composition gL^{-1} Casein enzymic hydrolysate-10, yeast extract-5, Nacl-10. A loop full of culture from the slant was inoculated into 100ml.of sterilized screening medium in 250ml.Erlenmeyer flask supplemented with filter sterilized (0.22-μm) Reactive Red 5B (20mgL^{-1}) and incubated on rotary shaker (130rpm) at 30°C for 96h [18]. At regular intervals, 5ml.sample was withdrawn aseptically and centrifuged at 10,000 rpm for 20min.The cell free supernatant was used to determine the percentage decolorization of Reactive Red 5B.

Identification mad Characterisation of VITEF1, VITEF2 and VITEF3: The identification and characterisation of the bacterial isolates VITEF1, VITEF2 and VITEF3 was done by gram staining, motility, presence of spores, spore position and spore morphology and by following biochemical tests as described in Bergey's manual of determinative bacteriology (Indole, Methyl Red, Voges-Proskauer test, Citrate, Catalase, Oxidase, Nitrate Reduction test, Hydrolysis of Casein, Starch, Urea and Gelatine). Assimilation of various sugars such as D-glucose, D-fructose, galactose, mannitol and D-maltose as sole carbon source was determined by inoculating the isolates into carbohydrate broth supplemented with respective carbon source. After inoculation the tubes were incubated at 37°C for 24-48h.

Decolorization Assay: The decolorizing activity was expressed in terms of percentage decolorization and was determined by monitoring the decrease in absorbance at absorption maxima (ëmax.) of the dye (i.e.513nm. for Reactive Red 5B). The uninoculated Screening Medium supplemented with respective dye was used as reference [19]. Decolourization activity (%) was calculated by the formula:

$$\%\ \text{decolourization} = \left\{\frac{\text{Initial Absorbance} - \text{Final Absorbance}}{\text{Initial Absorbance}}\right\} \times 100$$

Physico-Chemical Characterization of Soil Samples: Total Four soil samples were collected from the textile effluent contaminated sites and were analyzed for various physico-chemical parameters like pH, colour, organic matter, electrical conductivity, Cation Exchange Capactiy (CEC), K saturation, Ca saturation, Mg saturation and Na saturation. The various Micro nutrients (Iron, Manganese, Copper, Boron and Zinc) and Macro nutrients (Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sulphur and Sodium) have been also determined. The obtained data was analyzed using one-way ANOVA.

Statistical analysis: The results obtained from the physico-chemical analysis of four replicates of soil sediments have been expressed in terms of mean (average) and standard error (S.E). Data was statistically defined by one-way ANOVA using Microsoft excel. Results in each experiment were interpreted depending upon probabilities. Probability (p-value) was less than 0.05 which was found to be significant.

RESULTS

The effluent sample collected from the Winner Dyeing Industry in Tiruppur, Tamil Nadu, India, was black in colour, with pungent smell and pH of 8.9 which was within the permissible limits. The temperature of the effluent was very high (41°C). Electrical Conductivity (EC) (μs/m) of the effluent was quite low (3.65 μs/m). Total Suspended Solids (TSS) in the textile effluent was very high (15398.5 mg l^{-1}). Total Dissolved Solids (TDS) was also high in the sample (3672.5 mg l^{-1}). There was a high load of Chemical Oxygen Demand (COD) (876 mg l^{-1}) and Biological Oxygen Demand (BOD) (485.5 mg l^{-1}) in the collected sample. A high value of BOD and COD will cause depletion of Dissolved oxygen in water. The analysis of the effluent for heavy metals had shown their amounts to be considerably high Cu^{2+} (3.98 mg l^{-1}), Cd^{2+} (0.6 mg l^{-1}), Zn^{2+}(1.1 mg l^{-1}), Fe^{2+}(6.78 mg l^{-1}), Cr^{3+}(1.46 mg l^{-1}), Mn^{2+}(5.1 mg l^{-1}), Ni^{2+}(0.6 mg l^{-1}), Pb^{2+}(0.33 mg l^{-1}). The Total Hardness of water which is a combined effect of Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) metal cations present in effluent was also found to be high. The Chloride (Cl^-) content was found to be remarkably high (1830 mg l^{-1}) in the effluent where as the sulphate ion (SO_4^{2-}) content was found to be within permissible limits. The textile effluent showed phenolic contents greater than 0.1ppm which are though permissible but still toxic (Table 1). Different bacterial strains isolated from the textile effluent were screened for their ability to decolorize textile dye and the potential strains were morphologically and biochemically characterized for identification. The bacterial count (CFU/ml) was significantly high (11.2×10^5 CFU/ml).

In the present study all the 30 strains obtained from the textile effluent were screened for dye decolorization activity and 3 isolates (VITEF1, VITEF2 and VITEF3) were selected for the decolorization study based on their higher potential to decolorize the dye Reactive RED 5B. The percentage Decolorization of VITEF1 was found to be 60%, where as VITEF2 and VITEF3 exhibited percentage decolorization of about 57% and 58% respectively in 96h (Table 2). The gram staining test showed that all three isolates are gram positive, rod-shaped bacteria. Motility was found in VITEF1 and VITEF2 where as VITEF3 was found to be non-motile. The presence of spores was found in all 3 isolates. The spore position differed in

Table 1: Physicochemical characterization of the Textile Effluent

S.No	Parameter	Units	Effluent Sample
1.	Colour	-	Black
2.	Smell	-	Pungent
3.	Temperature	° C	41
4.	pH	-	8.9
5.	EC	µs/m	3.65
6.	TSS	$mg\ l^{-1}$	15398.5
7.	TDS	$mg\ l^{-1}$	3672.5
8.	COD	$mg\ l^{-1}$	876
9.	BOD	$mg\ l^{-1}$	485.5
10.	Cu^{2+}	$mg\ l^{-1}$	3.98
11.	Cd^{2+}	$mg\ l^{-1}$	0.6
12.	Zn^{2+}	$mg\ l^{-1}$	1.1
13.	Fe^{2+}	$mg\ l^{-1}$	6.78
14.	Cr^{3+}	$mg\ l^{-1}$	1.4
15.	Mn^{2+}	$mg\ l^{-1}$	5.1
16.	Ni^{2+}	$mg\ l^{-1}$	0.6
17.	Pb^{2+}	$mg\ l^{-1}$	0.33
18.	Ca	$mg\ l^{-1}$	28.00
19.	Mg	$mg\ l^{-1}$	6.00
20.	Cl^{-}	$mg\ l^{-1}$	1830
21.	SO_4^{2-}	$mg\ l^{-1}$	247
22.	Phenol	$mg\ l^{-1}$	0.150
23.	Bacterial Count	CFU/ml	11.2×10^5

Table 2: Percentage decolorization of Reactive RED 5B (20mg L^{-1}) by Bacterial Isolates

	Decolorization%			
Isolate	24h	48h	72h	96h
VITEF1	31	43	56	60
VITEF2	27	41	52	57
VITEF3	33	45	50	58

Table 3: Morphological and Biochemical Characteristics of Isolates VITEF1, VITEF2 and VITEF3

Character	VITEF1	VITEF2	VITEF3
Gram staining	+	+	+
Morphology	Rods	Rods	Rods
Motility	+	+	-
Spore	+	+	+
Spore position	Terminal	Central	Terminal
Spore shape	ellipsoidal	Oval	ellipsoidal
Indole	-	-	-
Methyl Red	-	+	-
Voges-Proskauer	-	-	-
Citrate test	-	-	-
Catalase test	-	-	-
Nitrate Reduction test	-	-	-
Oxidase test	-	+	-
Hydrolysis of:			
casein	-	+	-
gelatin	-	-	-
starch	-	+	-
urea	-	-	-
Acid from:			
D-Maltose	+	+	+
Mannitol	+	+	+
D-Glucose	+	+	+
D-Fructose	+	+	+
Galactose	+	+	+

+, Positive; -, Negative

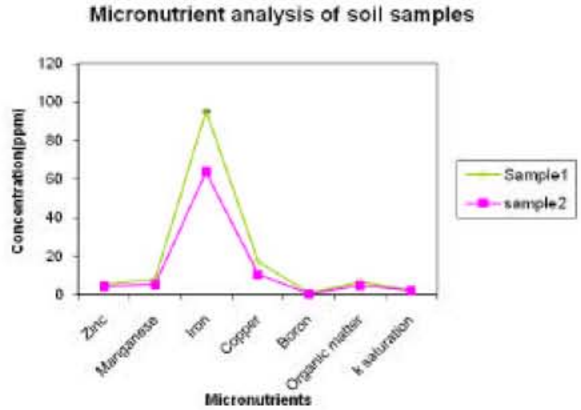

Fig. 1: Micronutrient analysis of Soil Samples
Micronutrient levels in the Textile Effluent contaminated soil sediments shows very low levels of Zinc (Zn), Manganese (Mn), Boron (B), Potassium (K) and Organic matter. Shows high levels of Iron (Fe).

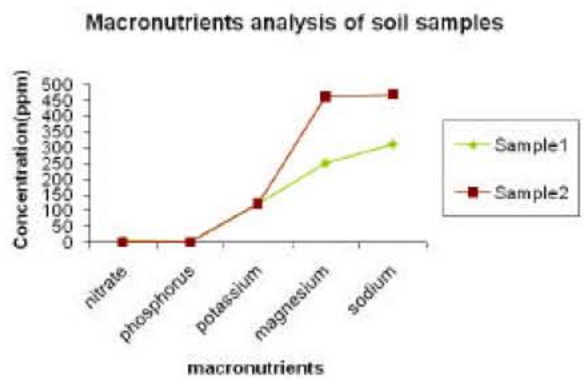

Fig. 2: Macronutrient analysis of Soil Samples.
Macronutrient levels in the Textile Effluent contaminated soil sediments shows very low levels of Nitrate and Phosphorus. Levels of Potassium, Magnesium and Sodium are high.

these isolates with VITEF1 and VITEF3 having terminal spores and VITEF2 having a central spore. The spore shape was found to be ellipsoidal in VITEF1 and VITEF3 where as it is oval in shape in VITEF2.T he Biochemical characterization of these 3 isolates revealed them to be negative for Indole, Voges-Proskauer, Citrate, Catalase and Nitrate Reduction test. VITEF2 was positive for Methyl Red test and oxidase test with VITEF1 and VITEF3 being negative. The isolates showed negative result for the hydrolysis of gelatine and urea.VITEF2 was positive for casein and starch and VITEF1 and VITEF3 were negative towards casein and starch. All 3 isolates utilised various sugars, D-Maltose, D-Glucose, D-Fructose, Mannitol and Galactose as sole carbon sources and were noted to be positive (Table 3). The results of Morphological and Biochemical tests indicate that the 3 isolates belonged to the genus *Bacillus sp.*

The physico-chemical analysis of the textile effluent contaminated soil sediments showed the impact of the wastewater upon the physical and chemical characteristics of soil. The pH of the soil samples was typical of arid area soil (slightly alkaline). The Micro and Macro nutrients were analysed in all the four replicates of soil samples. The level of micronutrients (Zinc, Manganese, Boron and Potassium) in soil sample 1 and 2 was found to be very low. The level of Iron was found to be high in both the soil samples (Fig.1). The Macronutrients level (Nitrate and Phosphorus) in soil sample 1 and 2 was noted to be very low, compared to the high levels of Potassium, Magnesium and Sodium (Fig.2). The significance level was assessed based on one way ANOVA.Statistical analysis shows significant value based on 95% confidence level($p<0.05$).

DISCUSSION

Many of the South Asian countries are experiencing severe environmental problems due to rapid industrialization. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing, etc.thrive as clusters. The effluent discharged by these industries leads to serious pollution of surface water sources, groundwater and soils and ultimately affects the livelihood of the poor [20]. The physico-chemical condition of the collected textile effluent sample revealed a high load of pollution indicators. Colour is contributed to a water body by the dissolved compounds (dyes and pigments). The Colour of the effluent was black due to mixture of dyes used in the dyeing process. [21]. In the present study the pH of the effluent sample was slightly alkaline when compared to the acidic pH of the dyeing effluent in a previous study [22]. The pH of the effluents affects the physico-chemical attributes of water which in turn adversely affects aquatic life, plant and humans. This also changes soil permeability which results in polluting underground resources of water [23]. In the present study the temperature of the effluent was considerably high in comparison with the reports of effluent temperature by others [24]. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD/COD. High values of BOD and COD were noted in the present sample in comparison to the very high values of BOD and COD in one effluent study. This high value demands significant amount of dissolved oxygen for enhanced intrinsic remediation of wastewater.

The Electrical Conductivity, TDS and TSS values of Tiruppur effluent sample are higher than the permissible limits but when compared to a textile effluent collected from a mill near Hisar (Haryana) was found to be low[25]. These high values predict the presence of excess of materials and dissolved matter in textile effluents. Higher values of Total Dissolved Solids are one of the major sources of sediments which reduce the light penetration into water and ultimately decrease the photosynthesis. The decrease in photosynthetic rate reduces the DO level of wastewater which results in decreased purification of wastewater by microorganisms [26]. The presence of heavy metals in the current sample was found to be high which is of the same order of magnitude reported in another effluent sample [27]. A higher value of heavy metal ions in the effluent severely affects the soil fertility and depletes the soil of its nutrients. Heavy metal ions at high concentrations were reported in both Algae and higher plants exposed to effluents [28].

The Textile effluent had very high values of chloride content and low values of sulphate, which on par to the value of one of the dyeing effluent sample from Faisalabad, Pakistan [29]. High chloride contents are harmful for metallic pipes as well as for agricultural crops if such wastes containing high chlorides are used for irrigation purposes. Moreover, high chloride contents also kill some microorganisms which are important in some food chains of aquatic life [30]. Majority of the textile effluent samples have permissible limits of sulphate ions. The effluent showed phenolic contents greater than 0.1ppm which is though permissible limit of the phenolic compounds still these compounds are very toxic to fish even at very low concentrations [31]. The bleaching and dyeing process are the main causes of pollutants which include caustic soda, hypochlorite and peroxides.

Three bacterial strains (VITEF1, VITEF2 and VITEF3) out of 30 isolated from the textile effluent in Tiruppur were found to possess the potential to decolorize the textile dye Reactive RED 5B in aerobic conditions.The percentage decolourization was 60% for VITEF1, 57% for VITEF2 and 58% VITEF3 respectively. This percentage decolourization was lower when compared to decolorization of Reactive blue 59 exhibited by *Bacillus odyssey, Morganella morganii and Proteus sp.* SUK 7 (89%,90% and 82%) in a similar study[32]. This shows the adaptability of the strains to the severe conditions of the effluent and their survival in the highly contaminated water. The ability of the isolates to decolorize textile dye was also been attributed to their adaptability to the xenobiotic compounds, by their biological activity and by chemical structure of dyes [33]. The individual strains may attack the dye molecule at different positions or may use degradation products produced by another strain for further degradation [34]. The possibility of use of various physical and chemical methods that can be used for the treatment of textile wastewater has been reported. But all of these methods have their own drawbacks such as they are economically feasible and high cost or operating expenses, lack of effective colour reduction, particularly for sulphonated azo dyes, sensitivity to a variable wastewater input and production of huge amount of sludge. The treatment of textile wastewater by purely biological processes may be possible even without the inclusion of other carbon sources, e.g. municipal wastewater [35]. Therefore microbial decolourization of the textile effluent has been proposed as a less expensive and less environmentally intrusive alternative.

In the present study, analysis of soil sediments for their physico chemical parameters revealed that they were silt to sandy loam, slightly alkaline and were low in organic matter, organic carbon and low in macronutrients (nitrogen and phosphorus). The micronutrients were also found to be below normal level (boron, zinc).They were found to be rich in potassium and sodium. Alkaline soils tend to have Ca, Mg and K in high concentrations. These results were in accordance with the observed values in a study on a soil contaminated with textile effluent in Pakistan [36]. All these results demonstrated metal contamination of soil surrounding the textile effluent. This study demonstrates clearly that soil adjacent to the flowing textile effluent experiences changes in physicochemical parameters. These changes can be attributed to high content of metal ions in dye and mordants such as HRB 38, nickel-pthalocyanine complex, HRV5 copper containing azo dye, zinc yellow pigment, iron blue pigment, chrome yellow and green pigment, are being discharged into the wastewater and thus accumulated in the soil [37].

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