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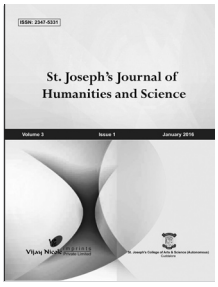

Isolation, Characterization and Screening of Decolorizing Bacteria from Textile Dye Effluent, Bhuvanagiri, Cuddalore District, Tamilnadu

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ABSTRACT

In the present research, textile dye effluent was collected from small scale textile industries, Bhuvanagiri. The physico-chemical properties of the dye effluent were analysed. The effluent showed reddish brown in colour and the values of TDS, TSS, BOD were beyond the limit prescribed by TNPCB. Nine different bacterial isolates were isolated from the effluent and their morphological and characteristics were studied. Two strains DT02 and DT07 were tentatively identified as Bacillus sp. Among the nine isolates, the isolate DT09 showed better results decolorization of the medium containing dye. The experiment of biodegradation was conducted by inoculating the isolate DT09 into medium containing 50 ppm of textile dye 4GL and methyl red. The biodegradation rate of the isolate TD09 was higher for the dye 4GL (33.76 %) than the dye methyl red (27.78 %)

Keywords: Bacteria, Decolorization, Textile Dyes, Degradation

1.0 INTRODUCTION

From the beginning of mankind, people have been painting and dyeing their surroundings, their skins and clothes with colorants. Up to the end of the nineteenth century natural dyes, obtained mainly from plants were used as main colorants for dyeing procedure. Nowadays, there are several chemically synthesized dyes used in dyeing industries. Over 0.7 million tons of synthetic dyes are produced annually, worldwide and approximately 10,000 different dyes and pigments are used industrially.

Among the different industries, the textile industry accounts for the largest consumption of dyestuffs, at nearly 80%. Especially textile industries produced more than 70% of the total quantity of waste in India[1]. From the textile industries, huge amount of waste water is released into natural reservoirs. Textile wastewater accounts for 22 % of the total effluent released from different types of industries.

Dyes are difficult to degrade because of its complex aromatic molecular structures and synthetic origin which make them stable. Due to low degradability of dyes, conventional effluent treatment systems are inefficient in treating waste water. Microorganisms which are able to survive in high concentration of pollutants are of great interest to degrade those pollutants. The increasing interest in bacteria is a result of their potential to degrade and mineralize most textile dyes effectively; bacteria also have a rapid growth rate

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and are straightforward to culture. Keeping the above points in mind, the present research designed to isolate bacteria form textile dye effluent and to assess the degradation ability of those isolates.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Textile dye effluent sample was collected from the Vellar river, which receive the effluent from the small scale textile industries in Bhuvanagiri. Water sample was collected using 500 mL plastic bottles. Sampling bottles for the determination of physico-chemical parameters were cleaned and rinsed using distilled water followed by using textile dye effluent.

2.2 Analysis of Textile Dye Effluent Sample

Textile dye effluent sample was assessed for physico-chemical parameters like color, temperature, pH, BOD, TDS, TSS and TS. These were analyzed in Refsyn biosciences Pvt.Ltd., Puducherry and the methodologies were as follows:

2.3 pH

The pH of the effluent was determined by potentiometric method using pH meter already standardized by using buffer solutions of known value before analysis.

2.4 Estimation of Biological Oxygen Demand (BOD)

2.4.1 Winklers iodometric method [2]

Preparation of dilution water: 1.0 mL of calcium chloride, magnesium sulphate, ferric chloride and phosphate buffer solutions were added to one litre of aerated distilled water and mixed thoroughly. This is the standard dilution water, prepared freshly every time. Freshly settled raw sewage at 2.0 mL was added as seeding to one litre of dilution water. The test water samples were diluted with seeded dilution water sample (1%, 5% and 10%). Each dilution sample was taken in two sets of BOD bottles. In one set of flasks DO was determined immediately while the other set was kept for incubation at 20ºC for five days.

2.4.2 Determination of DO

To the contents of the BOD bottle 2.0 mL of magnesium sulphate solution and 2.0 mL of alkali-iodide-azide solution were added and mixed thoroughly. A brown precipitate was formed, which was allowed to settle completely leaving a clear supernatant liquid. Then, 2.0 mL of concentrated sulphuric acid was added along the sides of the bottle and mixed for complete dissolution. The contents were transferred to a 500 mL conical flask and titrated immediately against 0.025 N sodium thiosulphate using starch as an indicator.

2.4.3 Calculation for DO

Volume of 0.025 N sodium thiosulphate used in the titration = DO in mg/L

DO at 0ºC 760 mm pressure = DO × 0.07 mg/L

2.4.4 Calculation for BOD

BOD (5 days at 20ºC) = (DO_0 - DO_5 - BC) ×100 per cent sample.

2.4.5 DO_0 = Initial DO

DO_5 = DO after 20ºC incubation for 5 days.

BC = Blank correction i.e., the difference in DO of blank on the initial day and after 5 days incubation.

2.5 Total Suspended Solids (TSS)

Total suspended solids are the portion of solids that usually remains on the filter paper. Suspended solids consist of silt, clay, fine particles of organic and inorganic matter, which is regarded as a type of pollutant because water high in concentration of suspended solid may adversely affect growth and reproduction rates of aquatic fauna and flora. For TSS analysis, a known amount of sample was filtered through the previously weighed filter paper. Filter paper was then dried at 103-105° C. TSS was determined by using following formula [3].

TSS mg/L = (final wt - initial wt)/amount of sample taken ×1000

2.6 Total Dissolved Solids (TDS)

Total dissolved solids (TDS) are the measure of total inorganic salts and other substances that are dissolved in water. TDS was determined following the procedure [4] by using Electrical Conductivity (EC) meter.

TDS (mg/L) = EC µs/cm × 0.67

2.7 Enumeration of Total Microbial Population in Collected Effluent Sample

The collected effluent sample was serially diluted up to 10^{-7} dilution to determine the population of bacteria and fungi. 0.1 mL from the 10^{-6} dilutions was plated on sterile Petri plates containing Nutrient Agar (NA) medium and incubated at room temperature for two days for enumerating the bacterial population, 10^{-4} dilutions was plated on sterile petriplates containing Rose Bengal Agar medium (RBA) and incubated at room temperature for 3 days for enumerating fungal colonies. After incubation, the number of bacterial and fungal colonies in the respective plates were counted and the population was expressed in terms of cfu/ mL.

2.8 Isolation of Bacteria from Textile Dye Effluent

Nutrient Agar (NA) medium was prepared and sterilized by autoclaving. The effluent sample was serially diluted and directly transferred on Petri plates containing Nutrient Agar medium from 10^{-6} dilutions and incubated at room temperature for 24 hrs. After the incubation period, the plates were observed for the growth on the media.

2.9 Characterization and Identification of the Isolated Bacteria

2.9.1 Morphological Characterization of the Bacteria

Colony character, colony morphology, cell morphology, spore forming ability and motility of the bacterial isolates were studied by standard procedures

2.9.2 Biochemical Characterization of the Isolates

For identification, biochemical tests *viz*., Indole test, methyl red, Voges Proskauer test, urease test, triple sugar iron (TSI) agar test, citrate utilization test, oxidase test, catalase test, starch hydrolysis and casein hydrolysis were tested by standard protocols

2.10 Screening of the Isolates for their Dye Degradation Efficiency

Degradation efficiency of the isolates was tested by streaking the isolates on the NA medium prepared by adding 50 mg/L of the dye methyl red. Based on the decoloration efficiency, best isolate was selected for further studies. Among the nine isolates, the isolate DT09 decolourized the medium effectively, so it is selected for further studies.

2.11 Biodegradation Studies by Using the Isolate DT09

The bacterial inoculum was prepared by inoculating the bacterial isolate DT09 in 100 ml of nutrient broth. Then the flask was kept in shaker incubator at 200 rpm and at the temperature of 37° C. For biodegradation, 100 ml of nutrient broth was prepared in 250 ml conical flask. The dyes methyl red and 4 GL were separately added at the rate of 50 ppm to the nutrient broth. One ml of the inoculum was added into the nutrient broth previously prepared with 50 ppm of dyes. The flasks were kept in mechanical shaker under the condition of 200 rpm and incubated at 30°C. Samples were drawn after 24 hrs for observation. Samples were centrifuged at 10000 x for 10 minutes. Decolourization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wave length 530 nm. Two control flasks (dye + medium without inoculums) were also maintained.

The percentage of decolourization was calculated using following formula.

$$\%\ \text{Decolurization} = \frac{\text{Initial O.D} - \text{Final O.D}}{\text{Initial O.D}} \times 100$$

3.0 RESULTS AND DISCUSSION

3.1 Physico-Chemical Properties of the Textile Dye Effluent

The physico-chemical properties like pH, temperature, TSS, TDS, TS and BOD were analyzed and the results are given in the Table-1. The color of the dye effluent was reddish brown. There are various colors of the textile dye effluent which were reported by many researchers [5, 6]. The variation was due to the color of the dye used in the dyeing industries.

Table-1 Physico-chemical Properties of the Textile Dye Effluent Sample

S.No	Parameters	Values	TNPCB limits
1.	Color	Reddish brown	Colorless
2.	pH	7.94	5.5 – 9.0
3.	Temperature	30°C	40°C
4.	TDS	8401 (mg/l)	200 (mg/l)
5.	TSS	3467 (mg/l)	200 (mg/l)
6.	TS	11868 (mg/l)	-
7.	BOD	420 (mg/l)	30 (mg/l)

The pH of the effluent was 7.94 and the temperature was 30°C. This was within the limit prescribed by TNPCB standards. A similar trend in pH of dye effluent was reported [5]. But some researchers found that the textile dye effluent was more alkaline in nature [7] and this was due to presence bleaching agents, NaOH, surfactants, and sodium phosphate used in the processes are reasons [6].

In the present research, the temperature of the effluent was 30°C. A similar trend in temperature has also been reported for physiological characterization of industrial effluents [8, 9].

The total suspended solid (TSS) in the collected effluent was 3467 (mg/l) and it was found to be high when compared to the TNPCB limit. Similar report was found [10]. High values of TSS may be attributed to coloured effluents which eventually use different dye stuffs [11]. Contrastingly, very low values of TSS have been reported [12].

The TDS of the collected textile dye effluent was found to be 8401 (mg/l). This was 42 times more than the limit prescribed by TNPCB. Total dissolved solids (TDS) is a measure of solid content in water. A large number of salts are found in waters and they are carbonates, chlorides, sulphates, phosphates, magnesium, potassium, etc. [8]. Similar value has also been reported [6]. Some researchers found low values of TDS [5].

The BOD is a measure of the quantity of oxygen used by microorganisms for the degradation of organic matter and is the most important parameter which determines the pollutants load in the water. In the present research, the BOD of the collected effluent was 420 mg/l. Similar results were reported by many researchers ([13,14] High values of BOD also documented by some researchers [15]. In contrast, the lower values of BOD (32 mg/l)was also reported by [15].

The total solids (TS) in the collected sample was11,868 mg/l. the high TS may be attributed by the use of salts during dyeing processes.. Similar report was reported by Lokhande *[8]*.

[16] conducted a research and the results showed some parameters like TDS, BOD, COD etc. exceeds the TNPCB limits at significant levels.

3.2 Enumeration of Total Microbial Population (Bacteria & Fungi)

The total microbial population (bacteria and fungi) was enumerated and presented in the Table-2. The results showed that the bacterial population in the effluent was 7.9×10^6 cfu/ml and fungi was 6.0×10^4 cfu/ml.

Table- 2: Enumeration of Microorganisms in Collected Effluent Sample

S. No.	Microorganism	Population
1.	Bacteria (10^6 cfu/mL)	7.9
2.	Fungi (10^4 cfu/mL)	6.0

3.3 Isolation of Bacteria from Textile Dye Effluent

Nine bacterial isolates were isolated from the collected textile dye effluent and designated as DT01 - DT09. The morphological characterization of the isolates were studied and presented in the Table-3 and the biochemical characterization of the bacterial isolates were also studied and the results were given in the Table-4. From these isolates, three isolates were gram positive and the remaining six were gram negative. Several researchers isolated the bacteria from the textile dye effluent [17-19]. Among which, two isolates (DT02 & DT07) were tentatively identified as *Bacillus* sp. in a study, *Bacillus* sp. was also isolated from textile dye effluent [20].

Table-3: Morphological Characterization of Bacterial Isolates

S.No.	Isolate	Colony morphology	Gram staining	Spore staining	Motility
1.	DT01	Yellow, regular, mucoid, smooth	Gram (-) rods	Non spore formers	Non-motile
2.	DT02	White, irregular	Gram (+) bacilli	Spore formers	Motile
3.	DT03	Regular	Gram (+) cocci	Non-spore formers	Motile
4.	DT04	Smooth, regular	Gram (-) rods	Non-spore formers	Non-motile
5.	DT05	Pink coloured, regular, smooth	Gram (-) rods	Non-spore formers	Non-motile
6.	DT06	Yellow regular	Gram (-) cocci	Non-spore formers	Motile
7.	DT07	White, mucoid, regular	Gram (+) bacilli	Spore formers	Motile
8.	DT08	Irregular	Gram (-) cocci	Non-spore formers	Motile
9.	DT09	White, regular	Gram negative rods	Non-spore formers	Motile

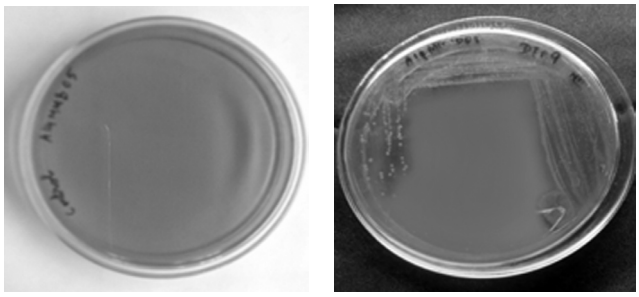
Table-4: Biochemical Characterization of the Bacterial Isolates

S. No.	Isolate	Indole	MR	VP	Urease	Citrate	TSI	Oxidase	Catalase	starch hydrolysis	Casein hydrolysis	Tentatively identified as
1	DT01	-	-	-	-	-	K/K	-	+	+	-	
2	DT02	-	+	-	+	-	K/K	+	+	+	+	Bacillus sp
3	DT03	-	-	-	-	-	K/A	+	-	+	+	-
4	DT04	-	+	-	+	-	K/K	+	+	+	+	-
5`	DT05	-	+	-	-	-	A/A	-	-	+	-	-
6	DT06	-	-	-	-	-	A/A Gas production	+	+	-	+	-
7	DT07	-	+	-	+	-	K/K	+	+	+	+	Bacillus sp
8	DT08	-	+	-	+	-	K/K	+	+	-	-	-
9	DT09	-	+	-	+	-	K/K	+	+	-	+	-

3.4 Screening of Bacterial Isolates for their Degradation Efficiency

Nine isolates were streaked on the nutrient agar supplemented with 50 ppm methyl red. Among which the isolate DT09 shows better in decolourizing the media (Fig-1), so it is selected for further studies. The isolate DT09 was a gram negative bacterium. Many researchers found that the gram negative bacteria were much efficient in dye degradation. Some of the important gram negative bacteria involved in dye degradation are *Pseudomonas* sp. [21-29].

Fig.-1: Screening of Bacterial Isolates on Dye Decolorization



Control **Effective Decolourization of bacterial isolate DT09**

3.5 Biodegradation of the Dye (methyl red and 4GL) using the Isolate DT09

In nutrient broth enriched with 50 ppm dye (methyl red and the textile dye 4GL), 1% culture was added and kept in orbital shaker at 200 rpm (Fig-2 & Fig-3). The flasks were incubated at 30°C. After one day, one ml of the sample was withdrawn and centrifuged at 1000 rpm. The OD vale of the supernatant are measured at 530 nm in UV-Spectrophotometer. The percentage of biodegradation was calculated and given in the Table-5. The isolate DT09 showed highest declourization percentage of 33.76 % on the textile dye 4GL followed by methyl red (27.78 %).

In the present research, single isolate (DT09) was used for biodegradation studies. Many reports were found on using single isolate in removing dye from the water [9, 30-32]. In contrast, some researchers found that the mixed cultures showed better performance in decolorizing textile dyes. For example, [33] isolated mixed bacterial culture from a domestic wastewater treatment plant which degraded 700 mg/l of methyl red within 18 hrs.

In our study, the biodegradation percentage of the isolate DT09 on the textile dye 4GL was higher (33.76%) than on methyl red (27.78). Similar result was found by [30], who reported that *Pseudomonas* sp. achieved 90% of decolorization of azo dye orange II but could decolorize only 35 % of azo dye orange I. The variation in the rate of decolorization of individual dyes could be attributed to the structural difference of the dyes.

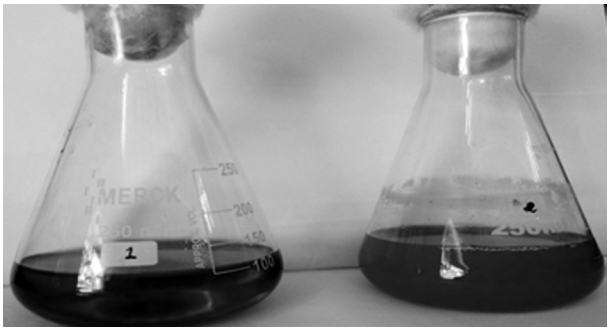
Table-5: Percentage of Biodegradation of Dyes (methyl red and 4GL) by the isolate DT09

S.No.	Dye	Initial OD (at 0 hr)	Final OD (after 24 hrs)	Decol-orization (%)
1	Methyl red	1.0562	0.7627	27.78
2	4GL	0.8331	0.5518	33.76

Fig.-2: Biodegradation of Textile dye 4GLby DT09

Treatment with DT09 **Control**

Fig.-3: Biodegradation of Methyl Red by DT09



Control **Treatment with DT09**

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