

Biodegradation of Textile Effluent Containing Azo Dye using Individual and Mixed Adapted Bacterial Strains

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ABSTRACT

Wastewater from the textile industries contains a variety of pollutants, particularly dyes. Azo dyes are the largest group of synthetic aromatic dye used in the textile industry for dyeing purpose and are highly water soluble in nature. The removal of azo dyes from industry effluents is desirable not only for aesthetic reasons but also because azo dyes and their breakdown products are toxic to aquatic life and mutagenic to humans. The treatment of these dyes have been carried out by many methods such as physical, chemical and biological as individual and in combination to reduce the effect of pollution. The biodegradation of azo dyes is difficult due to the complex structure, synthetic nature and some are highly resistant to microbial attack. To overcome this problem, this study aims in developing a consortium to degrade complex azo dyes present in the effluent. The current work aims in screening and identification of potent azo dye degrading bacteria, studying azo dye degradation efficiency of the isolates, preparing the microbial consortia and to determine the efficiency in decolourization and degradation in both chosen dye (Amido black 10 B) and effluent. The degradation efficiency of the effluent using mixed dyes were significantly higher than the individual strain which was evident from reduction in colour and COD which was 76 % and 79 % respectively. The degradation of the effluent using consortium was studied using FT-IR which revealed change in the peak characteristics.

KEY WORDS: AMIDO BLACK, AZO DYES, CONSORTIUM, DECOLOURIZATION, FT-IR

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INTRODUCTION

Textile industry is considered as one of the chief export earnings and employment providing sector of the nation. Though it helps developing the economy of the country it also burdens it by polluting the environment through the processes involved in its production especially the dyes and chemicals used. This industry also consumes a generous amount of water during its manufacturing processes especially in the dyeing and finishing operations of the plants. The textile wastewater is considered the most polluter among all the industrial sectors based on the volume generated and the composition of the effluent (Mansour et al 2012). Since, there is an increase demand for textile products the production also increases relatively and this has led to the use of synthetic dyes which in turn has led to be a source of severe pollution (Ogugbue and Sawidis 2011 Yin et al., 2019).

A wide range of dyes are being used in the textile industry among which synthetic dyes play an important role due to their cost effectiveness and dyeability. Synthetic dyes are polyaromatic molecules that give a permanent coloring to materials like textile fabrics. A variety of synthetic dyestuffs released by the textile industry pose a threat to environmental safety. The degree of coloring process continues even after the dyeing process, leading to effluents containing azo dye (Lu & Liu 2010). The chemical structure of the dyes makes it resistant to most types of physical, chemical and biological treatments (Mansour et al. 2011b). The removal of azo dyes from industry effluents is desirable not only for aesthetic reasons but also because azo dyes and their breakdown products are toxic to aquatic life and mutagenic to humans. Therefore, treatment of textile effluent is necessary before discharging in the environment. The treatment of textile effluents is also essential to protect the ecosystem and allow the subsequent recycling of the treated effluent for irrigation or reuse within the procedures of the textile plant (Yaseen and Scholz, 2019).

The treatment of these dyes have been carried out by many methods such as physical, chemical and biological as individual and in combination to reduce the effect of pollution. Physical or chemical methods for textile wastewater pretreatment are of high cost, extremely energy consuming, and environmentally low efficient and generate toxic sludge (Sakar *et al.*, 2017). These dyes are generally recalcitrant to biodegradation due to their xenobiotic nature. However microorganisms, being highly versatile, have developed enzyme systems for the decolorization and mineralization of azo dyes under certain environmental conditions. The complete mineralizations of organic pollutants by biological methods are cost effective and eco-friendly (Nachiyar et al., 2016)

The biodegradation of azo dyes is difficult due to the complex structure, synthetic nature and some are highly resistant to microbial attack. To overcome this problem, several studies using microbial consortia were attempted to achieve not only dye decolorization, but also degradation of the aromatic amines due to the efficiency of the microorganisms (Chan et al., 2011). Thus, this work aims in screening, identification of potent azo dye degrading bacteria, studying azo dye degradation efficiency of the isolates, preparing the microbial consortia and to determine the efficiency in decolorization and degradation in both chosen dye and effluent.

MATERIAL AND METHODS

Sample Collection: The textile effluent was collected from Chinnakkari Common Effluent Treatment Plant (CETP), Tiruppur and was used for the study.

Physiochemical analysis: The physiochemical parameters such as color, pH, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), TSS (Total Suspended Solids), TDS (Total Dissolved Solids) were determined immediately after being brought to the laboratory as per the standard APHA methods (Rice, 2012)

Isolation of autochthonous bacterial strains from effluent sample: The spread plate assay method was used for the enumeration of aerobic bacteria from the collected sample. This was done by serial dilution of the sample (10^{-1} to 10^{-8}), and placing 0.1ml of the diluted samples (10^{-6} , 10^{-7}) in an minimal media (64 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 15 g KH_2PO_4 , 2.5 g NaCl and 5.0 g NH_4Cl , 1M MgSO_4 and 1M CaCl_2) agar plate containing dyes. The dyes used for the study were Alizarine red, Amido black 10 B, Reactive red and Brilliant violet (SIGMA, India). The diluted samples were spread on the surface of agar plate using L- rod and was incubated at 37°C as for 48 hours. The cultures which showed a zone of clearance around their colonies were isolated and used for further screening (HeFang et al., 2004).

Screening of microorganisms capable of decolorizing and degrading the azo dye: The minimal broth containing four different dyes (100 mg/L) were prepared; the isolated organisms were inoculated and incubated at 37°C for 7 days. The degradation patterns were observed for each organism in different dyes using visible spectrophotometer in the wavelength ranges between 400 - 650 nm (Omar, 2008). The strains showing decolorization efficiency in chosen all four dyes, where studied for their potency in Amido Black 10 B dye and their percentage decolorization was recorded using the formulae below at 610 nm:

$$\% \text{ decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Identification of Selected Microorganisms: The screened bacterial strains were identified by using standard biochemical (Cappucino and Sherman, 2014) and microscopic techniques according to Bergey's Manual of Systemic Bacteriology.

Strain Identification - Molecular characterization: The three most active isolates were identified by 16S rRNA sequencing after extracting the genomic DNA. The 16S region of ribosomal rRNA gene was amplified using the universal primers 27F and 1492R primer. The PCR amplification was done by initial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 sec, 72°C for 60 sec and final extension at 72°C for 10 min. PCR purification was done by SolGent PCR Clean up kit (Millipore). The PCR product was sequenced using the 27F/1492R primers. Using ABI PRISM® BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), sequencing were performed. The sequences were assembled and edited using Bioedit software and deposited in the NCBI database for accession numbers. The sequences obtained were compared to find sequence similarity using GenBank program MUSCLE 3.7. PhyML 3.0 aLRT was used for phylogeny analysis. The program Tree Dyn 198.3 was used for tree rendering. The sequences obtained in this study were deposited in the GenBank database.

Test for synergism: In order to develop a bacterial consortium the selected strains must be tested for their compatibility. The test was performed as follows: three nutrient agar plates were taken and each plate was bored with two wells. The first plate was smeared with one of the three selected culture (TMB 2) and added with 10µl of the supernatant from TMB 6 and TMB 7 in the two wells respectively. The plates were then kept for incubation at 37°C for 24 hours. Absence of any zone of inhibition around the wells showed that the cultures are compatible. The test was repeated by changing the swabbed organisms with the other two selected bacterial isolates and changing the supernatants from the organisms added in the bored wells accordingly (Ammar *et al.*, 1998).

Development of Consortia for treatment textile effluent: Based on the mutuality of the isolates, the Cayley's tables were made. According to the Table-1, the culture consortium were developed and used for further study.

Treatment of effluent by individual strains and developed consortia: About 5ml of mixed culture was inoculated into 95 ml of textile effluent sample and incubated at 37°C in shaking condition for 5 days. The degradation patterns of the effluent were measured in spectrophotometer in the range of 423nm.

Table 1. Cayley's table for consortia preparation

Organisms	TMB 2	TMB 6	TMB 7
TMB 2	TMB 22	TMB 26	TMB 27
TMB 6	TMB 62	TMB 66	TMB 67
TMB 7	TMB 72	TMB 76	TMB 77

$$\% \text{ of decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Fourier Transformed Infrared Spectroscopy analysis: IR-spectra were obtained using a SHIMADZU FTIR 8400S. The untreated textile effluent (raw) and treated effluent with the potent consortia were assessed by FT-IR for the chemical modification. Effluent samples were recorded from 4000 – 400 as scanning range between wave number (cm⁻¹) and % Transmittance. All samples were run in triplicate and the data presented are the average of the three measurements.

RESULTS AND DISCUSSION

The textile effluent sample was collected in sterilized container from CETP, Chinnakarai, Tiruppur and stored at 4°C for further studies. The autochthonous bacterial strains were isolated in minimal media plate containing the azo dye using spread plate technique. The result showed around 7 different colonies had the ability to degrade the azo dye which was observed through the zone around the colonies. The obtained colonies were designated as TMB1, TMB 2, TMB 6, TMB7, TMB 8, TMB 10 and TMB 11. It was clear from the results that the natural adaption of microorganism enabled them to survive in the presence such recalcitrant compounds (azo dyes). The ability of the organisms to decolourize was due to the utilization of the toxic dye which resulted in the breakdown of chromophore of the dyes. The isolated strains were then subjected to quantitative screening by inoculating the isolated strains in minimal media broth containing azo dyes. The results revealed that among the 7 strains screened, three strains were found to have ability to degrade all the four azo dyes used in the study. These strains were chosen because they showed percentage reduction more than 45% in 48 hours of incubation in all the four dyes used for the study (Fig. 1). Therefore, the strains TMB2, TMB 6 and TMB 7 were chosen for further study and were identified. The isolated bacterial strains from effluent sample collected from the CETP were identified through morphologically different bacterial isolates cultures were preserved on nutrient agar medium at 4°C. The three organisms which showed significant decolourization of the dye degradation were

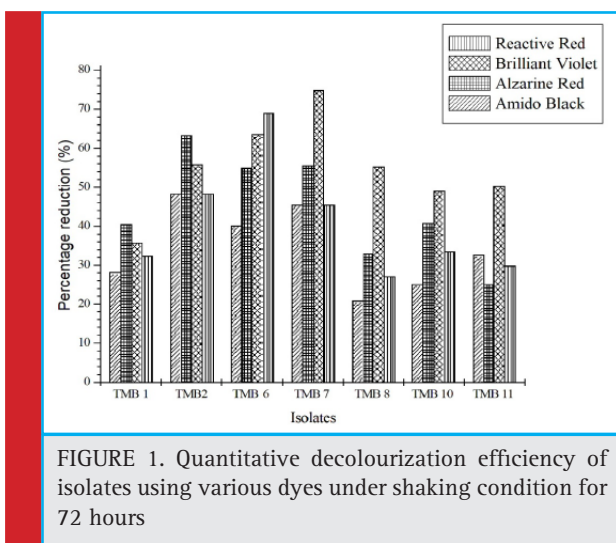


FIGURE 1. Quantitative decolourization efficiency of isolates using various dyes under shaking condition for 72 hours

identified through morphological and biochemical analysis. The isolated bacterial cultures – TMB2, TMB 6 and TMB 7 were tentatively identified to be *Pseudomonas* sp., *Klebsiella* sp., *Shigella* sp., through biochemical test.

This was reconfirmed with 16s rRNA sequencing after extracting DNA. The sequences obtained were compared using GenBank program-Basic Local Alignment Search Tool (BLAST). The phylogenetic trees based on 16S rRNA gene sequences were constructed by the neighbour-joining method. The sequences obtained in this study were deposited in the GenBank database. GenBank accession numbers for the nucleotide sequences are KY788338, KY788341 and KY789442. The isolates obtained were found to be *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella dysenteriae*. From the results it is clear that the obtained strain are pathogenic to human, but still have the potency to degrade the chromophore of the toxic dyes. Several studies have reported that these pathogenic strains have efficiency in degrading the azo compounds. The decolourization Direct Blue 6 by *Pseudomonas desmolyticum* in 72 h under microaerophilic conditions was reported by Kalme et al. (2007). Similarly the decolorization and biodegradation of four different azo dyes, Reactive Yellow 107 (RY107), Reactive Black 5 (RB5), Reactive Red 198 (RR198), and Direct Blue 71 (DB71), in a sequential microaerophilic- aerobic treatment in 72, 120, 96, and 168 h, respectively, by a facultative *Klebsiella* sp was accounted by Franciscon et al. (2009).

The recent findings of Dixit and Garg (2018) clearly state the pathogenic strain possess' potency to degrade the azo dye and completely mineralize them. In their study which is a two-stage sequential process that utilizes the isolated *Klebsiella pneumoniae* and successfully degrade the azo dyes and their metabolites completely in much less time as compared with the reported strains

Table 2. Physico - Chemical Characterization of Untreated Textile Effluent

Parameters	Value	TNPCB standards
pH	8.9	5.5-9.0
Temperature	35°C	40°C at the point of discharge
Conductivity (µS/cm)	13330	2250
Turbidity (NTU)	45.3	10
Color (Hazen units)	1200	400
COD (mg/L)	1160	250
BOD3 (mg/L)	70	30
Hardness	960	300
Total suspended solids (TSS) (mg/L)	3700	100
Total dissolved solids (TDS) (mg/L)	5970	2100
Chloride (mg/L)	2080	600
Phosphate (mg/L)	1.4	5
Sulphate (mg/L)	1745	1000

(Dixit and Garg, 2018). Decolourization of Acid Red 131 by using *Shigella* sp was reported by Sivaranjani et al (2013) in which they stated that 99% degradation was observed by the isolated strain (Sivaranjani et al., 2013). Therefore, further study of treating the effluent using the strains were carried out as to determine the efficiency of degradation and mineralization of azo dye containing effluents. The untreated textile effluent was characterized and its physico - chemical parameters was determined and tabulated below (Table 2).

The collected sample had an unpleasant fishy odor and was turbid with greenish color. Unpleasant smell and taste in water, perhaps due to declining vegetation, inorganic constituents / organic substances, wastewater discharge into water bodies (Mohabansi et al., 2011). As reported by Patel et al., (2015) the primary sample has fishy, secondary aerated and processed has rotten egg as well as stagnant sample has fortified odor (Patel et al., 2015). The strong coloration of the effluent was sometimes influenced by the pH and temperature of the dyeing process which strengths the chromophore group. The presence of highly colored components affects the dissolved oxygen of the water. pH of a effluent is very important in determination of water quality since, it affects other chemical reactions such as solubility and metal toxicity (Fakayode, 2005). The dissolved oxygen levels are found to be very low and hence a lot of oxygen has been used up, which shows the increased concentration of organic matter. The collected effluent sample had a BOD₃ 70 mg/l which implies the content of organic matter is too high and oxygen gets depleted rapidly. COD is a critical parameter in assessing the water

quality as it indicates the presence of non-biodegradable and organic matter present in the effluent. In the present study the investigations made indicate high COD value of around 1160 mg/l which is higher than the permissible level. As the physico-chemical parameters of the collected effluent are higher than the allowable limits, proper treatment is necessary before discharging. The elevated amount of industrial effluent pollution creates environmental issues that impact plant, animal and human life (Kolhe et al., 2011).

Analysis of compatibility was performed to verify whether the chosen strains used in biodegradation were suitable for effective bioremediation when used as a consortium. The results showed that there was no inhibition zone around the wells for any of the plates after incubation. The main reason for this compatible nature of the selected bacterial strains may be that they have co-existed for a longer period of time in a common environment. This clearly illustrates that the compatible nature of these strains would be effective in degrading the recalcitrant azo dyes and the complex organics present in the effluent. About 4 different consortia containing equal amount of the strains were prepared and designated as TMB 26, TMB 27, TMB 67 and TMB 267 and used for the study. On observing the reduction in the various physico-chemical parameters of the treated effluent sample using bacterial consortia, it could be concluded that the bioremediation efficiency of the consortium TMB 27 was found to be reduced to a significant extent (Table 3) compared to the other strains or devel-

oped consortia. This could be due to the fact that the consortium of adapted microorganism were able to exert more enzymes that have a wide spectral range which in turn could have degraded the complex organic compounds and dye content present in the effluent. Similarly investigation done by Puvaneshwari *et al.*, (2006) revealed that mixed bacterial strains showed an efficient degradation of the organic compounds.

FTIR gives information regarding the structural changes that occurs during biodegradation process with the help of functional groups present (Ladwani et al., 2016). IR Spectra of the untreated effluent showed many bands of 3394.72 cm^{-1} , 3174.83 cm^{-1} , 2607.76 cm^{-1} , 2110.12 cm^{-1} , and 1269.16 cm^{-1} which are representatives of functional group OH, N-H, O-H with strong and very broad intensity, and C-N. The peak position of 3498.12 cm^{-1} , 2858.51 cm^{-1} , 2607.76 cm^{-1} , 2144.84 cm^{-1} , 1597.059 cm^{-1} , 1435.04 cm^{-1} , 1095.57 cm^{-1} and 898.82 cm^{-1} which are the characteristics of O-H, C-H, O-H of strong and very broad intensity, C=C, ether C-O and C-Cl alkyl halide group respectively.

IR spectrum of the textile effluent treated with consortia shows difference in bandings when compared with the untreated sample. During the biodegradation of effluent, the IR spectrum of the treated sample shows variations such as appearance and disappearance of peaks 2754.34, 3429.43, 2144.34, 1693.50, 1435.03, 1269.16, 898.82 in treated sample. The absorption bands showed variation in 3930.27, 3853.77, 3722.61, 3649.31, 3251.98, 3194.12, 3140.11, 1577.77, 1107.14 cm^{-1} , due to N-H stretching, O-H stretching (alcohol), N-H stretching (Amide), N-H (bending), C-O stretching (Alcohol) respectively (Figure 2). From the above results it is clear that changes in the FTIR spectrum are evidence for the degradation of the dyes and complex organic matter present in the effluent into simpler molecules which is due to

Table 3. Percentage Reduction of Physicochemical Parameters of Effluent Treated using Individual Strains and Developed Bacterial Consortia

Treatments	Percentage of reduction in physico-chemical parameters (%) after 72 hours of treatment			
	COD	TDS	Colour	Turbidity
Untreated	-	-	-	-
TMB 2	71.1	48.07	65.7	39.2
TMB 6	64.9	29.6	50.5	49.2
TMB 7	62.8	27.1	52.6	38.1
TMB 26	62.8	35.5	57.8	53.6
TMB 27	79.3	59.7	73.6	66.8
TMB 67	73.1	53	68.4	67.9
TMB 267	75.2	56.4	71.05	56.9

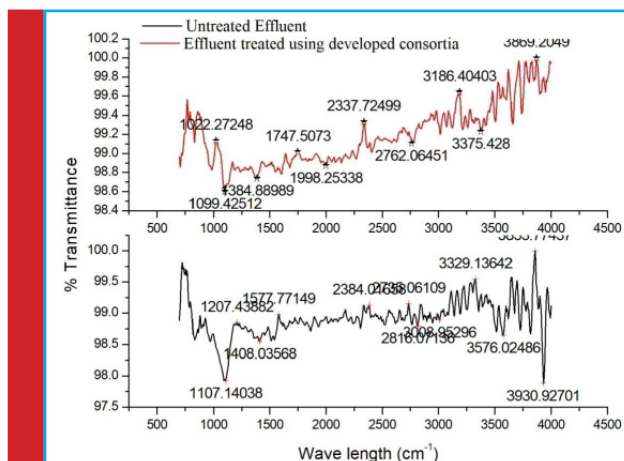


FIGURE 2. FTIR Spectra of untreated effluent and effluent treated using developed consortia

the metabolism of the mixed bacterial strains. Therefore, the study concludes that when mixed bacterial population when used in degradation of effluents containing recalcitrant compounds, the rate of degradation would be faster as more number of various enzymes produced by the strains would act on the molecules and aid in degradation. Similar studies on degradation of recalcitrant's by mixed microbial strains rather than individual have shown better degradation efficacy (Lade et al., 2015; Nachiyar et al., 2016; Chan et al., 2011) The strains isolated in the study are said to be pathogenic in nature which might pollute the environment further, in order to overcome this biotechnological tools can be used and the gene responsible for degradation can be cloned into non-pathogenic strains.

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