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Decolorization of Synthetic Dyes Using Bacteria Isolated from Textile Industry Effluent

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ABSTRACT

Bacteria have potential ability to decolorize synthetic commercial dyes used for textile dyeing. Effluents from textile and dyeing industries cause serious pollution of soil, water and environment. Therefore, this study was aimed to isolate potential dye degrading bacteria from textile effluents and to evaluate their capability to decolorize commercially used textile dyes. Effluent samples were collected from different discharge locations of one selected textile industry. Six bacteria were isolated using a modified nutrient media. Results of biochemical test parameters of these bacteria matched with *Bacillus subtilis* (isolate B2, B7 and C1), *Bacillus megaterium* (isolate D3), *Erysipelothrix* (isolate C4) and *Amphibacillus xylanus* (isolate A1). These isolates were cultured with three different concentrations of seven different textile dyes viz. Cibacron red FN-R, novacron blue, terasil green, novacron navy, novacron orange and novacron yellow. Degradation ability of dye stuffs by the isolates (0.01, 0.05, 0.1 mg L^{-1}) was observed by dye decolorization assay. Almost all dyes except novacron red were decolorized up to 99% by bacterial isolates after 3 days of incubation.

Key words: Bacteria, effluent environment, textile dyes, decolorizing ability, *Bacillus*, *Erysipelothrix*, *Amphibacillus*

INTRODUCTION

Textile industry generated waste water is a complex mixture of many pollutants such as heavy metals, chlorinated compounds, pigments and dyes (Saraswathi and Balakumar, 2009). It is estimated that approximately 15% of the dyestuffs are lost in the industrial effluents during manufacturing and processing operations (Khaled *et al*., 2009). The presence of textile dye even in low concentration in effluent is highly visible and undesirable (Nigam *et al*., 1996). There are more than 100,000 commercially available dyes with over 700,000 ton of dyestuff produced annually (Meyer, 1981). Synthetic dyes are chemically diverse and divided into azo, triphenylmethane or heterocyclic/polymeric structures (Cheunbarn *et al*., 2008). These dyes are designed to be stable and long lasting colorants and are usually recalcitrant in natural environment. After release into water bodies, these dyes have negative impact on photosynthesis of aquatic plants and the azo group (N = N) in dyes are converted to aromatic amines which are possible human carcinogens (Banat *et al*., 1996). Some dyes and their breakdown products also have strong toxic and mutagenic effect on living organisms (Pinheiro *et al*., 2004). Discharge of textile dyes without proper treatment may lead to bioaccumulation that may incorporate into food

chain and effect human health. Azo dyes inhibit the activity of tyrosinase enzyme that leads to inhibition of melanin synthesis and results in hypopigmentation (Dubey *et al*., 2007). Removal of dyes from effluents is usually by physicochemical means which are infrastructure intensive, costly and although the dyes are removed, accumulation of concentrated sludge creates disposal problem (Mahbub *et al*., 2011). On the other hand, biological treatment based on microbial transformation of textile dyes hold promises in providing a lower treatment cost and a more efficient mean of effluent treatment (Dos Santos *et al*., 2007). A number of microorganisms namely *Pseudomonas*, *Kurthia* (Zimmermann *et al*., 1982), *Aeromonas* (Chen *et al*., 2008), *Proteus mirabilis*, *Rhodococcus globerulus* (Joshi *et al*., 2008), *Bacillus* spp., *Micrococcus luteus*, *Staphylococcus aureus* (Mahbub *et al*., 2011) and white rot fungus *Phanerochaete* (Swamy and Ramsay, 1999) has already been reported of having the capability of decolorizing textile dye. The present study was undertaken to investigate the ability of dye degradation of bacteria those survive in local dye industry effluent.

MATERIALS AND METHODS

Collection of samples: Effluent samples were collected from eight different locations of discharge in a textile dyeing industry located in Gazipur, Dhaka, Bangladesh during June 2011. The color, pH and temperature of the collected samples were recorded at the time of sample collection. Samples were transported to the laboratory in sterile glass bottles and stored at 4°C before and after experiment.

Isolation of dye decolorizing bacteria: Effluent samples were enriched at 35°C for 48 h in Nutrient Broth (HiMiedia, India) containing 100 ppm cibacron red FN-R dye. After enrichment, 100 μL of enriched broth was spread on Nutrient Agar plate supplemented with 100 ppm cibacron red FN-R dye and incubated at 35°C for 48 h. After incubation, bacterial colonies showing clear zones were isolated as potential decolorizing bacteria as clear zones indicate the ability to degrade cibacron red FN-R.

Identification of selected bacterial isolates: The selected dye decolorizing isolates were identified on the basis of their morphological, physiological and biochemical characters as described in the Bergey's Manual of Determinative Bacteriology, 8th edition (Buchanan and Gibbons, 1974). The tests carried out were gram staining, spore staining, IMViC, starch hydrolysis, protein hydrolysis, catalase, oxidase, H_2S production, nitrate reduction, citrate utilization, gelatin liquefaction, growth response at different temperatures and salt concentrations, fermentation of glucose, lactose, arabinose, mannose, sucrose, mannitol, melibiose, trehalose and rhamnose.

Dye decolorization assay: The dyes used throughout the study were reactive azo dyes that are frequently used in most of the textile industries in Bangladesh. Seven textile dyes namely novacron orange, cibacron red FN-R, novacron yellow, novacron blue, novacron navy, terasil green and novacron red were used in the present study. The above mentioned dyes were chosen because they are the major types of dyes that produce the greatest variety of colors and are applicable at a wide range of temperature depending upon their chemical structures. These dyes were mixed with nutrient broth at a concentration of 0.01, 0.05 and 0.1 mg L^{-1}. These dye supplemented broths were dispensed into screw cap test tubes (10 mL per test tube) and autoclaved. These modified broths were inoculated with isolated test organisms individually and incubated at 35°C for 3 days

in static condition. After incubation period, decolorization of the dyes by selected isolates was determined at their respective maximum wavelength in the culture supernatant using a UV-Spectrophotometer. 1.5 mL of broth was centrifuged at 10000 rpm for 10 min. After centrifugation these supernatants were subjected to UV-spectrophotometry and absorbance was recorded. From this absorbance, residual dye concentration was calculated from standard curve plotted using different concentration of respective experimental dyes. The efficiency of color removal was expressed as the percentage ratio of the decolorized dye concentration to that of initial one based on the following equation:

$$\text{Decolorization (\%)} = \frac{\text{Initial dye concentration-Residual dye concentration}}{\text{Initial dye concentration}} \times 100$$

RESULTS AND DISCUSSION

Physicochemical and microbiological examination of samples: Physicochemical properties namely color, temperature, pH were analyzed and recorded at sampling sites and total viable bacteria were enumerated at the laboratory for all of the samples (Table 1). The effluent samples color was varied greatly depending upon their collection point that may be due to the variation of dye residual concentrations in the samples. During collection, temperatures of the samples were found to vary from 32-40°C, pH of the samples were recorded to range from 6.5-9.2. Increased pH is due to excessive use of carbonate, bicarbonate, H_2O_2 and NaOH during bleaching process (Colowick *et al*., 1988). Total viable bacteria were found in the samples ranging from 6.3×10^4-2.8×10^8 CFU mL^{-1}. The high bacterial counts reflect that the textile dyeing effluents are good sources of nutrients to facilitate the growth of certain bacteria (Mihir *et al*., 2006).

Characterization and identification of bacterial isolates: At the stage of preliminary screening, a total number of eighteen bacterial colonies having clear zone and distinct colony morphology were isolated from cibacron red FN-R dye supplemented nutrient agar plates. All the isolates were further screened on supplemented nutrient agar plates and six isolates namely A1, B2, B7, C1, C4 and D3 were found to be able to form clear zones on the plates. The selected six isolates were subjected to morphological, physiological and biochemical characterization. The colony characters on nutrient agar and microscopic features are presented on Table 2. The colony morphology was found to differ in color, form, margin, surface and elevation. These were found Gram positive, non acid fast, rod shaped with single, paired or short chain arrangement as showed in Fig. 1a. Except isolate C4, all other isolates were spore former (Fig. 1b). The spores were centrally located in the vegetative cell. The selected six isolates were tested to grow in different temperatures

Table 1: The color, temperature, pH and total viable bacterial count of the collected effluent samples

Place of the collection	Color	Temperature (°C)	pH	Total bacterial count (CFU mL^{-1})
Before discharge	Pink	40	7.5	2.8×10^8
After discharge	Ash	35	9.2	6.7×10^4
Distance (10 m)	Ash	37	7.4	6.3×10^4
Distance (30 m)	Ash	32	8.5	1.5×10^5
Nearby canal	Blackish	30	9.2	2.0×10^7
Before treatment	Orange	38	7.5	2.9×10^5
After treatment	Colorless	35	9.2	3.2×10^5
Effluent sludge	Ash	34	6.5	8.1×10^5

Table 2: Colony characters and morphology of the selected isolates

Isolate	Shape	Color	Elevation	Margin	Surface	Pigmentation	Morphology
A1	Irregular	Cream	Convex	Entire	Smooth	No	Short rod, single
B2	Irregular	Cream	Raised	Irregular	Smooth	No	Short rod, single, pair
B7	Irregular	Yellow	Raised	Entire	Smooth	No	Rod, long chain containing 19 to 20 cells
C1	Irregular	Cream	Raised	Irregular	Rough	No	Rod, long chain
C4	Irregular	Whitish	Convex	Irregular	Concentric	No	Short rod, single
D3	Irregular	Cream	Convex	Entire	Rough	No	Short rod, single

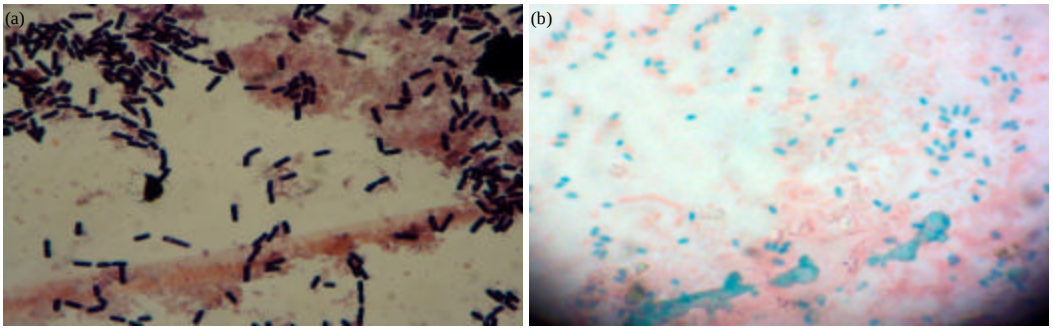

Fig. 1(a-b): (a) Vegetative cells of the isolate B7 and (b) Endospores of the isolate D3 in 100x magnification

using nutrient broth media. No growth was observed at 4 and 10°C. All isolates could grow at 27, 37 and 45°C except B2 and B7. Isolate B2 and B7 could not grow in 45 and 27°C, respectively. The isolates failed to tolerate higher concentrations of NaCl (7 and 8%) and all were able to grow at 2 and 4% NaCl except isolate B2.

Based on the results of biochemical test parameters (Table 3), the isolates were identified as *Bacillus subtilis* (B2, B7 and C1), *Bacillus megaterium* (D3), *Erysipelothrix* (C4) and *Amphibacillus xylanus* (A1). The identification was done by comparing the experimental results of biochemical test parameters with the limited descriptions in Bergey's Manual of Determinative Bacteriology (8th Edn.).

Decolorization of dye by the isolates: In the present investigation, isolates were tested for their ability to decolorize different concentrations (0.01, 0.05 and 0.1 mg L^{-1}) of seven textile dyes. After 3 days of incubation period, satisfactory dye decolorization by selected bacteria was seen. Decolorization pattern of 0.01 mg L^{-1} concentration of the experimental dyes are given in Table 4. The highest decolorization (99%) was found for the dyes novacron yellow and novacron orange by isolates A1 (*A. xylanus*) and B2 (*B. subtilis*). Almost all dyes in 0.01 mg L^{-1} concentration were found to be decolorized more than 80% by the tested isolates. Only novacron red could not be decolorized by isolates A1, B7 and C1 after three days of incubation.

Decolorization percentage of the experimental dyes at 0.05 mg L^{-1} concentration was found lower than 0.01 mg L^{-1} concentration. Decolorization percentages of experimental dyes by tested bacteria are showed in Table 5. All isolates failed to decolorize 0.05 mg L^{-1} concentration of

Table 3: Biochemical characteristics of the selected six isolates

	Biochemical test results of isolates					
Test parameters	A1	B2	B7	C1	C4	D3
Indole	-	-	+	+	-	-
MR	+	+	+	+	-	-
VP	-	-	-	-	-	-
Citrate utilization	-	+	+	+	+	-
Gelatin liquefaction	+	+	+	+	+	+
Glucose fermentation	+	-	-	+	-	-
Arabinose fermentation	-	+	+	-	-	+
Mannose fermentation	-	-	-	-	-	-
Sucrose fermentation	+	-	-	+	-	+
Mannitol fermentation	-	-	-	-	-	-
Xylose fermentation	-	+	+	-	-	+
Trehalose fermentation	+	+	-	-	+	+
Lactose fermentation	-	-	+	-	-	+
Melibiose fermentation	+	+	-	+	+	-
Rhamnose fermentation	-	-	-	-	+	-
Sorbitol fermentation	+	+	-	+	+	-
Starch hydrolysis	+	-	-	+	+	+
Oxidase	-	-	-	-	-	+
Proteolysis	-	+	+	-	+	-
Catalase	+	+	+	+	+	+
Isolates*	*Amphibacillus xylanus*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Erysipelothrix*	*Bacillus megaterium*

+: Positive reaction, -: Negative reaction, *Isolates identified on the basis of limited descriptions in Bergey's Manual of Determinative Bacteriology (9th Edn.)

Table 4: Decolorization of 0.01 mg L^{-1} concentration of experimental dyes by selected isolates at 35°C after 3 days of incubation

	Percentage of decolorization						
		Novacron					
Isolate	Cibacron orange FN-R	Blue	Yellow	Orange	Navy	Red	Terasil green
A1	94.8	70	88	99	96	0	92
B2	87.0	80	99	94	96	80	80
B7	80.0	50	91	91	97	0	90
C1	75.0	60	92	97	92	0	92
C4	80.0	50	89	94	94	20	90
D3	60.0	80	50	92	95	60	60

Table 5: Decolorization of 0.05 mg L^{-1} concentration of experimental dyes by selected isolates at 35°C after 3 days of incubation

	Percentage of decolorization						
		Novacron					
Isolate	Cibacron orange FN-R	Blue	Yellow	Orange	Navy	Red	Terasil green
A1	52	58	84	58	80	0	90
B2	80	25	89	60	75	0	80
B7	70	40	50	80	20	0	80
C1	70	39	92	90	85	0	91
C4	80	22	70	70	60	0	80
D3	60	40	0	80	60	0	50

Table 6: Decolorization of 0.1 mg L^{-1} concentration of experimental dyes by selected isolates at 35°C after 3 days of incubation

	Percentage of decolorization						
		Novacron					
Isolate	Cibacron orange FN-R	Blue	Yellow	Orange	Navy	Red	Terasil green
A1	50	10	40	20	71	0	54
B2	20	23	83	0	74	0	62
B7	40	37	50	40	0	0	62
C1	60	9	90	40	37	0	62
C4	50	0	0	60	76	0	54
D3	60	20	0	20	0	0	0

novacron red after three days of incubation. Decolorization of all other dyes except novacron red was satisfactory and the highest percentage of decolorization was observed greater than 90%.

Percentage of decolorization of the experimental dyes was found lower in the highest concentration 0.1 mg L^{-1} (Table 6). Decolorization rate of most of the dyes was moderate but decolorization percentage of novacron yellow by isolate B2 (*B. subtilis*) and C1 (*B. subtilis*) was found more than 80% even in their highest concentration.

In the present study, the isolates were unable to decolorize novacron red. Except novacron red, all other dyes were decolorized by at least 50% at their highest concentrations after 3 days of incubation. Similar decolorization potential of *Pseudomonas, Bacillus, Clostridium* and *Citrobacter* was reported previously (Sharnaik and Kaneker, 1995; Mihir *et al*., 2006; Sukumar *et al*., 2007; Wang *et al*., 2009). Many halophilic bacteria have been reported to be involved in dye decolorization (Uddin *et al*., 2007). Moderately halotolerant *Bacillus* was reported to decolorize azo dye red 2G in many studies (Oren *et al*., 1992; Ventosa *et al*., 1998). Azo-reductase is the enzyme which degrades azo-bond in textile dye and the azo-reductase gene has been identified in a number of bacteria namely *Azospirillum brasilense, B. subtilis, Bacillus stearothermophilus, Pseudomonas aeruginosa* and *Mycoplasma pneumonia* (Suzuki *et al*., 2001). In this study, most of the isolates were from *Bacillus* group which correlate the previous studies. The other two bacteria *Erysipelothrix* and *Amphibacillus xylanus* identified in our study are not still reported as decolorizing bacteria in literature and further investigation is needed to understand their decolorization capability.

CONCLUSION

The present study clearly demonstrates that the bacterial community in textile effluents of Bangladesh has the ability to degrade and decolorize various types of dyes used in such industries. The potential of these bacteria can be exploited to remove residual dye in textile wastes. Further detailed study is needed to optimize process parameters for bioremediation of textile effluents using these bacterial isolates.

REFERENCES

Banat, I.M., P. Nigam, D. Singh and R. Marchant, 1996. Microbial decolourations of textile dye containing effluents: A review. Bioresou. Technol., 58: 217-227.

Buchanan, R.E. and N.E. Gibbons, 1974. Bergey's Manual of Determinative Bacteriology. 8th Edn., Williams and Wilkins Co., Baltimore, MA, USA.

Chen, B.Y., W.M. Chen, F.L. Wu, P.K. Chen and C.Y. Yen, 2008. Revealing azo-dye decolorization of indigenous *Aeromonas hydrophila* from fountain spring in Northeast Taiwan. J. Chin. Inst. Chem. Eng., 39: 495-501.

Cheunbarn, T., S. Cheunbarn and T. Khumjai, 2008. Prospects of bacterial granule for treatment of real textile industrial wastewater. Int. J. Agric. Biol., 10: 689-692.

Colowick, S.P., N.O. Kaplan, W.A. Wood and S.T. Kellogg, 1988. Methods in Enzymology: Biomass: Part A: Cellulose and Hemicellulose. Vol. 160, Academic Press, London, UK., ISBN-13: 9780121820619, pp: 632-634.

Dos Santos, A.B., F.J. Cervantes and J.B. van Lier, 2007. Review paper on current technologies for decolourisation of textile wastewaters: Perspectives for anaerobic biotechnology. Bioresour. Technol., 98: 2369-2385.

Dubey, S.K., A. Pandey, A.K. Bajaj and K. Misra, 2007. Some commercial azo dyes as inhibitors of mushroom tyrosinase DOPA oxidase activity. J. Pharmacol. Toxicol., 2: 718-724.

Joshi, T., L. Iyengar, K. Singh and S. Garg, 2008. Isolation, identification and application of novel bacterial consortium TJ-1 for the decolourization of structurally different azo dyes. Bioresour. Technol., 99: 7115-7121.

Khaled, A., A. El-Nemr, A. El-Silkaily and O. Abdelwahab, 2009. Removal of direct N Blue-106 from artificial textile dye effluent using activated carbon from orange peel: adsorption isotherm and kinetic studies. J. Hazard. Mater., 165: 100-110.

Mahbub, K.R., J. Ferdousi and M.N. Anwar, 2011. Demonstration of decolorization of various dyes by some bacterial isolates recovered from textile effluents. Bangladesh J. Sci. Ind. Res., 46: 323-328.

Meyer, U., 1981. Biodegradation of Synthetic Organic Colorants. In: Microbial Degradation of Xenobiotics and Recalcitrant Compounds, Leisinger, T., R. Hutte, A.M. Cook and J. Nuesch (Eds.). Academic Press, London, UK., pp: 371-385.

Mihir, L.S., R.K. Mahbubar and I. Farida, 2006. Bacteria associated with textile dyeing industrial effluents and their depolarization potentiality. Bangladesh J. Microbiol., 23: 52-54.

Nigam, P., I.M. Banat, D. Singh and R. Marchant, 1996. Microbial process for the decolorization of textile effluent containing azo, diazo and reactive dyes. Process Biochem., 31: 435-442.

Oren, A., P. Gurevich, M. Azachi and Y. Henis, 1992. Microbial degradation of pollutants at high salt concentration. Biodegradation, 3: 387-398.

Pinheiro, H.M., E. Touraud and O. Thomas, 2004. Aromatic amines from azo dye reduction: Status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. Dyes Pigments, 61: 121-139.

Saraswathi, K. and S. Balakumar, 2009. Biodecolourization of azodye (pigmented red 208) using *Bacillus firmus* and *Bacillus laterosporus*. J. Biosci. Technol., 1: 1-7.

Sharnaik, S. and P. Kaneker, 1995. Bioremediation of colour of methyl violet and phenol from a dye-industry waste effluent using *Pseudomonas* spp. isolated from factory soil. J. Applied Bacteriol., 79: 459-469.

Sukumar, M., A. Sivasamy and G. Swaminathan, 2007. Decolorization of textile dye effluent by genetically improved bacterial strains. Applied Biochem. Biotechnol., 136: 53-62.

Suzuki, Y., T. Yoda, A. Ruhul and W. Sugiura, 2001. Molecular cloning and characterization of the gene coding for azoreductase from *Bacillus* sp. OY1-2 isolated from soil. J. Biol. Chem., 276: 9059-9065.

Swamy, J. and J.A. Ramsay, 1999. The evaluation of white rot fungi in the decolouration of textile dyes. Enz. Microb. Technol., 24: 130-137.

Uddin, M.S., J. Zhou, Y. Qu, J. Guo, P. Wang and L. Zhao, 2007. Biodecolorization of azo dye acid red B under high salinity condition. Bull. Environ. Contam. Toxicol., 79: 440-444.

Ventosa, A., J.N. Joaqin and A. Oren, 1998. Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev., 62: 504-544.

Wang, H., J.Q. Su, X.W. Zheng, Y. Tian, X.J. Xiong and T.L. Zheng, 2009. Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3. Int. Biodeterior. Biodegrad., 63: 395-399.

Zimmermann, T., H.G. Kulla and T. Leisinger, 1982. Properties of purified orange II azoreductase, the enzyme initiating azo dye degradation by *Pseudomonas* KF46. Eur. J. Biochem., 129: 197-203.