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Biodegradation of textile dyes by bacteria isolated from textile industry effluents

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A country like Bangladesh where textile industries are the main source of developing economy, pollution problem from such industries creates a huge risk for the environment. Textile industries discharge a huge amount of effluent containing various harmful chemicals including synthetic dyes that are very stable and threat to the living organisms. This study deals with the potential decolorization and biodegradation of Bemacron Yellow HP-2R (BY), Bemacron Red RS (BR) and Bemacron blue RS 01 (BB) dyes using bacteria isolated from textile effluent. The effluent and soil samples were collected from different locations of discharge point. Only two isolates were screened out after primary screening using dye supplemented nutrient agar media. Following colony morphology, physiology and biochemical analysis, they were presumptively identified as *Bacillus* sp. and *Staphylococcus aureus*. They were subjected to decolorization of 0.002 g/l BY, BR and BB dyes. *Bacillus* sp. showed superior decolorization potential of BR (71%) and BB (83%) dyes after 5 days of incubation. Whereas, *Staphylococcus aureus* showed 79% decolorization of BY dye after 5 days incubation. Decolorization efficacy can be further improved by optimizing environmental conditions and process parameters.

Keywords: Effluent, Textile dye, Biodegradation, Decolorization.

INTRODUCTION

Textile and clothing industries are the most pivotal sources of rapidly developing economy of Bangladesh. In the financial year 2016-2017, ready-made garments account for 80.7% of the foreign exchange earnings and 12.36% of the gross domestic products (1). All those huge number of textile industries require a large volume of water where 90% appears as waste water containing various types of chemicals including heavy metals, chlorinated compounds, pigments and textile dyes (2, 3). It is estimated that 10-15% of the dye are lost in the effluent during production and processing operations (4). Synthetic dyes are widely used in textile, leather, cosmetics, agriculture and food industries. These dyes are commonly used as these are available in broad range of color at low cost (5). Textile industries are reported to use the largest amount of dyes (60-70%) compared to other industries (6, 7).

Synthetic dyes are chemically diverse organic compounds containing unsaturated fatty acids as -C=C-, -N=N-, -C=N- in them. These properties of dyes allow them to fix firmly to the fibers and make them extremely stable to light and washing, and remain stable for long time in the environment (8). Due to their recalcitrant nature, they remain in the nature for long time and interfere with penetration of light and photosynthesis. Some dyes and their degraded products have toxic and mutagenic effects on flora and fauna (9). Strict attention should be taken in using dyes industrially. If effluents are released in the environment without treatment, bioaccumulation may occur in food chain and increase the concentration of harmful chemicals which ultimately, affect human and animal lives. It also may have adverse effect on chemical oxygen demand (COD) and biological oxygen demand (BOD) of aquatic environment (10, 11). All these have severe and long lasting impact on nature.

Industrial effluents containing variety of dyes can be treated by a number of physicochemical approaches as, filtration, adsorption, coagulation, chemical precipitation, chemical oxidation, reduction, photolysis, use of activated carbon, chemical flocculation etc. (2, 8, 12). However, these approaches are expensive, less efficient and produces enormous amount of sludge that are difficult to dispose off and consequently create land pollution (13). Biological approach using microorganisms are gaining interest due to their low cost, less sludge production and eco-friendly nature. Microorganisms can decolorize and even completely mineralize azo dyes that are widely used in various industries under certain environmental conditions (14). Moreover, consortia of *Proteus* spp., *Pseudomonas* spp. and *Acinetobacter* spp. were capable of degrading methyl red and carbol fuchsin dyes (8). The aim of this study is to isolate bacteria from textile industry effluents that can degrade Bemacron Yellow HP-2R (BY), Bemacron Red RS (BR) and Bemacron Blue RS 01 (BB) dyes utilized by the same industry for potential use in bioremediation of industrial effluents and to reduce the havoc to mankind.

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MATERIALS AND METHODS

Chemicals and Media. Textile dyes namely Bemacron Yellow HP-2R (BY), Bemacron Red RS (BR) and Bemacron Blue RS 01 (BB) (CHT, Switzerland) were collected directly from a textile industry located in Savar, Dhaka. The chemicals used in this work were of analytical grade. Microbiological media and medium ingredients were purchased from Oxoid, UK.

Sample collection. The untreated effluents and soil samples were collected from a discharge panel of a textile industry located in Savar, Dhaka. Three effluent samples namely effluent 1 (0 m), effluent 2 (5 m) and effluent 3 (10 m), and three nearby soil samples namely soil 1 (0 m), soil 2 (5 m), and soil 3 (10 m) were collected from different locations of drainage canal. The temperature, pH and color of the samples were recorded that were collected in early November, 2018. Samples were collected in sterile plastic bottles with sterile spatula and transported to the laboratory and stored at 4°C before and after experiment.

Screening and isolation of dye decolorizing bacteria. Before inoculation into solid media, the collected samples were enriched by inoculating 500 µl of samples into 10 ml of nutrient broth supplemented with 0.002 g/l BY, BR and BB dyes at room temperature for 24 h under shaking conditions (120 rpm). Following enrichment, samples were diluted and 100 µl of diluted enriched samples were spread on nutrient agar media supplemented with 0.002 g/l BY, BR and BB dyes. The plates were incubated at 37°C for 48 h to detect bacteria that can withstand the selected dye concentration. Morphologically distinct bacterial isolates were distinguished for further study and pure culture of those isolates was cultured on nutrient agar media for storage at 4°C (8).

Identification of selected bacterial isolates. The isolated bacteria were subjected to Gram staining, morphological and biochemical characterization as described by the Bergey's Manual of Determinative Bacteriology, 8th edition. The tests performed were IMViC, starch hydrolysis, catalase, oxidase, H_2S production, fermentation of lactose, dextrose and sucrose.

Dye decolorization assay. Decolorization experiment of those three selected dyes was performed by using 0.002 g/l of dye in 15 ml test tubes containing 10 ml of nutrient broth. A 100 µl of 24 h old bacterial culture corresponding to Mcfarland standard 0.5 was used as inoculum to inoculate the dye supplemented broth. The inoculated test tubes were incubated at 37°C for 1, 3 and 5 days to check the absorbance. Following incubation, decolorization of dyes by selected isolates was determined at their specific maximum wavelength in the culture supernatant using a UV-spectrophotometer. After incubation at each time period, samples were centrifuged at 10,000 rpm for 10 min and the supernatants were subjected to UV-spectrometry and the absorbance was recorded. The uninoculated media with BY, BR and BB dyes were used as respective blank for the dye decolorization assay. The percentage of dye decolorization was calculated as stated before (15).

$$\text{Decolorization (\%)} = \frac{\text{(Initial OD-Final OD)}}{\text{Initial OD}} \times 100$$

RESULTS

Physicochemical properties of samples. Physicochemical properties as color, temperature and pH of 3 effluent samples and 3 soil samples were recorded and presented in Table 1. The color of all the effluents was ash and soil samples was blackish due to the mixture of different types of organic and inorganic chemicals. The pH of all the samples varied from 6.9 to 7.8 that were near to neutral. The temperature of all the samples ranged from 32 to 37°C.

Table 1. The color, temperature and pH of the collected samples

Place of collection	Color	Temperature (°C)	pH
Effluent 1 (0 m)	Ash	37	7.8
Effluent 2 (5 m)	Ash	33	7.5
Effluent 3 (10 m)	Ash	32	7.5
Soil 1 (0 m)	Blackish	34	6.9
Soil 2 (5 m)	Blackish	33	7.0
Soil 3 (10 m)	Blackish	33	6.9

Isolation of dye degrading bacteria. During screening, only two bacterial isolates were identified from those 0.002 g/l dye supplemented nutrient agar plates. The isolates showed distinct morphological characteristics on the dye supplemented nutrient agar media. Subsequently, the isolates were subjected to morphological, physiological and biochemical characterizations. The microscopic features, colony characteristics on nutrient agar media and biochemical characters are shown in Table 2, Table 3 and Table 4.

Decolorization potential of the isolates. In the present study, selected isolates were tested independently for their ability to decolorize 0.002 g/l of the three textile dyes. The experiment was performed in a time-dependent manner for 1, 3 and 5 days. Decolorization percentage of the dyes with *Bacillus* sp. was shown in Figure 1. It was able to decolorize 60-83% of all dyes tested. Decolorization of BY, BR and BB dyes was observed in a time-dependent manner. After 5 days incubation, BY, BR and BB dyes showed nearly 72%, 71% and 82% decolorization, respectively.

Table 2. Morphology of the selected isolates

Microscopic Observation	*Bacillus* sp.	*Staphylococcus aureus*
Shape	Short rod	Cocci
Arrangement	Single, double and in cluster	Cluster
Gram reaction	Positive	Positive
Capsule staining	Present	-

Table 3. Colony characters of the selected isolates

Characteristics	*Bacillus* sp.	*Staphylococcus aureus*
Size	Moderate	Small
Shape	Circular	Circular
Margin	Entire	Entire
Elevation	Raised	Raised
Consistency	Butyrous	Butyrous
Texture	Smooth	Smooth
Opacity	Opaque	Opaque
Pigmentation	Creamy white	Golden yellow

Decolorization percentage by *Staphylococcus aureus* is presented in Figure 2. Here, *S. aureus* time-dependently

Table 4. Biochemical characteristics of the selected isolates

Media	Tests	Observation	
		***Bacillus* sp.**	***S. aureus***
Starch agar plate	Starch Hydrolysis	+ve	-ve
MYP media	*Bacillus* presence	+ve	-ve
Simmons citrate agar slant	Citrate utilization	-ve	-ve
GPB Broth	Methyl Red test	-ve	+ve
GPB Broth	Voges-Proskauer test	+ve	-ve
Indole test	Indole production	+ve	-ve
Lactose	Carbohydrate fermentation	+ve	-ve
Dextrose	Carbohydrate fermentation	+ve	-ve
Sucrose	Carbohydrate fermentation	+ve	-ve
Nutrient agar	Oxidase test	+ve	-ve
Nutrient agar	Catalase test	+ve	+ve
TSI	H_2S production	+ve	-ve

MYP denotes for Mannitol egg Yolk Polymyxin, GPB for Glucose Phosphate Broth, TSI for Triple Sugar Iron, +ve for Positive, –ve for Negative and *S. aureus* for *Staphylococcus aureus*.

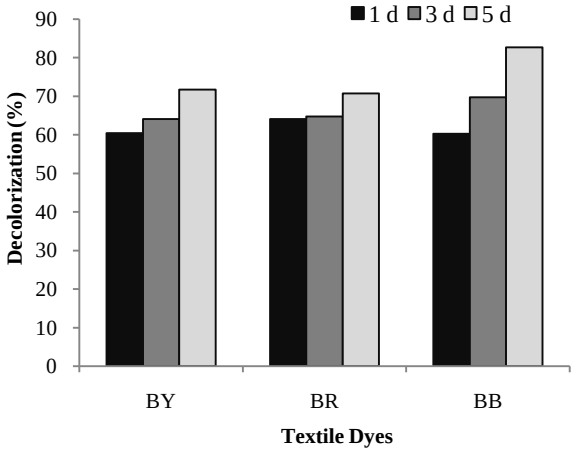

Figure 1: Decolorization of BY, BR and BB dyes by *Bacillus* sp. after 1, 3 and 5 days.

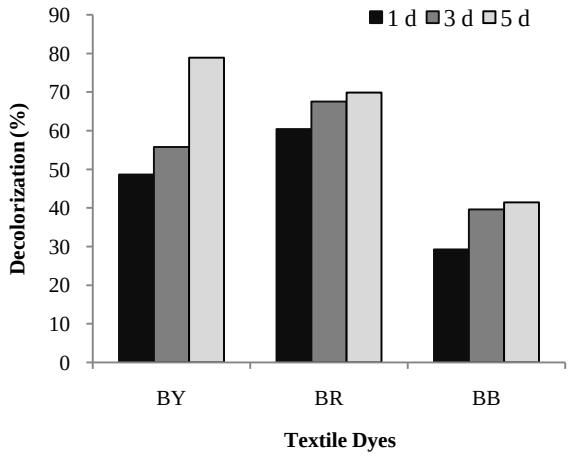

Figure 2: Decolorization of BY, BR and BB dyes by *Staphylococcus aureus* following 1, 3 and 5 days.

induced dye decolorization from approximately 49% to 79% for BY dye, 60% to 70% for BR dye and 29% to 41% for BB dye. Isolate A1 showed increased dye decolorization ability than Isolate A2 in the given environmental conditions. Percentage of decolorization of BB dye by Isolate A1 was found to be the highest and that was nearly 83% after 5 days. For isolate A2, the highest decolorization was observed for BY dye and that was 79% following 5 days of incubation.

DISCUSSION

Different effluents and soil samples from a discharge of textile industries located in Savar, Dhaka were collected to isolate dye degrading organisms. Only two isolates were found to grow profusely in all the three dye supplemented nutrient agar plates. According to colony morphology, physiology and biochemical characterization, those isolates were presumptively identified as *Bacillus* sp. and *Staphylococcus aureus*. Between the two isolates, *Bacillus* sp. is commonly found to degrade various textile dyes isolated from effluents of textile and printing press industries (16, 17). *Staphylococcus aureus* was previously isolated from textile effluent and reported to degrade cibacron orange FN-R, cibacron yellow F-4G, cibacron blue FN-R, cibacron navy FN-B, terasil black WNS and terasil red W-FS dyes (18). Both the isolates showed potential of all the three textile dye degrading capability. Further optimization of environmental condition and incubation period can improve the performance of these bacteria. Effect of pH, temperature, initial dye concentration and inoculum size greatly influences dye degradation competence. It was observed that dye degradation rate was remarkably decreased when higher concentrations of dye was used. Similarly, with reducing inoculum size, the degradation rate also reduced and most significant result was obtained when 10% inoculum was used (19).

In our study, dye decolorization was synonymous to dye degradation as following centrifugation, bacterial isolates were pelleted and retained its original color that indicated that the color of the dyes did not adsorb onto bacterial cells. Therefore, decolorization was due to degradation not because of adsorption onto bacterial isolates. There was neither growth nor decolorization showed in the control tubes, thus, the decolorization was due to the metabolic activity of the isolates.

Our spectrophotometric data showed a great potential of BY, BR and BB dye decolorization by both of the isolates. Only few organisms are capable of utilizing the synthetic dyes present in the enviorenment. The decolorization rate can be induced by performing the optimization experiment with various time, temperature, pH, inoculum size and can achieve a optimal condition for performing the experiment with great success. Furthermore,

consortium of isolates showed increased decolorization of methyl red and carbol fuchsin to 100% and 96%, respectively within only 24 h (12). Therefore, there is a chance to increase dye decolorization by applying mixed consortium of microorganisms. Here, co-metabolic activities might be involved to make dyes accessible to one organism that otherwise be unable to degrade through partial metabolism by other organisms.

CONCLUSION

The natural color is replaced by synthetic dyes due to rapid industrialization of variety of commercial products. They are used in tanning, textile, printing, leather and many more industries. Their extensive usage causes severe water pollution which is harmful for survival of micro- and macro-biota. There are containment to expose those harmful effluent in the nature after physical and chemical treatment. But, many industries dispose their effluent with no treatment or after partial physical and chemical treatment. These problems can be reduced by introducing microbes to degrade the residual dyes present in the effluent with minimal cost. The potential of isolated dye degrading microbes can be utilized in bioremidiation of such pollutants. Detailed studies on process parameters for bioremediation and genetic manipulation are required to improve the potential of these organisms to solve the pollution problems caused by synthetic dyes.

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