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ISOLATION AND SCREENING OF DYE DEGRADING MICROORGANISMS FROM INDUSTRIAL WASTE OF DHAKA CITY

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Abstract– Textile and dying industrial effluents are the major environmental concern for the present world. Untreated or partially treated effluent can contain toxins for environment and human beings. And bioremediation is one of most advance and ecofriendly approach for effluent treatment. In this study, different effluent and soil samples were collected from textile industry of Dhaka city. Here total 12 bacteria were isolated from collected samples using spread plate method. Among them *Staphylococcus* spp., *Bacillus* spp. and *Enterobacter* spp. are predominated which is confirmed by biochemical methods. All thus isolates were used for the further studies like dye degrading ability using decolorizing activity. Here, decolorizing activities of the isolated bacteria were measured using a photo electric colorimeter after aerobic incubation in different time intervals of the isolates in direct red in nutrient broth containing 0.1% of dye. In this study, some of isolates have capabilities to degrade dyes completely within eight days. So, the degrading efficacy of isolated bacteria indicate that these bacteria can be used in large scale treatment in textile industry.

INTRODUCTION

Textile industries are the major economic sector in Bangladesh but they are becoming problematic day by day because these industries release large amount of untreated or improperly treated discharge into the environment (Hossain *et al.,* 2018, Sakamoto *et al.,* 2019). During dyeing processes, approximately 2%–50% of dyes released in the discharge that don't bind to the fiber (Cui MH *et al.* 2016). Most of these dyes are potentially toxic to aquatic life (Abe *et al.,* 2019) and some are even carcinogenic and mutagenic to humans (Balakrishnan *et al.,* 2016; Brüschweiler and Merlot, 2017). Furthermore, color of the dyestuff interrupts the aquatic environment by reducing light penetration, gas solubility and interference of phytoplankton's photosynthesis (Saranaik *et al.,* 1995).

Azo dye is one of the main constituent of the textile and paper dyes that hold almost 70% of these dyes (Carliell *et al.,* 1995). These dyes are mostly used in the textile industry for their extensive variety of color shades and brilliant colors. Textile dyes pollute the environment heavily and contribute to different diseases like Contact dermatitis, Pigmented purpuric dermatoses, Schamberg disease, Lichen aureus and Majocchi disease (Ozcan *et al.,* 2019; Peralta-Zamora *et al.,* 2003; Rajaguru *et al.,* 2002; Umbuzeiro *et al.,* 2005). Azo dyes are recalcitrant xenobiotics and most of these compounds are carcinogenic or contain mutagenic properties (Kunz *et al.,* 2002; Wang *et al.,* 2009). Methods like chemical precipitation, adsorption, and flocculation have substantial disadvantages, which include complex structural set-up, huge chemical and power consumption and formation of a large volume of sludge and 90% of textile dyes could remain unaffected by conventional treatment methods (McMullan *et al.,* 2001; Peralta-Zamora *et al.,* 2003). Hence, bioremediation is most advanced and environmentally friendly approach to treat these toxic dyes before discharge where different dye degrading bacteria will be key player to remove the toxic dyes from the environment.

Many microorganisms have been reported for their ability to decolorize azo dyes such as bacteria (Parmar and Shukla, 2018), fungi (Zhang *et al.,* 2016; Šlosarèíková *et al*., 2017), actinomycetes (Linde *et al*., 2014) and algae (Otto and Schlosser, 2014). Different study on industry effluent proved that using microbes is the best solution to decolorize and detoxify a wide range of toxic dye where various bacterial species including *Enterobacter* spp., *Bacillus* spp., *Pseudomonas* spp., *Aeromonas* spp. and *Halobacter* spp. have been accounted to breakdown different dyes (Salar *et al.,* 2012; Feng *et al.,* 2012; Mendes *et al*., 2011; Telke *et al.,* 2008). In most cases, bacteria disintegrate azo bonds of the dyes, which result in the formation of colorless amines and subsequently simpler compounds (Stolz *et al.,* 2001).

This study endeavors to isolate and distinguish bacterial strains having strong dye decolorizing capability, which can be potential applicant agent for the remediation of textile dye effluent.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from 6 (six) distinct discharge points of dying industry in Dhaka, Gazipur and Naraynganj District of Bangladesh. The Samples were brought to the laboratory using sterile Plastic Polybag and Plastic Bottle in a cooler box and stored at 4 °C.

Chemicals source

Fresh dyes were collected from Maa Color Plus Dyeing House, Islampur Road, Dhaka, Bangladesh.

Screening of Dye-Decolorizing Bacteria

Soil samples were enriched by co-incubating in nutrient broth containing 0.01% of each dye at 35 °C for 24 hours. 1 mL of enrichment culture was put onto 100 mL nutrient broth medium supplemented with 0.01% of respective dye following incubation at 35 °C for 48 hours. The resulting dyes are exhibiting decolorization.

Physiochemical and microbial analysis of textile wastes

The collected waste soil samples have been analyzed to determine its Physical parameters. The various parameters such as pH, Color and Odor were analyzed as described in standard methods for textile waste analysis and analyzed Total Viable bacterial count (TVBC).

Isolation of pure colony and preparation of pure culture

In this study, 10^{-1} to 10^{-8} dilutions of the different samples were used for preliminary isolation of dye decolorizing organisms. In order to isolate pure colony for this experiment, collected six samples were directly plated on nutrient agar plate in the presence of 0.01% synthetic dye and incubated at 37 °C for 24-72 hours. After incubation period each of plates was observed separately to find any sort of growth on the media. The particular colonies were randomly isolated and streaked on nutrient agar plate to purify. Pure culture of 12 different isolates were prepared and stored.

Identification of bacteria

For primary determination of dye degrading bacteria, a loop of each sample was inoculated onto Eosin Methylene Blue (EMB), MacConkey agar media, Cetrimide Agar media, Mannitol Salt Agar (MSA), Mannitol Egg Yolk Polymyxin Agar (MYP), Starch Agar media and incubated at 37 °C for 24 hours. After corroboration from cultural methods, all positive isolates of selected bacteria were exposed to different biochemical tests such as Gram staining, Shape, Catalase, Oxidase, Citrate test, TSI (Slant/Butt), VP test, MR test, Indole test according to Bergey's Manual of Bacteriology (Buchanan and Gibbons, 1974).

Stock culture preparation

After isolation of concerned organisms, each of the organisms were picked and inoculated into TSB broth and stocked with 20% glycerol at -80 °C into an eppendorf.

Dye decolorization assay

Nutrient broth containing 0.1% different dyes were autoclaved at 121°C at 15 psi pressure for 15 minutes. The test was initiated by incubating 5% (v/v) of the inoculums to the decolorization medium at 37 °C and 100 RPM. At defined intervals of 2nd, 4th and 6th day, the culture was withdrawn, centrifuged at 10,000 RPM for 15 min, and the supernatant was examined for absorbance at 550 nm for Acid orange 488 nm for Direct red and 520 nm for Congo red under visible light in a spectrophotometer (UV-VIS RS spectrophotometer, LaboMed. Inc.). The extent of decolorization was expressed as percent (%) decolorization and estimated as $(A_i - A_t)\ DA_i \times 100$, where initial absorbance of the dye solution and

absorbance at cultivation time denoted by A_i and A_t respectively.

RESULTS AND DISCUSSION

Physical characteristics and microbial evaluation of textile dye waste samples

The collected sample has been analyzed to determine the some physicochemical characteristics like pH, temperature, color, odor and TVBC of industrial wastes. Table 1 indicates that the waste samples color varied enormously depending upon their collection point that may be due to the variation of dye and other residual compounds' concentrations in the samples (Table 1).

Indigenous Bacteria of soil sample

It has long been reported that bacteria inhabit in industrial effluents utilizing its constituents as their source of energy. Similarly, soil samples nearby distinct discharge points examined in this study was appeared to harbor a various community of microorganisms. A total of 12 bacterial strains were isolated from soil samples. It thus provides evidence that soil nearby discharge points harbor a wide range of bacterial species that may even degrade the dyes to obtain their essential elements (Table 1).

Bacteria Capable of Decolorizing Azo Dyes

Screening of the bacterial isolates was done to identify the isolate capable of degrading textile azo dyes namely, Acid orange, Direct red and Congo red in nutrient broth containing 0.1% of respective dye. Almost all bacterial strains capable of decolorizing selected dyes were screened and considered as potential candidates (Table 2).

Decolorization (% per day) of selected textile dye observed by spectrophotometer

The highest percentage of decolorization of the Acid orange dye was about 70.29% (Isolate 10) whereas the lowest decolorization was 10.68% (Isolate 1) at second day while at the sixth day, Isolate 8 showed the highest Decolorization was about 97.93% while Isolate 1 revealed the lowest decolorization was recorded about 53.71%.

The local dye of Acid orange was decolorized up to 32.86%, 22.57%, 33.71%, 35.43%, 20.57%, 41.14%, 68.87%, 46.29%, 47.43%, and 67.14% respectively by Isolate 2, Isolate 3, Isolate 4, Isolate 5, Isolate 6,Isolate 7, Isolate 8, Isolate 9, Isolate 11, and Isolate 12 at the second day. Then at the sixth day 88.24%, 86.00%, 72.31%, 86.20%, 65.54%, 78.00%, 93.70%, 79.53%, 63.18%, and 94.63% were accounted respectively by Isolate 2, Isolate 3, Isolate 4, Isolate 5, Isolate 6, Isolate 7, Isolate 9, Isolate 10, Isolate 11 and Isolate 12 (Table 2).

Table 2. Evaluation of Acid Orange Dye Decolourization of isolated bacteria

No. of Isolates	Degradation (%)		
	Day 2	Day 4	Day 6
Isolate 1	10.86%	51.71%	53.71%
Isolate 2	32.86%	70.29%	88.24%
Isolate 3	22.57%	68.86%	86.00%
Isolate 4	33.71%	65.71%	72.31%
Isolate 5	35.43%	53.71%	68.20%
Isolate 6	20.57%	57.71%	65.54%
Isolate 7	41.14%	62.29%	78.00%
Isolate 8	68.87%	87.71%	97.93%
Isolate 9	46.29%	61.43%	93.70%
Isolate 10	70.29%	74.29%	79.53%
Isolate 11	47.43%	56.00%	63.18%
Isolate 12	67.14%	78.29%	94.63%

Absorbance at 550nm

The highest percentage of decolorization of the Direct red dye was about 19.01% (Isolate 9) whereas the lowest decolorization was 0.30% (Isolate 1) at second day while at the sixth day, Isolate 12 showed the highest decolorization was about 99.02% while Isolate 1 revealed the lowest decolorization was recorded about 11.51%.

The local dye of Direct red was decolorized up to

Table 1. Physicochemical and microbial assessment of textile waste samples

Samples	pH	Temperature °C	Colour	Odor	TVBC/g
S1	7.64	27.4	Black-Grey	Unpleasant	1.9×10^8
S2	7.75	27.5	Greenish	Unpleasant	2.2×10^7
S3	7.34	27.5	Greenish	Unpleasant	9.1×10^7
S4	6.89	27.9	Ash	Unpleasant	1.8×10^8
S5	7.87	27.6	Black-Gray	Unpleasant	1.6×10^8
S6	7.63	27.8	Greenish	Unpleasant	2.2×10^7

9.77%, 14.12%, 13.23%, 14.22%, 13.82%, 1.88%, 14.12%, 13.92%, 15.07%, and 6.52% respectively by Isolate 2, Isolate 3, Isolate 4, Isolate 5, Isolate 6, Isolate 7, Isolate 8, Isolate 10, Isolate 11 and Isolate 12 at the second day. Then at the sixth day 65.43%, 63.26%, 62.00%, 59.53%, 55.72%, 32.37%, 73.44%, 92.71%, 52.20%, and 61.48% were accounted respectively by Isolate 2, Isolate 3, Isolate 4, Isolate 5, Isolate 6, Isolate 7, Isolate 8, Isolate 9, Isolate 10 and Isolate 11 (Table 3).

Table 3. Evaluation of Direct Red Dye Decolourization of isolated bacteria

No. of Isolates	Degradation (%)		
	Day 2	Day 4	Day 6
Isolate 1	0.30%	8.39%	11.51%
Isolate 2	9.77%	31.30%	65.43%
Isolate 3	14.12%	47.58%	63.26%
Isolate 4	13.23%	19.74%	62.00%
Isolate 5	14.22%	36.62%	59.53%
Isolate 6	13.82%	47.88%	55.72%
Isolate 7	1.88%	15.80%	32.37%
Isolate 8	14.12%	51.92%	73.44%
Isolate 9	19.01%	34.85%	92.71%
Isolate 10	13.92%	43.75%	52.20%
Isolate 11	15.07%	19.64%	61.48%
Isolate 12	6.52%	32.77%	99.02%

Absorbance at 488nm

The highest percentage of decolorization of the Congo red dye was about 69.18% (Isolate 12) whereas the lowest decolorization was 7.56% (Isolate 1) at second day while at the sixth day, Isolate 9 showed the highest Decolorization was about 98.73% while Isolate 1 revealed the lowest decolorization was recorded about 19.63%.

Table 4. Evaluation of Congo Red Dye Decolourization of isolated bacteria

No. of Isolates	Degradation (%)		
	Day 2	Day 4	Day 6
Isolate 1	7.56%	16.89%	19.63%
Isolate 2	29.74%	67.74%	83.54%
Isolate 3	17.56%	44.84%	67.86%
Isolate 4	41.33%	67.42%	75.39%
Isolate 5	12.57%	32.18%	48.64%
Isolate 6	22.46%	58.23%	67.21%
Isolate 7	14.21%	34.58%	43.73%
Isolate 8	67.53%	78.62%	89.67%
Isolate 9	74.67%	86.31%	98.73%
Isolate 10	18.53%	52.12%	68.47%
Isolate 11	12.77%	46.25%	56.34%
Isolate 12	69.18%	80.46%	95.86%

Absorbance at 520nm

The local dye of Congo red was decolorized up to 29.74%, 17.56%, 41.33%, 12.57%, 22.46%, 14.21%, 67.53%, 74.67%, 18.53% and 12.77% respectively by Isolate 2, Isolate 3, Isolate 4, Isolate 5, Isolate 6, Isolate 7, Isolate 8, Isolate 9, Isolate 10 and Isolate 11 at the second day. Then at the sixth day 83.54%, 67.86%, 75.39%, 48.64%, 67.21%, 43.73%, 89.67%, 68.47%, 56.34%, and 95.86% were accounted respectively by Isolate 2, Isolate 3, Isolate 4, Isolate 5, Isolate 6, Isolate 7, Isolate 8, Isolate 10, Isolate 11 and Isolate 12 (Table 4).

As shown in (Figure 1), the maximum decolorization ability ware Isolate 8, Isolate 9 and Isolate 12 almost 100% against all the selected dyes, however Isolate 1 accounted the minimum decolorization ability against respective dye after last day.

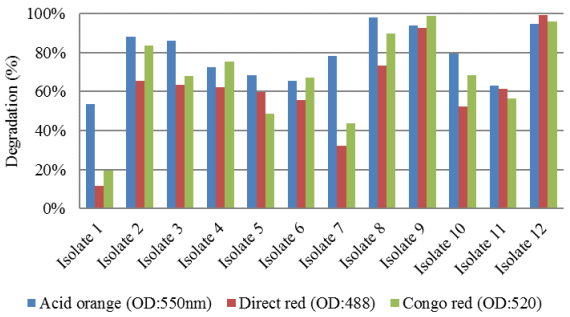

Fig. 1. Graphical presentation of dye decolorization (%) after six days by the tested isolates.

Identification of Dye Decolorizing Bacteria: The bacterial strains expressing strong decolorizing activity was determine by their morphological, cultural, physiological and biochemical features. After scrutinizing the properties with that described in Bergey's Manual, the bacterial strains were identified as *Pseudomonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Enterobacter* spp., *Klebsiella* spp. and *Escherichia coli* (Table 5).

DISCUSSION

Till now researchers are trying to find out an effective eco-friendly approach to treat the textile dye but it is still a very big challenge (Dos Santos *et al.*, 2007). There are three kinds of techniques for the treatment of textile dye effluent namely physical, chemical and biological that have been widely reviewed (Hao *et al.*, 2000; Robinson *et al.*, 2001).

Table 5. Summary of result exemplified by biochemical analysis on different isolates.

Isolates Name	Gram staining	Shape	Oxidase	Catalase	TSI (Slant/ Butt)	Citrate test	VP test	MR test	Indole test	Possible pathogens
Isolate 1	- ve	Rods	+ ve	+ ve	K/K	+ve	-ve	- ve	- ve	*Pseudomonas* spp.
Isolate 2	- ve	Rods	-ve	+ve	A/A	-ve	-ve	+ve	+ve	*Escherichia coli*
Isolate 3	+ ve	Cocci	- ve	+ ve	A/A	-ve	+ ve	+ ve	- ve	*Staphylococcus* spp.
Isolate 4	+ ve	Cocci	+ ve	+ ve	A/A	+ ve	+ ve	- ve	- ve	*Bacillus* spp.
Isolate 5	+ ve	Cocci	- ve	+ ve	A/A	-ve	+ ve	+ ve	- ve	*Staphylococcus* spp.
Isolate 6	- ve	Rods	- ve	+ ve	A/A	- ve	- ve	+ ve	+ ve	*Enterobacter* spp.
Isolate 7	- ve	Short rods	+ ve	+ ve	A/A	+ ve	+ ve	- ve	- ve	*Klebsiella* spp.
Isolate 8	- ve	Rods	- ve	+ ve	A/A	- ve	- ve	+ ve	+ ve	*Enterobacter* spp.
Isolate 9	+ ve	Cocci	- ve	+ ve	A/A	-ve	+ ve	+ ve	- ve	*Staphylococcus* spp.
Isolate 10	+ ve	Rods	+ ve	+ ve	A/A	+ ve	+ ve	- ve	- ve	*Bacillus* spp.
Isolate 11	- ve	Short rods	+ ve	+ ve	A/A	+ ve	+ ve	- ve	- ve	*Klebsiella* spp.
Isolate 12	+ ve	Rods	+ ve	+ ve	K/A	+ ve	+ ve	- ve	- ve	*Bacillus* spp.

M: Motile; NM: Non-Motile; + ve: Positive; -ve: Negative; A/A: Acidic Slant/ Acidic Butt; K/K: Alkaline Slant/ Alkaline Butt; K/A: Alkaline Slant/ Acidic Butt.

Among the three techniques of treatment biological treatment is more reliable which results, less toxic and more eco-friendly treatment (Saraswathy *et al*., 2010). Different bacterial species have been reported to be capable of color removal and have the ability to decolorize various dyes efficiently compared to others (Saraswathy *et al*., 2010; Fu and Viraraghavan, 2001).

In this study, 12 isolates were screened for decolorization of different dyes. The used local dyes in this study belong to the azo dye group. They were chosen for this study because of their extensive and common use in the cotton textile industry in Bangladesh and worldwide.

The biodecolorization of azo dyes vary depending on the presence of very specific changes in their molecular structures (Park *et al*., 2007) and the various mechanisms of decolorization of the dyes which followed by diverse bacterial groups (Wilkolazka *et al*., 2002). Keeping all these perspectives, the present study was attempted with the main aim of determination of Physio-chemical parameters, isolation and identification of dye degrading bacteria, the decolorization percentage of textile azo dye with the help of spectrophotometer.

CONCLUSION

All of the above result suggests that *Pseudomonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Enterobacter* spp., *Klebsiella* spp. and *Escherichia coli* can flourish in the toxic dye environment by utilizing them as their source of energy when other sources are limited or unavailable. All of the isolates in this study have impressive dye degrading capability and *Staphylococcus* spp., *Bacillus* spp. and *Enterobacter* spp. being the best of them. Therefore, these microbes can be used as incredible way for the bioremediation of textile dyes which have been proved to be the smartest way to wrestle with dye effluent related pollution. Further molecular study on their enzymatic property and degradation procedure could express them as a significant textile dye degrader.

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