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Azo and Phthalocyanine Dyes Degradation by Bacteria Isolated from Textile Industrial Waste

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ABSTRACT

The textile industry poses serious threat to the environment. Huge amount of untreated dyes is being discharged in the industrial effluents. Azo dyes are of major concern due to their extensive use and carcinogenic properties. Here we have studied the microbial degradation of four textile azo (C.I reactive black 5, yellow 145, and red 195) and phthalocyanine dyes (C.I reactive blue 21). Thirty-five bacteria and one yeast isolated from textile industry wastewater were tested for their tolerance to and degradation of the four dyes. Most of the bacteria showed maximum dye tolerance at 1000 ppm. Dye degradation monitored by measuring the absorbance of dye solutions at their λ_{max} (592 nm for black dye, 614 nm for blue dye, 423 nm for yellow dye and 523 nm for red dye) before and after bacterial treatment for 5 days and showed 83% removal of black 5, 49% removal of blue 21, 84% removal of yellow 145 and 85% removal of red 195 by *Jeotagalicoccus huakuii. Comamonas aquatica* could degrade 79% of black 5, 42% of blue 21, 83% of yellow 145 and 87% of red 195. *Bacillus subtilis* degraded 84%, 41%, 82%, 85%; *Moraxella* sp. degraded 82%, 28%, 81%, 77% and *Aeromonas veronii* degraded 73%, 30%, 80%, 76% black, blue yellow and red dyes, respectively. To determine the toxicity of degraded dye products a hemolytic assay was performed which showed a variable decrease or increase in red blood cells cytotoxicity caused by bacterial treated or untreated dye solutions. The current study suggests that some of the bacteria have the potential to degrade a number of textile industry dyes and could be exploited for xenobiotic removal.

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KT performed experiments and manuscript writing. MSM planned and conceptualized the research. MA and AK interpreted the results and performed critical analysis.

Key words
Microbial degradation, Industrial wastewater, Azo dyes, Phthalocyanine dye

INTRODUCTION

Textile production is a big industry in Pakistan. Dyes are among the most prolific compounds discharged from the textile industry as waste (Phugare *et al*., 2011). Azo dyes are being widely used in the textile sector due to their brighter colors and intense shades (Wang *et al*., 2009) but their complex aromatic ring structures with one or more azo (–N=N–) bonds make them hard to degrade (Vandevivere *et al*., 1998). Their persistence in water bodies may lead to serious environmental and health issues. These dyes biologically magnify in the food chain of freshwater organisms like algae and fish (Hossain *et al*., 2018), disturb the photosynthesis of aquatic plants, reduce the oxygen content and cause the eutrophication in water bodies (Rawat *et al*., 2018). Serious health issues like carcinogenesis, mutagenesis, skin irritations, allergies and dermatitis are also caused by azo dyes (Sarwajith *et al*., 2018). Their by-products like aromatic amines are responsible for disturbed blood formation (Carmen and Daniela, 2012). Furthermore, studies have suggested that 60-70% of azo dyes are toxic and withstand the conventional treatment procedures. The phthalocyanine dyes (e.g. reactive blue 21) are water soluble metal complexes, usually containing copper, that have also been reported as toxic and non-degradable compounds under both aerobic and anaerobic conditions (El-Aggadi and El-Hourch, 2021).

Coagulation and flocculation techniques are also used for dye extermination from the environment but these processes need chemicals in bulk leaving behind a huge amount of sludge. An enzymatic dye degradation is also of limited use because of high cost, low enzymatic stability and product inhibition (Husain, 2010). Biodegradation and bioremediation are natural processes in which microbes break, reduce and simplify the harmful waste materials and use the resulting byproducts for their routine metabolic activities (White *et al*., 2006).

There is a huge number of microorganisms responsible for bioremediation of many toxic compounds.

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For a long time, bacteria like *Bacillus subtilis*, *Bacillus cereus* and *Aeromonas hydrophila* have been considered as bioremediators. *A. hydrophila* has the ability to detoxify toxic aromatic amines produced during the anoxic dye degradation process. In recent studies *Pseudomonas luteola, Proteus mirabilis* and other *Pesudomonas* species have been reported to remediate azo dyes in anoxic conditions (Jamee and Siddique, 2019; Thanavel *et al*., 2019). *Proteus vulgaris* has also been observed growing and decoloring azo dyes faster than many bacteria (Britos *et al*., 2018). The genus *Zoogloea* is an important member of bio-remediators that helps settling down the organic pollutants in a secondary treatment system (Tortora *et al*., 2008). There are certain bacteria, like *Pseudomonas desmolyticum, Pseudomonas* sp., *Micrococcus glutamicus*, *Micrococcus* sp., *Enterococcus gallinarum, Pseudomonas putida, Lysinibacillus* sp., *Pseudomonas pulmonicola* and *Klebsiella* sp. which can degrade dyes when their enzymatic genes are either innately or over expressed in response to the presence of toxic dyes (Vikrant *et al*., 2018; Mittal *et al*., 2018).

Although novel microbes for energy efficient and cost effective bioremediation of recalcitrant dyes have been reported, but still there is a need to search for new environment friendly microbes that are highly specialized for removal of a large variety of dyes without being exhausted. In the present study we have determined the efficiency of microorganisms isolated from industrial effluent to degrade azo and phthalocyanine dyes.

MATERIALS AND METHODS

Sample collection

Textile industrial waste samples were obtained along the main Paharang drain in Faisalabad, which receives a huge amount of textile effluent and domestic waste water. Samples of water and sludge were collected at 0 meter, > 10 meters and > 1000 meters away from the industrial outfall along the main drain. Only one soil sample was collected at 0 meter. All water samples were mixed to make a composite sample and the same was done for sludge samples. Besides sampling, the temperature and pH of effluent, their electrical conductivity (EC) and total dissolved solids (TDS) were also determined (APHA, 1998).

Microbiological analysis

0.1 ml aliquots of ten-fold serially diluted samples were inoculated separately on a series of respective Nutrient Agar and Sabauroud Agar petri plates using the spread plate method. Plates were then incubated at 37 ºC for 24 h for bacterial growth. After 24 h of incubation, bacterial colonies were counted as cfu/ml and later purified by the streak plate method. Pure bacterial and yeast isolates were then subjected to standard microbiological testing for their identification (Cappuccino and Sherman, 2014).

Assessment of bacterial tolerance against dyes

For assessing bacterial tolerance against dyes, pure bacterial isolates were grown in the presence of variable amounts (30-1000 mg/l) of the dyes. 1% stock solution of reactive red 195 (Aksakal and Ucunb, 2010), reactive yellow 145 (Patil and Shukla, 2015), reactive black 5 (El-Bouraie *et al*., 2016), and reactive blue 21 (Silva *et al*., 2012) were used which were procured from Sitara Chemicals, Lahore. Nutrient Agar plates containing varying concentrations (30-1000 mg/l) of the dyes were inoculated with the respective bacterial isolates and incubated at 37ºC for 24-48 h. The dye tolerance of bacteria was recorded by observing the presence or absence of bacterial growth at the different dye concentrations in the petri plates.

Assessment of bacterial ability to bio-degrade/de-color the dyes

In each test tube containing 8.7 ml of autoclaved nutrient broth, a 300 µl (300 ppm) aliquot of filter sterilized respective dye stock solution (reactive black 5, reactive red 195, reactive yellow 145 or reactive blue 21) was added individually. The test tubes were then inoculated with 1 ml of individual bacterial (1-36) isolates to make the final volume of 10 ml. Test tubes were then incubated at 37 ºC under static conditions for 5 and 10 days. Un-inoculated tubes containing dye and nutrient broth were incubated under the same conditions for assessment of abiotic de-colorization in each dye. After incubation, bacteria were separated from the culture broth by centrifugation at 3500-4000 rpm for 20 min. Measurement of de-colorization of supernatant after removal of the bacterial cell pellet was done using a UV-Visible spectrophotometer (SkanIt Software RE 4.1, Thermo Fisher Scientific Oy) at λ max values of 423, 523, 592 and 614 nm for reactive yellow 145, reactive red 195, reactive black 5 and reactive blue 21, respectively (Khalid *et al*., 2008).

De-colorization was assessed by the difference in absorbance readings in the presence or absence of bacterial treatment of a specific dye at its respective λ max. For this, 96-well polystyrene, flat bottom micro-titer plates were used with the addition of 100 µl dye solution to each well. The formula used for percentage de-colorization was: De-colorization %= $At_0 - At_f / At_0 \times 100$, where At_0 is the initial absorbance and At_f is the final absorbance after incubation (Khalid *et al*., 2008).

Cytotoxicity assessment of dye solutions after bacterial treatment by hemolytic activity

The cytotoxicity of bacterial treated dye solutions was

determined by the method of Powell *et al*. (2000). Three-ml of human red blood cells (RBCs) were poured gently into a 15 ml sterile falcon tube and washed three times with 5 ml chilled phosphate-buffered saline (PBS) by centrifuging the tube each time for 5 min at approximately 3000 rpm. After washing, 180 µl of RBCs suspension and 20 µl of dye solution were mixed together in a 2 ml micro-centrifuge tube. The tube was again centrifuged for 5 min and 100 µl of supernatant was transferred to another micro-centrifuge tube containing 900 µl chilled PBS for dilution. Triton X-100 (0.1 %) and PBS were used as positive and negative control, respectively. For all other solutions of dyes (treated or untreated with bacteria), this same method was followed. On an ELISA plate absorbance at 576 nm was recorded. Cytotoxicity was measured as % lysis of RBCs using the following formula:

$$\% \text{ Hemolysis} = \frac{\text{Absorbance of sample} - \text{Absorbance of negative control}}{\text{Absorbance of positive control}} \times 100$$

RESULTS

Physical parameters

During sampling, the wastewater temperature ranged from 28 to 38°C, pH ranged from 7.5 to 10.5, EC readings had the range 4206-8745 µS/cm and TDS had the range 2523-5247 ppm. These readings were measured in water samples only.

Microbiological isolation and identification

Table I shows colony forming units (CFU/mL or CFU/g) measured. Two types of agars (Nutrient Agar and Sabauroud Agar) were used for this purpose. Thirty-five different bacterial isolates and one yeast isolate were obtained. Most of the isolated bacteria were Gram positive rods and a few were coccii (spherical) in shape. Some Gram negative bacteria were also obtained. Bacterial identification results were obtained using different standard microbiological tests (Cappuccino and Sherman, 2014).

Assessment of bacterial tolerance against dyes

All of the bacteria, which were subjected to varying concentrations of four individual dyes up to 1000 ppm in the nutrient agar plates, showed different tolerance levels. Most of the bacteria grew at 1000 ppm dye concentration. The least dye tolerance was seen in case of *Corynebacterium flavescens* and *Bacillus safensis.* It was noted that the bacteria which were not able to grow up-to 1000 ppm dye concentration, were also not able to degrade dyes effectively as their % dye removal capacity was also lower than the bacteria which showed tolerance at 1000 ppm of dyes (Table II).

Assessment of biodegradation of dyes

After the incubation of 10 days all of the dye degradation results were obtained under aerobic environment without agitation. All of the isolated bacteria were able to degrade the tested dyes to different extents (Table III).

The most efficient degradation (91.94%) of black dye was done by the bacterium *Micrococcus luteus*, whereas the other bacteria such as *Bacillus subtilis, Bacillus paralicheniformis*, *Jeotagalicoccus huakuii* and *Comamonas aquatica* degraded the dye 90.20%, 89.25%, 88.38% and 87.30% respectively. *Clavibacter michiganensis*, *Staphylococcus sciuri, Bacillus subtilis, Aeromonas veronii* and *Paenibacillus residui* degraded 51.89%, 49.40%, 42.90%, 40.00% and 41.42% of blue dye, respectively. Likewise, the most prominent degradation of yellow dye was observed by the bacteria such as *Bacillus subtilis (*84.00%), *Bacillus pumilus (*82.70%), *Jeotagalicoccus huakui* (82.30%), *Comamonas aquatica (*81.53%) and *Staphylococcus sciuri (*78.00%). *Bacillus paralicheniformis* (88.00%), *Bacillus subtilis* (86.50%), *Staphylococcus sciuri* (86.10%), *Comamonas aquatica* (84.00%) and *Aeromonas veronii* (82.00%) showed red dye degradation. *Corynebacterium* spp. and *Bacillus safensis* were observed having least biodegradation ability.

Five days incubation period for the degradation of colors was also assessed to know the proficiency of the bacteria (Table IV). Accordingly, more or less the same percentages of degradation were observed as those of 10 days.

Table I. Effect of Nutrient Agar and Sabauroud Agar on number of colonies per g or per mL (CFU/mL).

	Sample type	Soil	Sludge	Water
On nutrient agar	Number of bacterial colonies	26 in 10^{-4} dilution	68 in 10^{-3} dilution	158 in 10^{-2} dilution
	CFU/g or CFU/ml	2,600,000	680,000	158,000
	Number of fungal colonies	———	———	———
On Sabouraud agar	Number of bacterial colonies	84 in 10^{-2} dilution	25 in 10^{-2} dilution	17 in 10^{-2} dilution
	CFU/g or CFU/ml	84,000	25,000	17,000
	Number of fungal colonies	14 in 10^{-4} dilution	1 in 10^{-4} dilution	2 in 10^{-3} dilution

Table II. Maximum tolerance levels determination of bacterial strains against dyes in ppm.

Bacterial isolates	Black dye	Blue dye	Yellow dye	Red dye
B. mycoides; Moraxella sp.; *Corynebacterium kutscheri*; *Clavibacter michiganensis*; *B. megaterium (SS)*; *Exiguobacterium aestuarii*; *Jeotagalicoccus huakuii*; *B. paralicheniformis*; *Aeromonas veronii*; *Solibacills silvestris*; *B. mycoides*; *Micrococcus luteus*; *Staphylococcus sciuri*; *Cedecea neteri*; *B. subtilis*; *B. mycoides*; *B. velezensis*; *B. subtilis (GS)*; *B. atrophaeus*; *B. subtilis (JS)*; *B. licheniformis (NS)*; *Bacillus* sp.; *B. megaterium*; *B. licheniformis (RS)*	1000	1000	1000	1000
Actinobacillus capsulatus; *Comamonas aquatica*; *Micrococcus variance*; *Corynebacterium pilosum*; *Paenibacillus residui*; *Kluyvera intermedia*; *Escherichia coli*	800	800	800	800
Corynebacterium flavescens	400	400	400	400
Bacillus safensis	500	400	400	400
Corynebacterium bovis	700	300	700	700
Bacillus pumilus	700	700	700	700
Candida albicans	1000	400	1000	1000

Table III. Degradation of dyes (% decrease in dye concentration) by different bacterial isolates, 10 days after inoculation.

Isolate ID	Isolate name	Black dye	Blue dye	Yellow dye	Red dye
A	*Bacillus mycoides*	86.20 %	12.00%	71.75%	80.60%
D	*Comamonas aquatica*	87.30%	40.40%	81.53%	84.00%
G	*Corynebacterium flavescens*	70.70%	24.46%	77.80%	71.49%
H	*Corynebacterium kutscheri*	67.0%	15.10%	31.40%	34.50%
I	*Clavibacter michiganensis*	80.50%	51.89%	68.24%	76.00%
K	*B. safensis*	62.25%	6.00%	29.00%	29.30%
L	*Exiguobacterium aestuarii*	85.20%	18.16%	73.79%	82.90%
M	*Jeotagalicoccus huakuii*	88.38%	37.00%	82.30%	82.30%
N	*B. paralicheniformis*	89.25%	37.80%	75.40%	88.00%
O	*Aeromonas veronii*	79.70%	42.00%	63.20%	82.80%
S	*Solibacillus silvestris*	71.90%	14.73%	75.98%	51.75%
T	*B. mycoides*	87.37%	31.70%	71.20%	82.80%
V	*Micrococcus luteus*	91.94%	13.60%	47.20%	75.80%
Z	*Paenibacillus residui*	79.31%	41.42%	69.90%	68.10%
AA	*B. pumilus*	72.40%	15.60%	82.70%	81.50%
AS	*Cedecea neteri*	75.20%	26.30%	61.89%	67.00%
BS	*B. subtilis*	90.20%	42.90%	84.50%	86.50%
ES	*Candida albicans*	81.80%	31.78%	72.90%	73.56%
GS	*B. subtilis*	47.95%	4.54%	-13.00%	13.90%
HS	*B. atrophaeus*	29.00%	8.98%	18.39%	14.80%
JS	*B. subtilis*	39.69%	20.80%	12.00%	40.44%
LS	*Escherichia coli*	79.78%	30.30%	51.50%	42.50%
NS	*B. licheniformis*	73.13%	26.20%	49.19%	49.89%
PS	*B. species*	33.90%	29.70%	78.40%	15.60%
QS	*B. megaterium*	69.53%	27.15%	58.00%	54.45%
RS	*B. licheniformis*	86.43%	39.20%	72.00%	81.78%
SS	*B. megaterium*	49.49%	9.91%	15.76%	11.00%
TS	*Staphylococcus sciuri*	84.75%	49.40%	78.60%	86.10%
XS	*B. velezensis*	56.90%	1.00%	5.69%	33.60%

Table IV. Degradation of dyes (% decrease in dye concentration) by different bacterial isolates, 5 and 10 days after inoculation.

ID	Isolate name	Black dye		Blue dye		Yellow dye		Red dye	
		5d	10d	5d	10d	5d	10d	5d	10d
D	*Comamonas aquatica*	79.47	87.30	42.20	40.40	82.75	81.53	85.70	84.00
BS	*Bacillus subtilis*	84.78	90.20	41.00	42.90	82.75	84.50	85.70	86.50
NS	*B. licheniformis*	29.20	73.13	26.00	26.20	43.39	49.19	27.80	49.89
M	*Jeotagalicoccus huakuii*	83.37	88.38	49.90	37.00	84.77	82.30	85.64	82.30
ES	*Candida albicans*	71.86	81.80	25.00	31.78	81.50	72.90	61.85	73.56
N	*B. paralicheniformis*	16.87	89.25	30.30	37.80	21.76	75.40	7.00	88.00
O	*Aeromonas veronii*	73.14	79.70	30.59	42.00	80.90	63.20	76.62	82.80
TS	*Staphylococcus sciuri*	55.30	84.70	26.34	49.40	41.93	78.60	22.47	86.10
B	*Moraxella species*	82.20	---	28.60	---	81.64	---	77.93	---

Cytotoxicity of dyes

A hemolytic assay was performed to determine the percentage of final cytotoxicity (Table V). Dye solutions showing prominent de-colorization were visually selected for their toxicity evaluation. Prominent percent cytotoxicity decrease of dye solutions was observed after treatment with *Bacillus subtilis, Jeotagalicoccus huakuii, Comamonas aquatica, Staphylococcus sciuri, Bacillus pumilus and Candida albicans* in case of yellow, black, yellow, yellow, yellow and black dyes, respectively. Percent cytotoxicity increase was also observed in case of *Bacillus paralicheniformis* and *Aeromonas veronii.*

Table V. Hemolytic assay showing % final cytotoxicity.

Isolates names	Black	Blue	Yellow	Red
Control	10.289	10.434	16.014	8.768
Bacillus subtilis	---	---	7.174	10.652
Jeotagalicoccus huakuii	6.594	---	10.362	7.318
Comamonas aquatica	---	---	4.710	9.492
Staphylococcus sciuri	13.478	---	5.507	---
Aeromonas veronii	12.753	10.217	12.101	7.826
B. paralicheniformis	15.579	14.927	16.376	11.956
B. pumilus	---	---	3.188	17.681
Bacillus sp.	---	---	7.898	---
Candida albicans	3.840	---	14.710	---

--- = not determined.

DISCUSSION

In the present study only aerobic conditions were provided for dye degradation. Two degradation trials with different bacteria were performed, one with five days of incubation and other with ten days of incubation. Generally, it was noted that with the increase of incubation from 5 to 10 days, no obvious increase in overall degradation potential of wastewater acquired bacteria was observed. It can be suggested that maximum degradation can be achieved within 5 days of incubation, even less than this period. This trial pattern expressed that five days of incubation was sufficient to attain maximum degradation results.

Many bacteria showed very encouraging bioremediation potential, but when the test tubes containing test dyes and bacteria were closely observed, the bottom of the tubes were fully de-colored whereas at the upper portion of test tube solution some dye was seen, which was suggestive of anaerobic dye degradation. *A. veronii* showed aerobic degradation of reactive blue 21 dye as decoloration was observed at the upper portion of nutrient broth solution in the test tube. It has been previously reported that azo dye degradation occurs more effectively in anaerobic conditions (Kulla, 1981). Furthermore, phthalocyanine dyes (reactive blue 21) have been reported to be non-degradable under aerobic and anaerobic conditions (El-Aggadi and El-Hourch, 2021). In the current study, aerobic biodegradation of the reactive blue 21 dye was observed, but still it was the most resilient dye for degradation among tested dyes. Whereas reactive black 5 dye was the most easily degradable dye (Table III).

J. huakuii in the current study presented very good bio-degradation potential. It was proved to be among the best candidates for bio-removal of the tested dyes. Visually it showed complete black dye degradation from the medium in 10 days (Table III). This organism was shown swarming in Nutrient Agar rich medium. This organism, from the family Staphylococcaceae, is Gram-positive and coccus in shape, and it is moderately halophilic, being able

to grow in 0–23% NaCl (Guo *et al*., 2010). In addition to its already reported halo-tolerance it showed high dye tolerance and dye degradation.

C. aquatica showed good degradation of all four dyes. Especially, it degraded the reactive blue 21 dye more efficiently than most of the bacteria, except for *C. michiganensis* that showed the highest percent removal of the reactive blue 21 dye. Furthermore, it degraded dyes more quickly. CorreÃa (2008) and Khanna and Srivastava (2005) described that *Comamonas* spp. could degrade large polyhydroxyalkonates (PHAs) using their secretory extracellular hydrolases. These enzymes breakdown the PHA polymers into smaller fragments that can be easily taken up by the bacteria for utilization and assimilation inside the bacterial cells.

B. subtilis (ID: BS) showed the strongest capability to degrade all four of the tested dyes during incubation for 10 days as compared to other isolates. It has been reported that *B. subtilis* is able to remediate 98% of some azo dyes by its enzyme systems like laccase, azo-reductase and peroxidase in just 20 hours (Kumar *et al*., 2015). This bacterium also has the ability to remove sulphonated azo dyes not only by simple absorbing or adsorbing to the cell wall but by proper degradation (Mabrouk and Yusef, 2008).

Another microorganism, *A. veronii* showed promising degradation results for all four dyes. This organism was also observed growing at a low temperature of around 25 ºC (room temperature) without an incubator during the winter season. It is a Gram-negative rod that can be isolated from freshwater, soil and clinical sources (Sinha *et al*., 2004). It shows resistance towards antibiotics like tetracyclines and ciprofloxacin. It may cause pathogenesis in the skin, soft tissues and gastrointestinal tracts of humans and fish (Skwor *et al*., 2014). *Aeromonas* species have been well recognized as good bioremediators since 1970s (Thanavel *et al*., 2019). Current study results comply with this reported information.

S. sciurii obtained from textile wastewater showed good bio-remediating ability as illustrated by its percentage effect of dye de-colorization (Table III). This microorganism can be found in a number of hosts including animals and humans, and in the environment. This bacterium signifies some special features such as presence of multiple virulence genes and resistance genes and it further acts as a source of toxin and virulence genes for other Staphylococci members. Regardless of being a carrier of such traits, this microorganism is considered someway harmless (Nemeghaire *et al*., 2014). Many genes for resistance can be exploited for the bio-degradation of certain chemicals like heavy metals and dyes. It is known that the more a bacterium has special traits like resistant genes or enzyme coding genes, the greater would be its potential in bioremediation (Das *et al*., 2016). Results comply with the established facts in case of *S. sciuri.*

In the current study, *C. albicans* was seen growing at 37 ºC as yeast form having oval cells and mold-like form at room temperature. This yeast grew well in nutrient broth, the same as bacteria, and even displayed dye (aromatic complexes) removal potential. Many types of yeasts can consume aromatic complexes as growth substrates, but for co-metabolism they use aromatic compounds more effectively (Mörtberg and Neujahr, 1985). The observed bioremediation potential of *C. albicans* complies with already mentioned research.

All of the bacterial isolates named with xS, like ES, NS etc, were proficient in growing capably on Sabouraud Agar (Table I). This agar is suggested to be used for fungal growth and its pH was adjusted to 5. So organisms with xS IDs were able to grow under a wide range of pH as they were collected from alkaline (textile) water (pH of 9-10) and were also seen growing in acidic medium. The pH of textile wastewater is towards alkaline, and it contains halo-tolerant bacteria (Asad *et al*., 2007). It meant that microorganisms isolated from such an environment have more resilient properties, which enabled them to survive in highly polluted environment.

Bacillus, Staphylococcus, Corynebacterium, Escherichia and some other bacteria are among the best hydrocarbon degraders (Kafilzadeh *et al*., 2011). In the current study these bacteria showed good potential for removing dyes, except in the case of *Corynebacterium* species which showed little growth in broth media and less degradation of dyes. *Bacillus* species are well known for the remediation of aromatic compounds (dyes) (Cybulski *et al*., 2003). Results of *Bacillus* spp. of present study were in accordance with this except in case of *Bacillus safensis* which was observed as weak bioremediation candidate. Furthermore, *Bacillus* spp. manifest bio-surfactant producing abilities (Abed *et al*., 2014). Bio-surfactant production by these bacteria is helpful to decrease the surface tension of pollutant molecules at the surface of wastewater. The bio-degradation pathways for *Bacillus* and *Aeromonas* species have been well described by Mrozik *et al*. (2003).

Dye solutions visually showing prominent de-colorization were selected for their toxicity evaluation (Table V). An increase in toxicity of bacteria treated solutions may have been due to the production of aromatic amines resulting from azo dye breakage (O'Neill *et al*., 2000) or may be due to extracellular toxins secreted by certain bacteria. The increase in toxicity may be due to the prolonged incubation. The decrease in toxicity of bacterial treated solutions of dyes, when compared with the control group, suggested that bacterial treatments were

good for dye bioremediation as they not only removed the dye content but also decreased the toxic effects of the resultant products of dye degradation. Such bacteria can be used as potential candidates for the removal of not only azo dyes but also for the removal of more toxic aromatic amines. According to the cytotoxicity results, most easily detoxified dye was C.I reactive yellow 145 dye than other three dyes.

CONCLUSION

Bacteria and yeast isolated from industrial wastewater were capable of degrading azo and phthalocyanine dyes effectively. The double azo dye class (reactive black 5 dye) was easy target for bacterial degradation whereas the phthalocyanine dye (reactive blue 21) was more difficult to be degraded. Among 36 isolates, *Jeotagalicoccus, Comamonas, Bacillus, Moraxella, Aeromonas* and *Staphylococcus* showed very promising dye degradation. In context of decrease in toxicity of bacteria treated products of dyes among these five best isolates, *C. aquatica* was ranked first with 70% cytotoxicity decrease, *S. sciuri* was ranked second with 65% cytotoxicity decrease and *B. subtilis* was ranked third with 55% cytotoxicity decrease.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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