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Degradation and detoxification of reactive azo dyes by native bacterial communities

Vivekanandan Neelambari*, Vishwanathan Maharani, Shanmugam Vijayalakshmi and Thangavel Balasubramanian

CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai–608502, Tamilnadu, India.

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The initial selections of bacterial strains were based on effective decolourization of the medium by visible observation and UV-Vis spectral analysis. The operational parameters for dye degradation by the bacterial strains was observed from 6.5 to 7.5 pH range, 35 to 45°C with 100 mg/l dye concentration in static condition for 3 days incubation. The FTIR analysis reveals the modification of the functional groups after bacterial treatment. The reduction of toxicity after bacterial treatment was confirmed using *Vigna radiata* phytotoxicity study. It was concluded that the native bacterial strains isolated from the sludge environment possess high dye degrading ability. Further characterization of these bacterial strains and their enzymes are necessary for intense field application study.

Key words: Azo dyes, native bacteria, FTIR, toxicity, *Vigna radiata*, optimization.

INTRODUCTION

The textile industrial developments have originated increasing use of extensive synthetic dyes. It is estimated that over 2, 80,000 tons of textile dyes are discharged in industrial effluent every year, worldwide. Therefore, pollution from these discharge contaminated with dyestuff is becoming alarming (Pandey et al., 2007; Jin et al., 2007). Among all chromogenic groups of dyes, the reactive groups of azo dyes are widely used in the textile dyeing process due to the superior fastness for the fabric, high photolytic stability and resistance to microbial degradation. They have been increasingly used (more than I million tons worldwide) because of the ease and cost-effectiveness of their synthesis, stability and variety of colors available in comparison to natural dyes (Stolz, 2001). However, the serious environmental problems as a result of large production and utilization of dyes have attracted extensive concerns (Pandey et al., 2007). Azo dyes are widely known dyestuff used in industries and hence commonly released in the environment (Chang et al., 2001). This disposal in aqueous ecosystems leads to reduction in sunlight penetration hence decrease photosynthetic activity, DO, water quality and depicts severe environmental problems (Vandevivere et al., 1998). In addition to this, impact in terms of chemical oxygen demand (COD), many synthetic dyes show toxic, carcinogenic and genotoxic effects (Ozfer et al., 2003). Various physical and chemical technologies methods were employed for the remediation of the hazardous effluent but remains as drawbacks. As an alternative in use, bioremediation is an effective technique, which is ecofriendly, cost effective, has less sludge producing properties (Singh et al., 2008). Organisms utilizing azo dyes have been the target, due to their role in the treatment of waste containing azo dyes (Sponza and Isik, 2002). Microbes (especially bacteria, fungi) and plants generally replace the present engineering and chemical treatment processes. Thus, the present study was undertaken to isolate and identify textile dye degrading

*Corresponding author. E-mail: nilbr23@gmail.com. Tel: +91 9976451092.

bacteria from dye contaminated sludge environment. The degradation and detoxification was confirmed by FTIR analysis and phytotoxicity study.

MATERIALS AND METHODS

Dyes, chemicals and microbiological media

The reactive azo dyes used for the degradation study (100 mgl^{-1}) were Yellow MR, Blue MR, T Blue G, Red MSB and Orange M2K. The mineral salt medium (MSM) (composition: K_2HPO_4 1.6 g, KH_2PO_4 0.2 g, $(NH_4)_2SO_4$ 1 g, $MgSO_4.7H_2O$ 0.2 g, $FeSO_4.7H_2O$ 0.01 g, NaCl 0.1 g, $CaCl_2.2H_2O$ 0.02 g, dye 100 mg/l, glucose 3 g and yeast extract 1 g) was used for bacterial isolation (Nachiyar and Rajakumar, 2006). All other chemicals used were of technical grade.

Enrichment, isolation and characterization of reactive dyes degrading bacteria

Isolation of the bacterial strains was carried out from soil collected in effluent discharge site of textile dyeing industry, Perundurai, Tamil Nadu. For enrichment study, 1 g soil was added to MSM containing 5 different dyes separately and incubated at 37°C. The decolorized culture was transferred to the fresh medium to obtain successive pure strains by spread plate technique in agar medium (Supaka et al., 2004). The pure bacterial strains were inoculated in MSM amended dyes and the potential degrader showing maximum decolourization was isolated for further degradation study. The isolated colonies were transferred to the dye containing broth and selected on the basis of rapid decolourization. The strains were identified based on the morphological, physiological and biochemical approach.

Decolourization study by UV-Vis analysis

Decolourization was determined by measuring maximum absorbance (200 to 800 nm) of the respective dyes (kmax: Yellow MR, Blue MR, T Blue G, Red MS B and Orange M2K) and the concentration of culture supernatant using UV-Visible spectrophotometer (Shimadzu software UV Probe 2.33). All the experiments were performed in triplicates and the decolourization activity was expressed in terms of percentage decolourization using the following formula (Phugare et al., 2011).

$$D\% = \frac{Ai - At}{Ai} \times 100$$

Where 'D'- Decolourization in %, Ai - initial absorbance and At - absorbance at incubation time *t*.

Influence of physiochemical parameters on dye decolourization

The optimum decolourization of reactive dyes by bacterial strains was studied at different parameters. Decolourization was analysed at varying parameters such as pH (3, 4, 5, 6, 7 and 8), temperature (25, 30, 35, 40, 45 and 50°C), different dye concentration (50, 100, 150 and 200 mgl^{-1}), additional carbon source (1% glucose, sucrose and mannose), salt concentration (0.5, 1, 1.5, 2, 2.5 and 3%), shaking (150 rpm) versus static conditions and incubation period for effective decolourization. All the experiments were carried out in triplicates (Saratale et al., 2011).

Degradation study by FTIR analysis

Degradation products of the dyes were monitored by FTIR analysis. The culture supernatant was treated with equal amount of ethyl acetate and dried over sodium sulphate and dissolved in HPLC grade methanol for FTIR analysis. The control and samples were dried and mixed with KBr (1:20; 0.02 g of sample with K Br at a final weight of 0.4 g).

The samples were ground, desorbed at 60°C and pressed to obtain IR- transparent pellets. The absorbance FT-IR spectra of the samples were recorded using an FTIR (Spectrum 2000 Perkin-Elmer spectrometer). The spectra were collected within a scanning range of 400 to 4000 cm^{-1}. The FTIR was first calibrated for background signal scanning with a control sample of pure KBr and then the experiment sample was scanned. The FTIR spectrum of the non-degraded control was finally subtracted from the spectra of degraded dyes (Saratale et al., 2009).

Phytotoxicity study

The phytotoxicity study was carried out at room temperature using *Vigna radiata* plant seeds. The plant seeds were tested with wide range of dyes (Yellow MR, T Blue G, Blue MR, Red MS B and Orange M2 K) and its phytotoxic nature was analysed. Then the seeds were tested with the dye degraded metabolites and toxicity was analysed. The control was carried out using plain water at the same time. Experiments were carried out in triplicates. Germination (%), length of plumule (shoot) and radicle (root) was recorded after 7 days (Phugare et al., 2011).

RESULTS AND DISCUSSION

Isolation and screening of dye degrading bacterial strains

A total of 67 different bacterial strains were isolated from the sediments of textile industrial discharge. The bacterial strains for each selective dye was identified as *Pseudomonas aeruginosa* (Yellow MR), *Alcaligenes faecalis* (Blue MR), *Proteus mirabilis* (T Blue G), *Serratia marcescens* (Red MS B) and *Bacillus licheniformis* (Orange M2K) by morphological, physiological and biochemical characters (Table 1). The decolourization of reactive dyes by bacterial strains was efficient from pH 6 to 7, temperature of 35 to 40°C, 1% glucose, about 30% salt concentration with 100 mgl^{-1} dye concentration at static condition of about 48 h. The isolated potential strains could bring >80% decolourization of the reactive dyes (100 mgl^{-1}) at static condition within 48 h in a low nutrient medium (Figure 1). FTIR spectral comparison between dyes and its products formed after decolourization by bacterial strains, confirmed biodegradation of the dye into different metabolites. The present phytotoxicity study confirmed good germination rate as well as significant growth of plumule and radical for *V. radiata* seeds observed in degraded metabolites and plain water as compared to the dye sample.

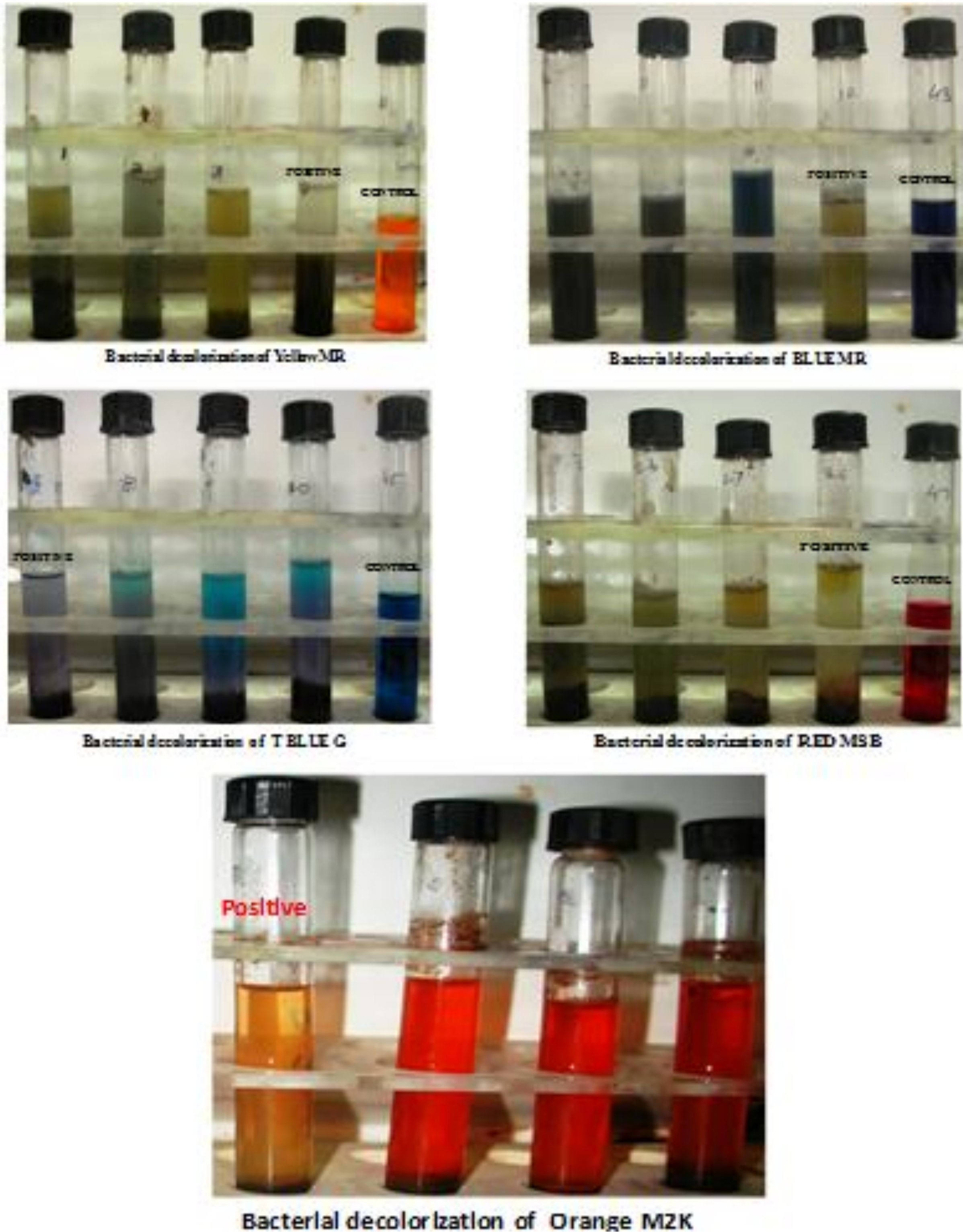

Figure 1. Decolourization of different dyes by bacterial strains.

Decolourization with physicochemical parameters

The decolourization of reactive dyes by bacterial strains was efficient from pH 6 to 7 (Figure 2). The temperature was found to be good from 35 to 40°C for bacterial strain in decolourization of azo dyes (Figure 3). Further changes in pH and temperature showed decrease in decolourization activity. The optimum concentration for dye

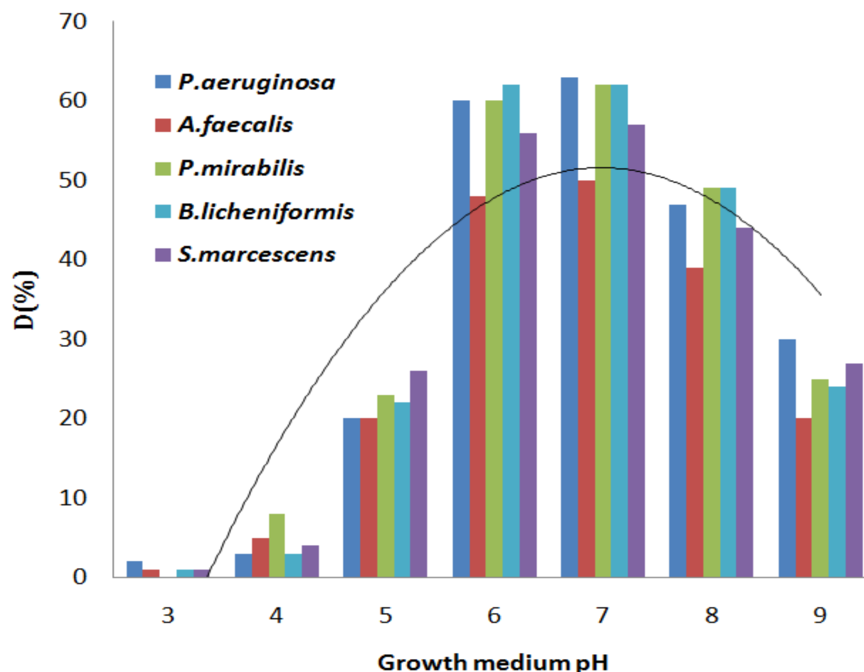

Figure 2. Effect of medium pH on dye decolourization by bacterial strains.

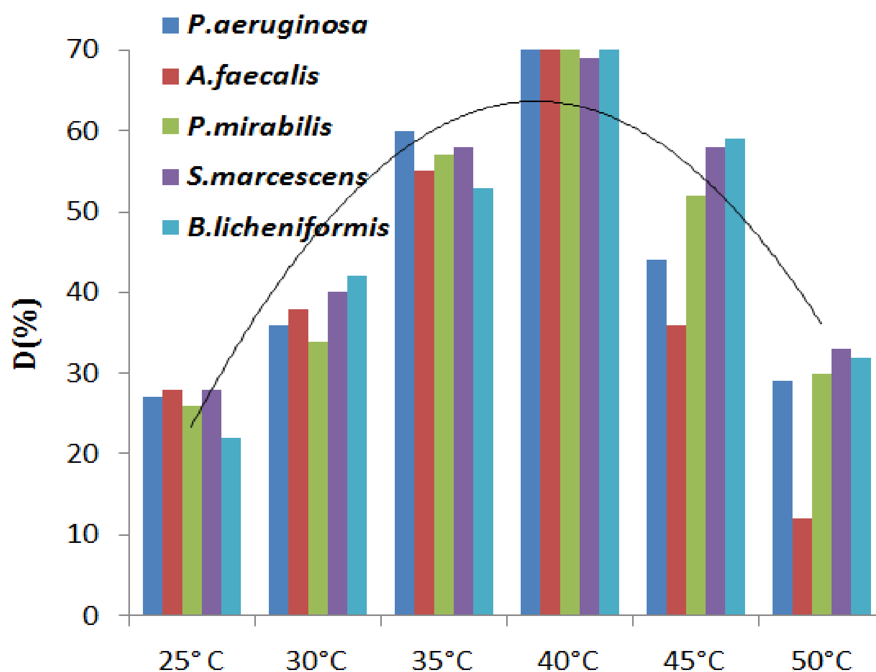

Figure 3. Effect of varying temperature on dye decolourization by bacterial strains.

decolourization by bacterial strains was about 100 mgl^{-1} (Figure 4). The decolourization activity of bacterial strains was successful at static condition (Figure 5) and decrease in decolourization was observed at shaking condition (150 rpm). But more turbidity was observed in medium incubated in shaking condition. This may confirm the effective bacterial growth reciprocal to decolourization. The 1% glucose was confirmed as the best carbon source for efficient dye decolourization by bacterial strains when compared with sucrose and mannose (Figure

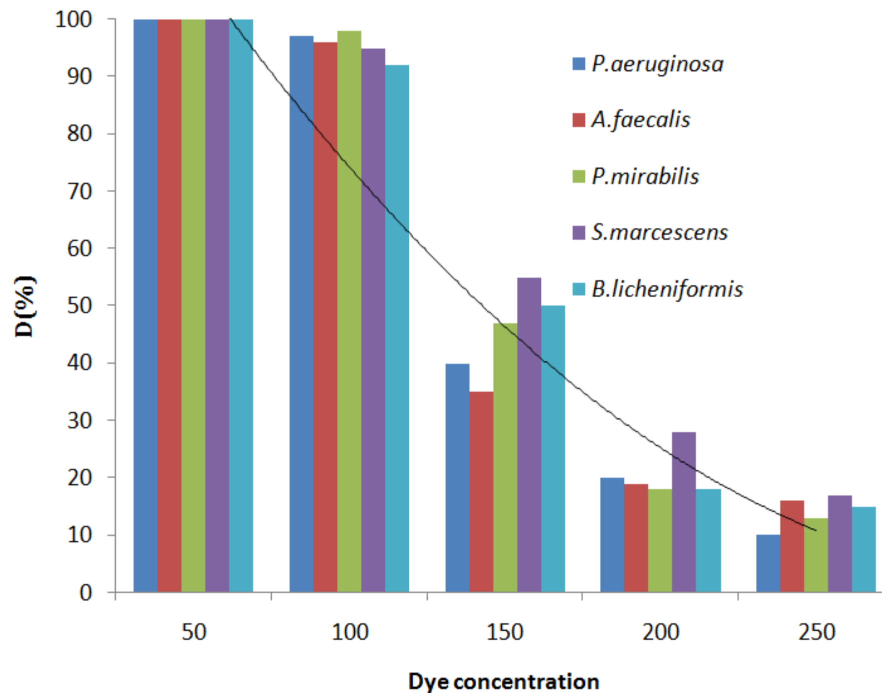

Figure 4. Dye concentration versus dye decolourization by bacterial strains.

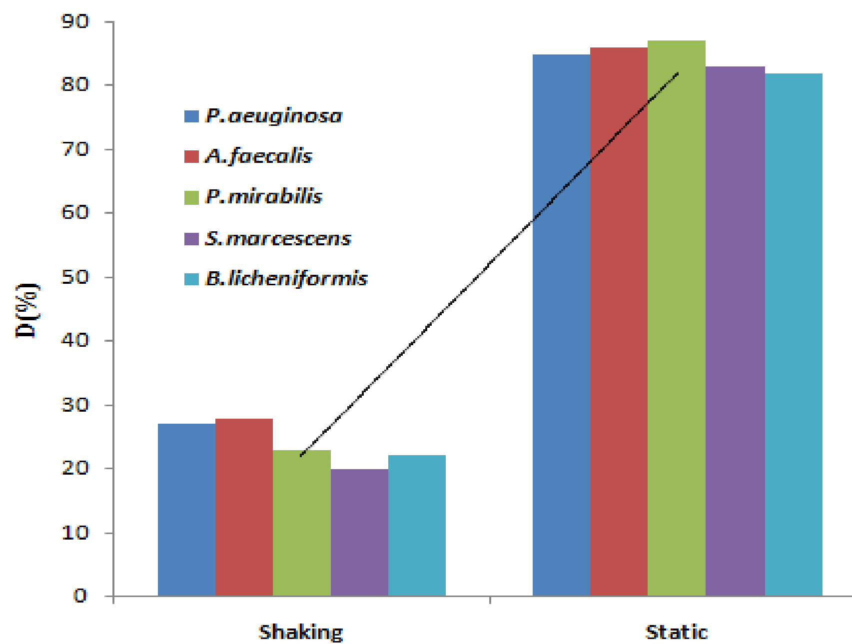

Figure 5. Effect of shaking and static condition on dye decolourization by bacterial strains.

6). The bacterial strains decolourized efficiently at about 3% salt concentration (Figure 7). The decolourization was found to be completed at about 48 h (Figure 8) incubation, respectively. The isolated potential strains could bring >80% decolourization of the reactive dyes (100 mgl^{-1}) at static condition within 48 h in a low nutrient medium (Table 2). In connection to the literature, Khalid et al. (2008) and Tamboli et al. (2010) reported that shaking condition delays the degradation of textile dyes which supports our observation. Similarly, previous

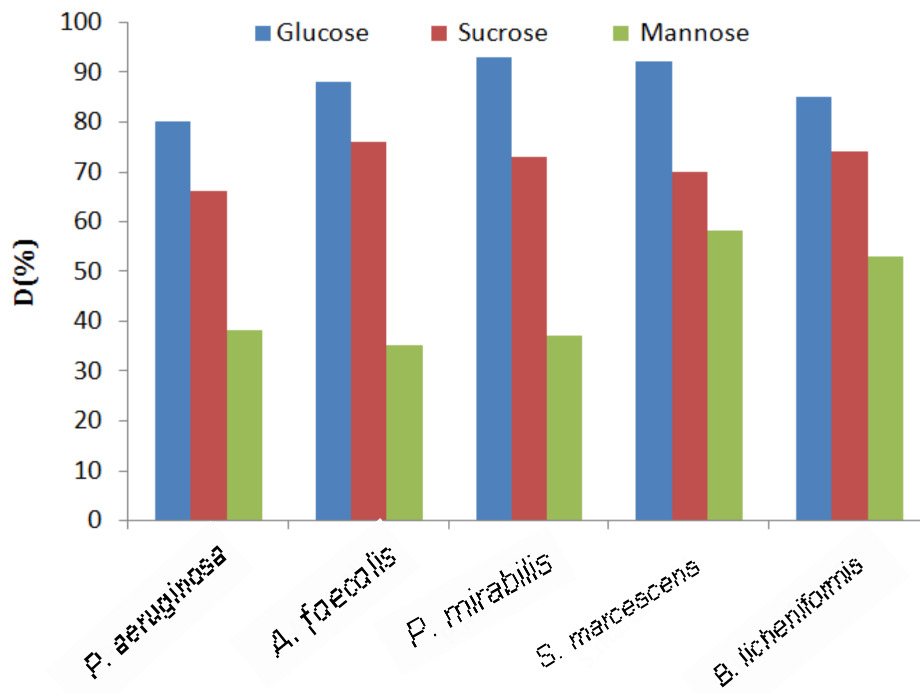

Figure 6. Effect of carbon sources on dye decolourization by bacterial strains.

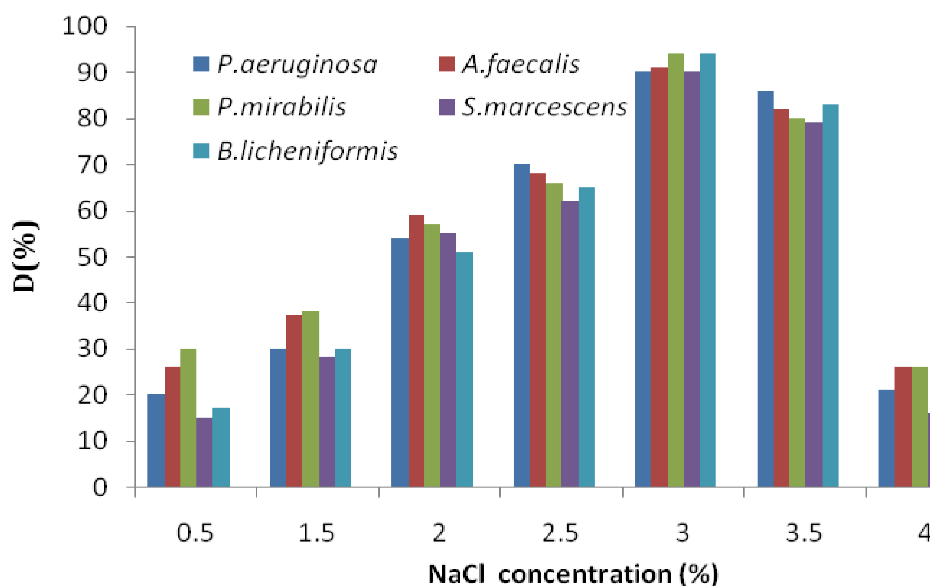

Figure 7. Effect of salinity on dye decolourization by bacterial strains.

reports by Parshetti et al. (2011) stated that, the percentage of decolourization was decreased with increasing dye concentration and time required for 100% decolourization of 10, 30, 50, 70 and 100 mgl^{-1} Crystal Violet by *A. radiobacter* was 8, 24, 36, 48 and 86 h, respectively. Jadhav et al. (2010) reported that the concerted metabolic activity of bacterial consortium DAS led to complete decolourization of Reactive Orange 16 (100 mg l^{-1}) within 48 h at pH 7 and 30°C.

Infrared spectrum analysis

FTIR spectral comparison between dyes and its products

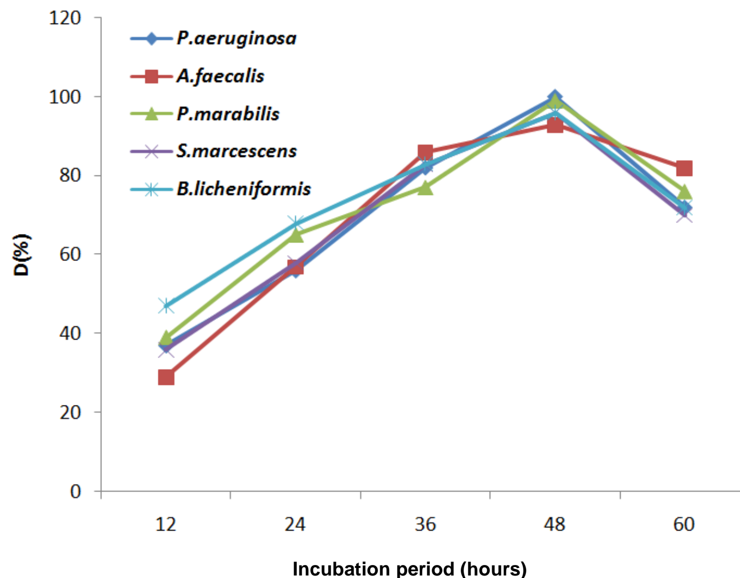

Figure 8. Effect of incubation time on dye decolourization by bacterial strains.

Table 1. Morphological, physiological and biochemical characteristics of dye degrading bacteria.

Characteristics	*Pseudomonas aeruginosa*	*Alcaligenes faecalis*	*Proteus mirabilis*	*Serratia marcescens*	*Bacillus licheniformis*
Gram reaction	Gram -ve	G -ve	G -ve	G -ve	G +ve
Cell shape	Coccobacilli	Bacilli	Bacillus	bacilli	Bacilli
Colony morphology	Mucoid, umbonate elevation			Entire margin	Irregular and variable colony
Pigment production	Pyocyanin and pyoverdine	-		Red pigment	
Indole test	-	-		-	
MR test	-		+	-	
VP test	-		-	+	
Citrate	+	+	+		
Motility	+	+	+	+	+
H_2S	-				
Nitrate reduction	+	-	+	+	
Glucose	-	-	+	+	
Sucrose	+			+	+
Lactose	-			-	+
Urease	-		+		-
Xylose	-				-
Raffinose	+				
Maltose	+				+
Mannitol	-				
Arabinose	-				-
Oxidase test	+	+	-	-	-
Catalase	+	+	+	+	
Arginine	+				+
Ornithine	-				
Oxygen requirement	Aerobic, Facultative anaerobe	Obligate aerobe	Facultative anaerobe	Facultative anaerobe	Aerobic, Facultative anaerobe
Spore	-				+

Table 2. Reactive dyes decolourizing potential strains.

Reactive dyes (100mg/l)	nm	Bacterial strain	Decolourization (%)
Yellow MR	256	*P. aeruginosa*	90
Blue MR	223	*A. faecalis*	87.2
T Blue G	214	*P. mirabilis*	88.5
Red MS B	216	*S. marcescens*	90
Orange M2 K	210	*B. licheniformis*	92

Table 3. Phytotoxicity study of dyes before and after bacterial treatment using *V. radiate.*

Parameter		Yellow MR		Blue MR		T Blue G		Red MS B		Orange M2 K	
	A	B	C	B	C	B	C	B	C	B	C
Germination (%)	90	29	78	19	58	22	74	27	80	15	65
Radicle (cm)	7.5± 1.9	1.6±0.2	5.3 ± 0.6	0.4±0.2	5.6±0.9	1.6±0.6	5.7±0.5	1.7±0.5	5.8±0.4	1.5±0.4	5.0±0.7
Plumule (cm)	1.7±0.3	0.4±0.1	0.5±0.3	0	0.5±0.2	0.2±0.1	0.5±0.3	0.4±0.2	0.6±0.2	0.2±0.3	0.4±0.3

A, Water; B, reactive dyes and C degraded metabolites.

formed after decolourization by bacterial strains, confirmed biodegradation of the dye into different metabolites. The FTIR spectrum of extracted metabolites showed significant changes in position of peaks when compared with the control dye spectrum. The FTIR spectra obtained from the Yellow MR raw dye possesses sulfonic group and azo groups. The peak shows S - S stretching at 528.5 cm^{-1} for sulfonic groups, N = O stretching at 1519.91 cm^{-1} for nitro compounds, C=O stretching at 1637.56 cm^{-1} for alkanes, CH stretching at 2945.3 cm^{-1} and N-H stretching at 3454.5 cm^{-1}. After bacterial treatment the metabolites showed peaks at 615.29 and 1469.76 cm^{-1} for CH stretching in the case of alkynes and 1124.5 cm^{-1} for C=O stretching in the case of esters. The peak 1589.34 cm^{-1} is for C = C stretching. The absence of disulfide group and azo group signify the biodegradation of dye. The Blue MR raw dye shows peaks at 1570.06 cm^{-1} for NH bend, 3439 cm^{-1} for NH stretching, 1608.63 cm^{-1}, 1732.08 cm^{-1} for C=O stretching, 2933.73 cm^{-1} for CH stretching alkanes. The degraded sample shows peaks at 1124.5 cm^{-1} for C=O stretching, 2249 cm^{-1} for C=C stretching for the presence of alkynyl group, 2736.99 cm^{-1} for OH stretching. The spectral peaks obtained from the T Blue G possess N-H wag at 835.18 cm^{-1}, 1139.93 cm^{-1} for C-O stretching, C-H wag of alkyl halides at 1234.44 cm^{-1} and OH bend at 1396 cm^{-1} peak. The disappearances of the following peaks were observed in the bacterial treated dye. The Red MS B also shows disappearance of NH, C-Br and sulfonic group in treated dye. The Orange M2K dye shows FTIR spectral peaks at 1633.71 cm^{-1} for N-H bend, 542 cm^{-1} for C-Cl group, 1047.35, 1215.15cm^{-1} for C-N and C-H wag and 2931.8 cm^{-1} for C-H stretching for the presence of alkanes and 3450.65 cm^{-1} for OH group. The bacterial treated metabolites shows peaks at 623.01 cm^{-1} for CH bond, 1124.5 cm^{-1} for C = O stretching, 2924.09 cm^{-1} for OH stretching. The disappearance of NH bond C-Cl group was observed to confirm the changes of the product. In the present study, the FTIR spectrum of extracted metabolites showed significant changes in the position of peaks when compared with the control dye spectrum. Similar results were also reported by Jadhav et al. (2010), Parshetti et al. (2006).

Phytotoxicity study of dyes and its degradation product using *V. radiata*

The untreated dyeing effluent may be unsafe to the ecosystem, when directly used for agriculture. Thus, it was of serious concern to assess the toxicity of the effluent before and after bacterial treatment. The present phytotoxicity study confirmed good germination rate as well as significant growth of plumule and radical for *V. radiata* seeds observed in degraded metabolites and plain water as compared to the dye sample. The phytotoxicity assessment of the dyes using *V. radiata*, before and after degradation confirms the detoxification of the dye product after degradation (Table 3). In raw dyes, less germination (15 to 29%), radical (0.4 ± 0.2 - 1.7 ± 0.5 cm) and plumule growth (0 - 0.4 ± 0.2 cm) was observed. After bacterial treatment of dyes, the maximum germination (58 to 80%) radical (5.2 ± 0.7 - 5.8 ± 0.4 cm) and plumule growth (0.4 ± 0.2 - 0.6 ± 0.2 cm) was observed. The correlation results confirm the detoxification and changes in nature of dye after bacterial treatment. The metabolites generated after the bacterial degradation is less toxic as compared to the original dye. The phytotoxicity test thus confirmed the modification of the raw compound or detoxification of the reactive dyes after incubation. Kalyani et al. (2008) stated that, the phytotoxicity results Red BLI with seeds of *Sorghum vulgare*

and *Phaseolus mungo* showed more sensitivity towards dye, while the products obtained after dye decolourization have less inhibitory effects. Jadhav et al. (2010) observed that the *Pseudomonas aeruginosa* strain BCH was able to detoxify the dye, Direct Orange 39 (1000ppm each day) effectively which was tested with *Triticum aestivum* and *Phaseolus mungo*. Phugare et al. (2010) reported the phytotoxicity study using *P. mungo* and *T. asetivum* treated with the dye Red HE3B and its degraded metabolites by microbial consortium reveals detoxification of the dye after treatment. Parshetti et al. (2011) reported the decrease in toxicity of crystal violet degradation by *A. radiobacter* using seeds such as *Sorghum bicolour*, *V. radiata, Lens culinaris* and *T. aestivum* plants.

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