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Decolourisation of Textile Dyes and Biological stains by Bacterial strains isolated from Industrial effluents

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ABSTRACT

Pseudomonas aeruginosa and Brevibacillus choshinensis were enriched and isolated from effluent sample of a textile processing unit using Sterile Minimal medium containing 10% Glucose and incubated at 28 ± 2° C for 7 days. The isolates were identified using standard biochemical tests carried out by KEM Hospital, Pune, India. These isolates were checked for decolourisation of textile dyes and biological stain solutions. It was found that both the bacterial isolates were able to degrade the textile dyes upto 250 ppm concentration. Pseudomonas aeruginosa was further studied for decolourisation of biological stain solutions and was found to decolorize upto 100 ppm of the stain solution. Phytotoxicity of the textile dyes was estimated by measuring the relative changes in seed germination of three plants: Triticum spp. (Wheat), Vigna radiate (Moong seeds) and Brassica juncea (Mustards seeds). Radicle and Plumule growth (length) were recorded after 6 days of exposure to different concentrations of dyes. Toxicity of the degraded dye was also checked which indicated that after decolourization the toxicity of the dye reduced. This property of these bacterial isolates shows a potential that can be utilized for the bioremediation of various textile industrial effluents thus saving the ecosystem from harmful effects of various dyes.

Key Words: Textile dyes, Biological stains, Microbial decolourization, Seed germination, Dye detoxification.

INTRODUCTION

Rapid industrialization and urbanization that results in the discharge of large amount of waste in the environment, is one of the main reasons for pollution. Textile and dyeing industries – one of the major industries in India are the main sources for release of colored effluents (mainly consisting of dyes) to the environment. The dyes used are mainly synthetic in nature that makes them more stable and more difficult to be biodegraded [1, 2]

Appearance of the industrial waste water that includes its colour and turbidity developed due to dye contamination has to be removed before it is released into the environment **[**3**]** since it inhibits penetration of sunlight thereby affecting photosynthesis and consequently the productivity of the autotrophs. The treatment of textile effluents is of interest due to their toxic and aesthetic impacts on receiving waters. While much research has been carried out to develop effective treatment technologies for wastewater containing azo dyes, no single solution has been satisfactory for remediation of broad varieties of textile waste **[**2**].** Biotechnology presents opportunities to detoxify industrial effluents at a lower operating cost than other physical and chemical remediation processes such as adsorption, flocculation, membrane filtration, electrochemical techniques, ozonation, and coagulation **[**4, 5**]**. The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages since the process is relatively inexpensive and uses a simple method **[**6, 7, 8**].**

MATERIALS AND METHOD

Chemicals:
Textile dyes (Red SNR and Blue SNR) were procured from Clarient, Ciba Company, New Delhi, India. Biological stains like Methylene Blue, Malachite Green and Crystal Violet were procured from S.D.fine- Chem .Ltd, Mumbai and Saffranine from Loba Chemical Pvt. Ltd, Mumbai

Isolation and identification the microorganisms:
Effluent samples were collected from the vicinity of a textile processing unit. The samples (1 ml) were inoculated using Sterile Minimal medium containing Na_2HPO_4 (0.6 g), KH_2PO_4 (0.4 g), NaCl (0.5 g), NH_4Cl (0.1 g), 1M $MgSO_4.7H_2O$ (0.1 ml), 0.1 % Vitamin B_1 solution (0.1 ml), 0.1 N $CaCl_2.2H_2O$ (0.1 ml) and 10% Glucose (1.0 ml) (pH 7.4) along with 25ppm of dye and incubated at 28 ± 2° C for 1 week at static conditions. The enriched broth was plated on sterile Minimal medium agar plates and incubated at 28 ± 2° C for 24 hours. The isolated organisms were identified using standard biochemical tests carried out by KEM Hospital, Pune, India.

Textile dye tolerance: Two textile dyes - Red SNR & Blue SNR and the biological stains- Methylene Blue, Malachite Green, Saffranine & Crystal Violet, were used for tolerance study. Stock solution (10,000 ppm) of each dye was prepared in distilled water. The Minimum Inhibitory Concentrations (MIC) of the dyes/stains for the isolated bacteria was determined by growing the pure culture of the bacterial isolate in increasing amount of the dye. The inoculated tubes were incubated at 28 ± 2° C for 24 hours. The tolerance was measured based on growth observed within 24 hours (Turbidometry method).

Decolourization test: The isolated bacteria were further tested to check their ability to decolorize the textile dyes. The bacterial isolates were grown in two different media-

1. **Medium A**- Sterile Minimal medium containing Na_2HPO_4 (0.6 g), KH_2PO_4 (0.4 g), NaCl (0.5 g), NH_4Cl (0.1 g), 1M $MgSO_4.7H_2O$ (0.1 ml), 0.1 % Vitamin B_1 solution (0.1 ml), 0.1 N $CaCl_2.2H_2O$ (0.1 ml) and supplemented with 1.0 ml of 10 % Glucose (pH: 7.4).
2. **Medium B**- Sterile Minimal medium containing Na_2HPO_4 (0.6 g), KH_2PO_4 (0.4 g), NaCl (0.5 g), NH_4Cl (0.1 g), 1M $MgSO_4.7H_2O$ (0.1 ml), 0.1 % Vitamin B_1 solution (0.1 ml), 0.1 N $CaCl_2.2H_2O$ (0.1 ml) and supplemented with 1.0 ml of 10 % Casamino acids (pH: 7.4).

Each medium was supplemented with increasing concentrations of the dye (50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm). The flasks were incubated at 28 ± 2° C for 24 hrs under static conditions. After incubation the solution was centrifuged at 5000 rpm for 10 mins. The optical density was taken at the appropriate wavelength (λ max).

Percentage decolourization was calculated by the formula- **[**8]

$$\text{Percentage decolourization} = \frac{\text{Initial O.D. - Final O.D.}}{\text{Initial O.D.}} \times 100$$

Bioassay for dye toxicity (**Seed germination test):** In this experiment, the effect of four different concentrations of dyes was evaluated on germination of seeds of 3 plants: *Triticum* spp., *Vigna radiata* and *Brassica juncea*. The seeds were germinated in sterile Petri dishes. Three sets of ten seeds each of *Triticum* spp., *Vigna radiata* and *Brassica juncea* were treated with 2 ml of dye solutions (concentrations of 500 ppm, 1000 ppm, 5000 ppm and 10,000 ppm respectively). The range of the used concentration covers possible dye discharge into water streams. Seeds germinated in petri dishes containing distilled water were used as a control. All dishes were kept in room temperature (28±2 ° C) for the period of 6 days. Germination of seeds was daily recorded. At the end of the germination experiment, the shooting percentage and root length of seedlings was measured. **[**9, 10**]**

In another independent experiment, 100 ppm of the Red SNR dye was subjected to degradation by *Pseudomonas aeruginosa*. The inoculations were made as follows:
1. 100 ppm of dye in plain water
2. 100 ppm of dye in plain water & autoclaved
3. 100 ppm of dye in plain water, inoculated, incubated at 28°C for 2 weeks, centrifuged
4. 100 ppm of dye in plain water, inoculated, incubated at 28°C for 2 weeks, autoclaved

The toxicity of the degraded dye was checked by checking the germination pattern on *Triticum* spp., *Vigna radiata* and *Brassica juncea* seeds. The seeds were germinated in sterile Petri dishes, layered with sterile circular filter paper

(diameter-10cm). 10 Seeds each of *Triticum* spp., *Vigna radiata* and *Brassica juncea* were irrigated with 2 ml of samples described in the table above. Every day 2 ml of the same dye solution was applied to the surface of the filter paper. Each treatment was replicated three times. Seeds germinated in petri dishes containing distilled water were used as a control. All dishes were kept in room temperature (28±2°C) for the period of 6 days. Germination of seeds was daily recorded. At the end of the germination experiment, the shooting percentage and root length of seedlings was measured.

RESULTS AND DISCUSSION

The two bacterial isolates were obtained from effluent samples taken from textile industries by enriching these samples and they were identified by biochemical tests (KEM Hospital, Pune, India) as *Pseudomonas aeruginosa* and *Brevibacillus choshinensis.* (Table 1a and 1b)

Sr. No.	Biochemical test	Result	Sr. No.	Biochemical test	Result
1.	Beta xylosidase	-	2.	D-Mannitol	-
3.	L-Lysine -Arylamidase	-	4.	D-Mannose	-
5.	L-Asparatate Arylamidase	-	6.	D-Melezitose	-
7.	Leucine Arylamidase	+	8.	N-Acetyl-D-Glucosamine	-
9.	Phenylalanine Arylamidase	+	10.	Palatinose	-
11.	L-Proline Arylamidase	-	12.	L-Rhamnose	-
13.	Beta galactosidase	-	14.	Beta-glucosidase	-
15.	L-Pyrrolydonyl -Arylamidase	+	16.	Beta-Mannosidase	-
17.	Alpha galactosidase	-	18.	Phosphoryl choline	-
19.	Alanine arylamidase	+	20.	Pyruvate	-
21.	Tyrosine arylamidase	+	22.	Alpha-glucosidase	-
23.	Beta- N -Acetyl- Glucosaminidase	+	24.	D- tagatose	-
25.	Ala-Phe-Pro-Arylamidase	+	26.	D-trehalose	-
27.	Cyclodextrine	-	28.	Inulin	-
29.	D-galactose	-	30.	D-glucose	-
31.	Glycogene	-	32.	D-ribose	-
33.	Myo-inositol	-	34.	Putrescine (assimilation)	-
35.	Methyl-A-D-Glucopyranoside	-	36.	Growth in 6.5%NaCl	-
37.	Ellman	-	38.	Kanamycin resistance	-
39.	Methyl-D-xyloside	-	40.	Oleandomycine resistance	-
41.	Alpha-Mannosidase	-	42.	Esculin hydrolyse	+
43.	Maltotriose	-	44.	Tetrazolium red	+
45.	Glycine arylamidase	-	46.	Polymixin B resistance	-

Table 1a: Biochemical tests for *Brevibacillus choshinensis* identification (KEM, Pune)

Sr. No.	Biochemical test	Result	Sr. No.	Biochemical test	Result
1.	Ala- Phe- pro- Arylamidase	-	2.	Saccharose/sucrose	-
3.	Adonitol	-	4.	D- tagatose	-
5.	L-Pyrrolydonyl-Arylamidase	-	6.	D- trehalose	-
7.	L-Arabitol	-	8.	Citrate (Sodium)	+
9.	D-Cellobiose	-	10.	Malonate	+
11.	Beta-Galactosidase	-	12.	5-Keto-D- Gluconate	-
13.	H2S Production	-	14.	L –Lactate alkalinisation	+
15.	Beta-N-Acetyl-Glucosaminidase	-	16.	Alpha- Glucosidase	-
17.	Glutamyl Arylamidase pNA	-	18.	Succinate alkalinisation	+
19.	D- Glucose	+	20.	Beta- N-Acetyl- Galactosaminidase	-
21.	Gamma-Glutamyl Transferase	+	22.	Alpha- Galactosidase	-
23.	Fermentation/ Glucose	-	24.	Phosphatase	-
25.	Beta- glucosidase	-	26.	Glycine arylamidase	-
27.	D- maltose	-	28.	Ornithine Decarboxylase	-
29.	D- mannitol	+	30.	Lysine decarboxylase	-
31.	Beta- Xylosidase	+	32.	Decarboxylase base	-
33.	Beta – Alanine Arylamidase pNA	-	34.	L-Histidine assimilation	+
35.	L- Proline arylamidase	+	36.	Courmarate	+
37.	Lipase	+	38.	Beta- glucoronidase	-
39.	Palatinose	-	40.	O/129 Resistnace (Comp.*Vibrio*)	+
41.	Tyrosine arylamidase	+	42.	Glu-Gly-Arg-Arylamidase	-
43.	Urease	-	44.	L-malate assimilation	+
45.	D-sorbitol	-	46.	Ellman	**-s**
47.	L-Lactate assimilation	+			

Table 1b: Biochemical tests for identification *Pseudomonas aeruginosa* (KEM, Pune)

These isolates were checked for their dye decolourization potential by growing them in Medium A and Medium B (Sterile Minimal broth containing Glucose and Sterile Minimal broth containing Casamino acids) containing various concentrations of the textile dye. It was seen that both the organisms could effectively decolorize the dye when grown in Medium A. The decolourization in Medium B was less in comparison with Medium A when the absorbance was checked suggesting that the dyes may be acting as Nitrogen source in the medium supplemented with glucose acting as Carbon source.

The absorbance was found out for both the dyes at the appropriate wavelength *i.e.* Red SNR dye at 523.20 nm and for Blue SNR dye at 611.60 nm using UV-Visible spectrophotometer. The percentage decolourization was found out using the formula-

$$\text{Percentage decolourization} = \frac{\text{Initial O.D. - Final O.D.}}{\text{Initial O.D.}} \times 100$$

Highest percentage decolourization was shown by *Pseudomonas aeruginosa* when grown in Medium A (Table 2).

Sr. No	Name of the dye	λ max (nm)		Percentage Decolourization:			
				100 ppm	150ppm	200 ppm	250 ppm
1.	Red SNR	523.2	GP	90.42	91.45	93.12	93.68
			GB	83.42	78.90	84.02	86.74
			CP	02.04	23.63	17.49	17.62
			CB	07.55	04.13	06.87	11.68
2.	Blue SNR	611.6	GP	75.99	76.53	80.18	78.13
			GB	68.47	68.53	73.11	68.98
			CP	00.83	04.53	11.53	23.40
			CB	--	12.40	09.67	06.80

GP- Glucose with Pseudomonas aeruginosa
GB- Glucose with Brevibacillus choshinensis
CP- Casamino acids with Pseudomonas aeruginosa
CB- Casamino acids with Brevibacillus choshinensis

Table 2: Percentage decolourization of red dye and blue dye withPseudomonas aeruginosa and Brevibacillus choshinensis

Pseudomonas aeruginosa showed a percentage decolourization of 93.68 % and 78.13 % respectively when grown in Medium A containing 250 ppm solutions of the Red SNR dye and Blue SNR dye *w*hereas *Brevibacillus choshinensis* showed a percentage decolourization of 86.78 % and 68.98 % [Fig 1, 2, 3 and 4].

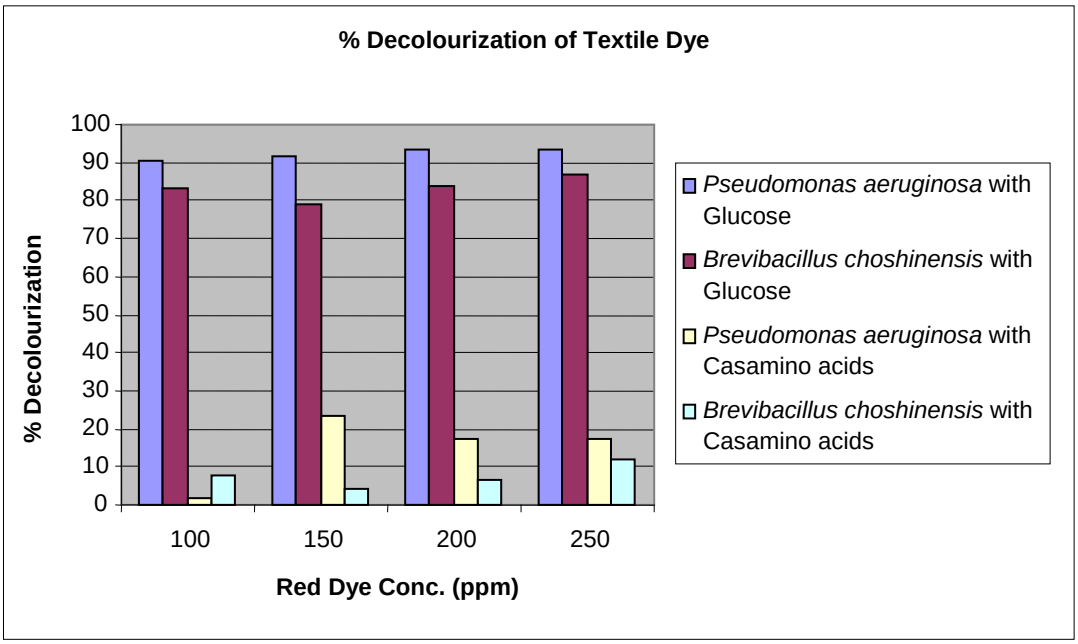


Fig 1: Percentage decolourization of Red SNR dye by two bacterial isolates grown in Medium A and Medium B

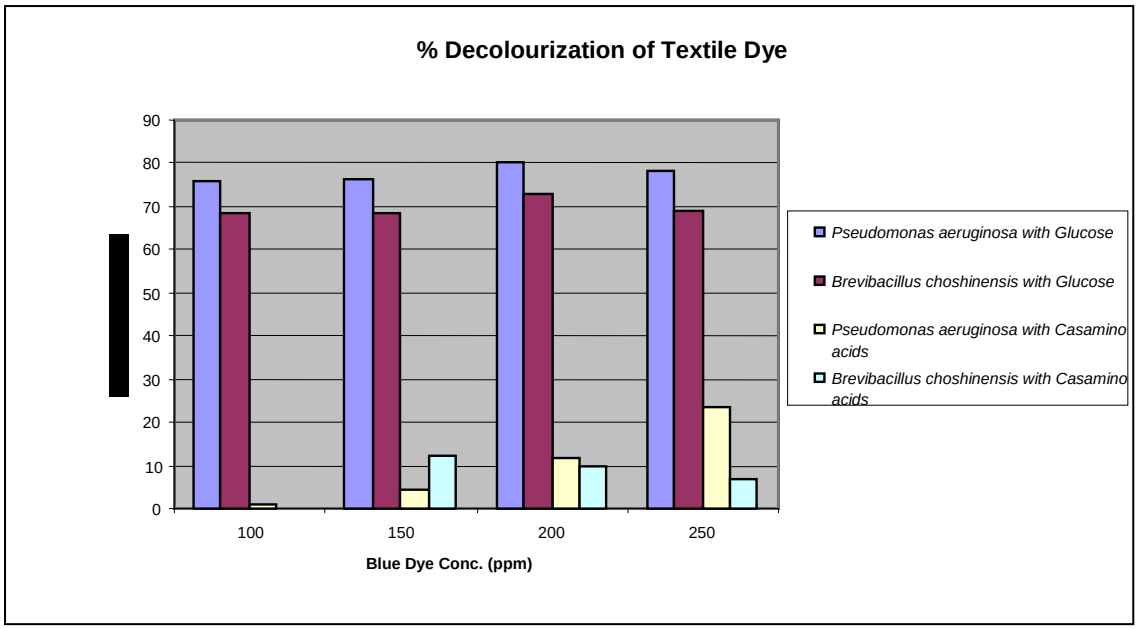


Fig 2: Percentage decolourization of Blue SNR dye by two isolates grown in Medium A and Medium B

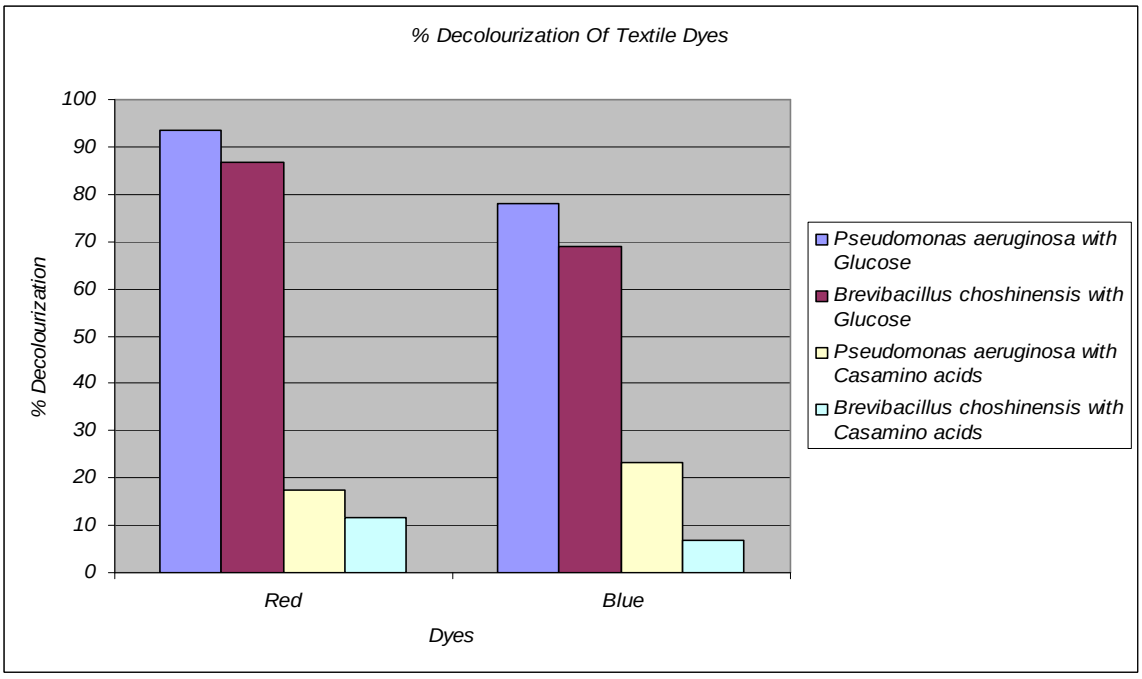
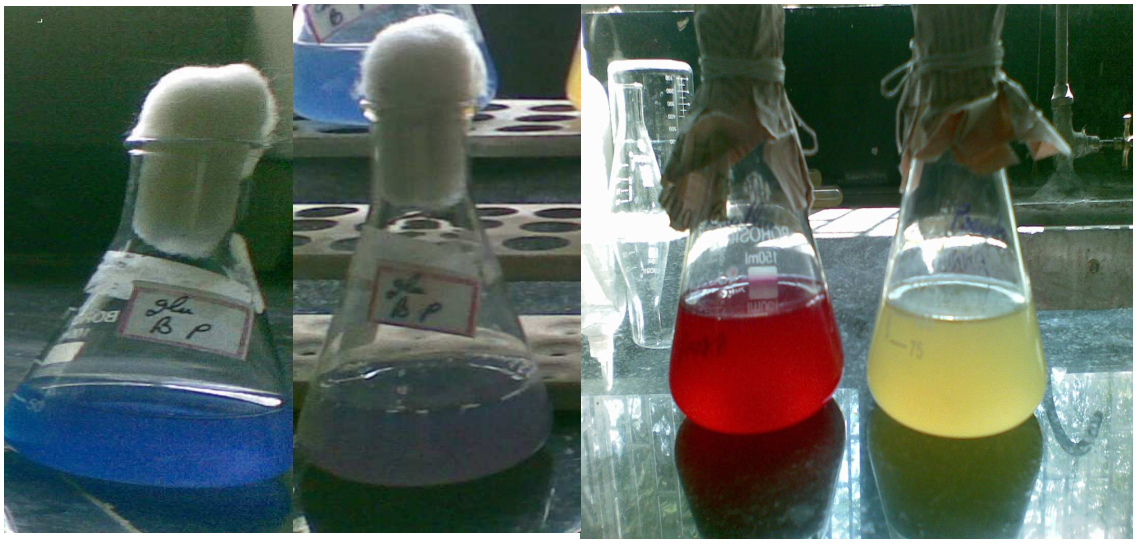


Fig 3: Comparison between the Percentage decolourization of Red SNR dye and Blue SNR dye



Before **After** **Before** **After**

Fig 4: Decolourisation of Blue dye and Red dye

When the medium was supplemented with Casamino acids (Medium B), the percentage decolourization of Red SNR dye was reduced from 93.68 % to 17.62 % for *Pseudomonas aeruginosa* and from 86.78 % to 11.68 % for *Brevibacillus choshinensis*.

The dye decolourization capacity of *Pseudomonas aeruginosa* was studied using biological stain solutions- Methylene Blue, Malachite Green, Saffranine & Crystal Violet and it was found that *Pseudomonas aeruginosa* was able to decolorize biological stains upto 100ppm concentration (Table 3).

Sr. No	Name of the Biological stain	λ max (nm)	Percentage Decolourization:	
			50 ppm	100 ppm
1.	Methylene blue	570	**77.99**	**60.98**
2.	Malachite green	570	**75.86**	**60.13**
3.	Saffranine	450	**40.50**	**49.35**
4.	Crystal violet	520	**24.10**	**40.50**

Table 3: **Percentage decolourization of Biological stains with *Pseudomonas aeruginosa* after 24 hours.**

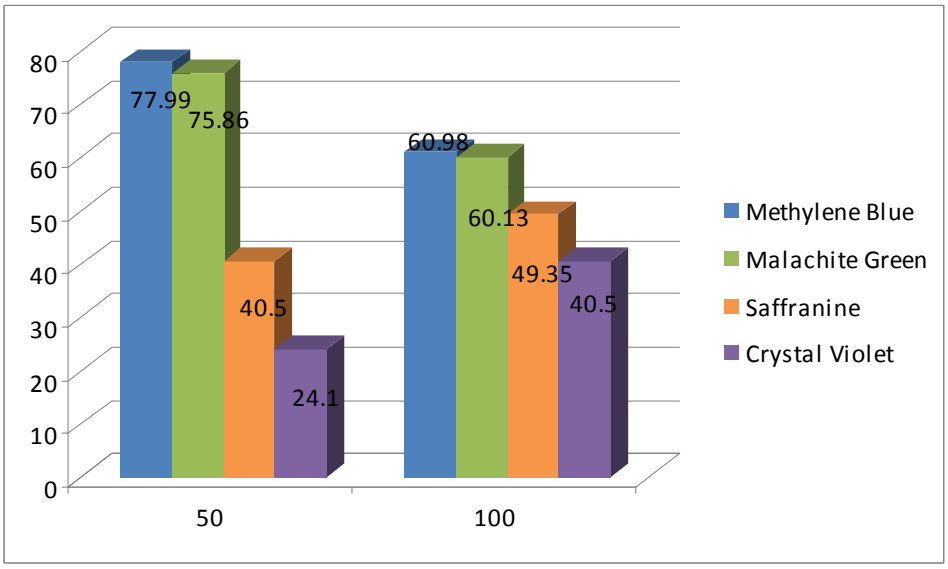


Fig 5: Percentage decolourization of Biological Stains by *Pseudomonas aeruginosa* after 24 hours.

After incubation of *Pseudomonas aeruginosa* with the individual biological stain solution for 24 hours, the percentage decolourization for Methylene Blue, Malachite Green, Saffranine and Crystal Violet was found to be 77.99 %, 75.86 %, 40.50 % and 24.10 % respectively (Fig 5 & 6).

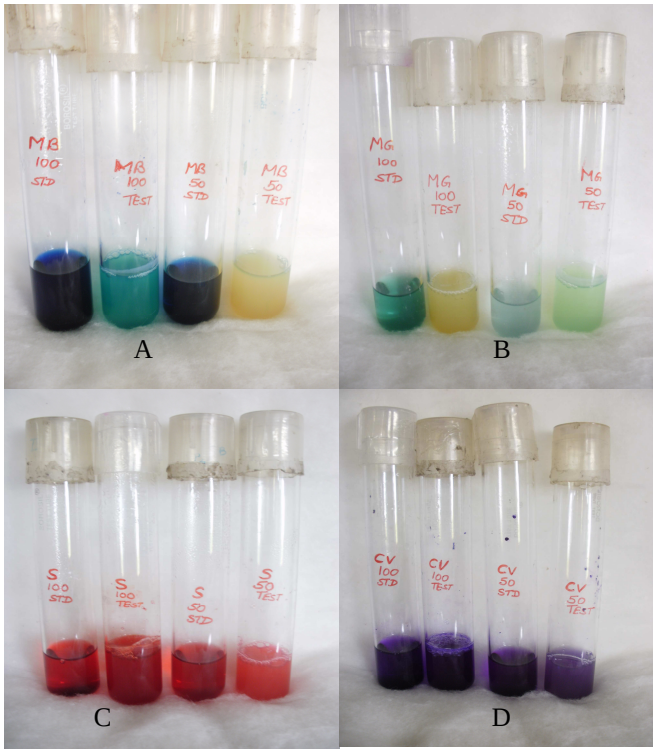


Fig 6: Biological stain decolourisation by *Pseudomonas aeruginosa*
A- Methylene blue
B- Malachite green
C- Saffranine
D- Crystal violet

In other dye degradation studies, it was seen that *Shewanella putrefaciens* AS96, isolated from activated sludge was capable of completely decolorizing

100 ppm dyes in just 4-8 hours of static incubation. *Shewanella putrefaciens* AS96 was capable of decolorizing dyes in media containing NaCl concentrations as high as 60 gm/lit **[**11**].** *Aspergillus niger* and *Penicillium spp* were also found to efficiently cause biodegradation of the colour Direct Red 81 dye and Reactive Red 120 dye respectively **[**12**].** Apohan and Yesilada (2005) reported that the toxicity of azo dyes could be removed by fungal treatment**.** Some researchers have also reported about reducing the toxicity of various dyes by fungal treatment [13, 14, 15]. In some cases anaerobic conditions are required for the dye decolourisation by S. *cerevisiae* **[**16**].** Aerobic *Staphylococcus aureus* was found to reduce the colour of the crude effluent by 37.5% after 90 days of incubation in the crude effluent. Similarly *Bacillus subtilis was* found to reduce the colour by 25**% [**2**].** Aerobic reduction has also been reported to occur in several bacteria such as *Pseudomonas sp*. **[17],** *Bacillus sp*. [18**]** and *Klebsiella pneumonia* **[**19**].** Studies show that *Acetobacter liquefaciens* is unique in that decolourisation of Methyl Red occurs sufficiently under both aerobic and anaerobic (static) conditions **[**20**].** *Enterococcus faecalis* YZ 66 was found to decolourize Reactive orange. It was found to decolorize 77.73% of the dye in 80 minutes [21]

The bioassay for dye toxicity in this study was based on measuring the effect of dye (Red SNR) on seed germination, plant shooting and root elongation. The phytotoxicity of different soluble textile dyes was estimated by measuring the relative changes of the aforementioned plant parameters using *Vigna radiata* (whole moong), *Triticum* spp (Wheat) and *Brassica juncea* (Mustard seeds) seeds as test plants.[Table 4]

Seed type	Dye (Red SNR) concentration in ppm	% germination	Average shoot length (cm)	Average Root length (cm)
***Triticum* spp (Wheat)**	Control	100 %	5.45	9.99
	500 ppm	100 %	5.02	8.76
	1000 ppm	100 %	3.89	4.87
	5000 ppm	No germination	--	--
	10,000 ppm	No germination	--	--
***Brassica juncea* (Mustard seeds)**	Control	100 %	5.88	4.98
	1000 ppm	100 %	4.78	3.58
	5000 ppm	40 %	1.80	2.99
	10,000 ppm	60 %	2.11	2.45
***Vigna radiata* (Moong seeds)**	Control	100 %	5.00	4.92
	500 ppm	100 %	4.53	4.00
	1000 ppm	100 %	4.18	3.64
	5000 ppm	100 %	No shooting	2.98
	10,000 ppm	100 %	No shooting	2.45

Table 4: Comparison between % germination, shoot and root length of different seeds at different dye concentrations.

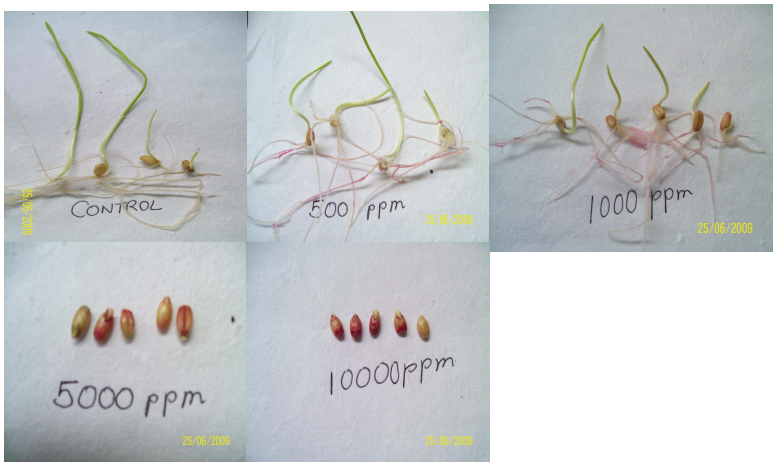


Fig 7: Effect of textile dye on *Triticum* spp (Wheat) germination

Fig 8: Effect of textile dye on *Brassica juncea* (Mustard seeds) germination

The results show that high concentrations of dyes were more toxic to seed germination as compared with the lower concentrations [Fig 7,8 & 9]. Similar study was conducted by Ren *et. al* (1996) who demonstrated the toxicity of Polycyclic Aromatic Hydrocarbons (PAHs), Anthracene (ANT), Benzo[a]Pyrene (BAP), and Fluoranthene to the duckweed *Lemna gibba L.* and *Brassica napus L.* seeds. These authors used the germination efficiency, root and shoot growth, and chlorophyll content, as a measurement for toxicity. Hassan Moawad *et. al*. (2003) showed that the textile dyes were toxic to the seed germination process of four plants: clover, Triticum spp (Wheat), tomato and lettuce.

The results of this study suggest that while the exposure of seeds to low concentration of dye during germination was less toxic to seed germination as compared with the higher concentrations, the low concentration of the dye could adversely affect the shooting percent significantly. This is in agreement with the previous work by Kosinkiewicz *et. al.* (1984) who reported that most dyes containing phenol ring were phytotoxic.

Fig 9: Effect of textile dye on *Vigna radiata* (Moong) seed germination

100 ppm of the dye was subjected to decolourisation by *Pseudomonas aeruginosa*. After incubation at 28°C for 2 weeks, the percentage decolourisation obtained was 68.75 %. The effect of the decolourised dye was checked by checking the germination pattern of *Vigna radiata* (Moong), *Triticum* spp (Wheat) and *Brassica juncea* (Mustard) seeds (Table 5).

Seed type		Percentage germination(%)	Average shoot length (cm)	Average Root length (cm)
Vigna radiate (Moong Seed)	Water control	100	14.44	13.64
	Dye	100	10.46	6.74
	Autoclaved dye	100	10.04	4.56
	Inoculated /autoclaved	100	14.0	6.07
	Inoculated /centrifuged	100	11.3	7.5
Brassica juncea (Mustard seeds) seeds	Water control	100	5.325	7.72
	Dye	100	4.46	6.28
	Autoclaved dye	100	4.3	4.14
	Inoculated /autoclaved	100	4.55	10.45
	Inoculated /centrifuged	100	5.87	5.65
Triticum spp (Wheat)	Water control	100	7.63	10.30
	Dye	100	7.0	8.8
	Autoclaved dye	100	5.76	7.33
	Inoculated /autoclaved	100	8.77	11.6
	Inoculated /centrifuged	100	8.2	8.03

Table 5: Comparison between % germination, shoot and root length of different seeds using decolorize dye.

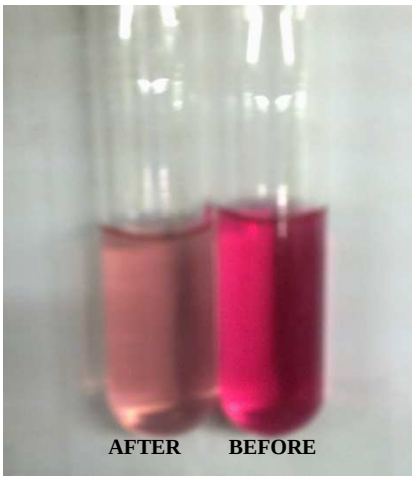


Fig 10: Decolourization of Textile Dye Red SNR by Pseudomonas aeruginosa after two weeks of incubation at room temperature.

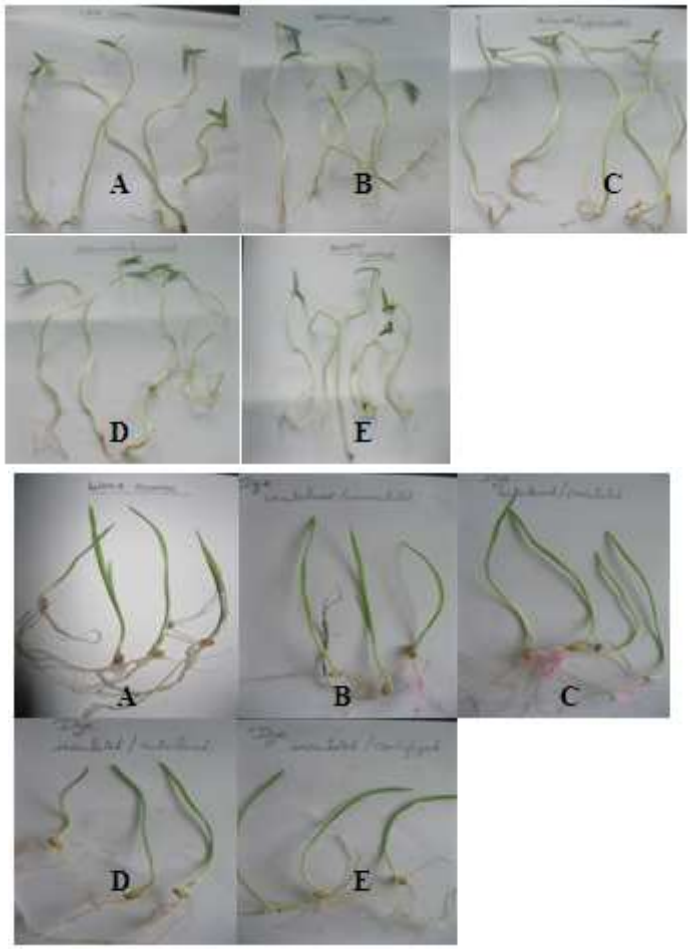


Fig 11: Effect of treated dye sample on *Vigna radiata* (Moong seeds), *Triticum* spp (Wheat), *Brassica juncea* (Mustard seeds)

A Water control
B 100 ppm of dye in plain water
C 100 ppm of dye in plain water & autoclaved
D 100 ppm of dye in plain water, inoculated, incubated at 28°C for 2 weeks, centrifuged
E 100 ppm of dye in plain water, inoculated, incubated at 28°C for 2 weeks, autoclaved

The results obtained showed that decolourisation of the dye resulted in reduction in the toxicity of the dye (Table 5, Fig 10 & 11). Hence when *Pseudomonas aeruginosa* was used to decolorize the textile dye RED SNR, it not only decolorized it but also reduced its toxicity.

CONCLUSION

Thus from the current study, it can be concluded, that the two isolates were capable of degrading textile dyes as well as biological stains and this property of the isolates can be further used as a basic tool for bioremediation of various textile effluents.

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