Decolourisation of Textile Dyes and Biological Stains by Bacterial Strains Isolated From Industrial Effluents

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Abstract

The color removal of textile wastewater is a major environmental concern. Color can be removed from wastewater by Physico-chemical methods such as adsorption, irradiation, ion exchange, oxidative process, ozonation, coagulation have been used to decolorize textile effluents, but these methods are costly, inefficient and sometimes produce hazardous by-product, it also affects the environment during the degradation process. The effluents discharged by different industries contain a high range of physico-chemical parameters like conductivity, hardness, alkalinity, COD, TSS, nitrates, nitrites, cations and anions. The effluent which is untreated is one of the major sources of consumed metal dyes, phenol, aromatic amines. Several aromatic amines are known mutagens and carcinogens to human beings. Among low cost,

viable alternatives, available for effluent treatment and decolorization, the biological systems are recognized. The present study was undertaken to explore with the Textile dyes effluents & Biological stains degraded by the bacterial strains isolated from the effluent sample. Phytotoxicity of the textile dyes was estimated by measuring the relative changes in seed germination of plants. Radicle and Plumule growth (length) were recorded after 6 days of exposure to different concentrations of dyes. Toxicity of dye was reduced after decolorization. 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 dye, Effluent, Decolourization, Bioremediation and Bacteria*

1 Introduction

Dyes make the world more beautiful through colored substances, but on the other hand they represent a serious pollution problem for the environment. Almost one million tons of dyes are annually produced in the world. Dyes are the most common synthetic colorants released to the environment via textile, pharmaceutical and chemical industries. Many of them may be carcinogenic to humans as well as to animals. The discharge of dyes in water bodies is problematic not only for aesthetic reasons, but also because

dyes and their cleavage products (aromatic amines) are carcinogenic [1].

Nowadays, water pollution has become a matter of great concern in our society. Most of the water pollutions are related to the industrial effluents. Textile industry is one of the most important industries all over the world and this industry uses large volumes of water in wet processing operations and thereby, generates substantial quantities of wastewater containing large amounts of dissolved dyestuffs and other products. Water is

essential for survival and existence of life on planet earth. The waste water and sewage are released from the industries that wastes are entering into the water bodies, it is one of major source of environment toxicity and it also affects the soil micro flora and aquatic ecosystem [2, 3]. The most environmental problem faced due to the textile dyeing industry is that the industry produces large volumes of high strength of aqueous waste effluents.

The colored effluents discharged from textile processing and dye-manufacturing industries contain a significant amount of unreacted dyes. Especially in textile industries produced more than 70% of the total quantity of waste in India [4]. India is the second largest exporter of dyestuffs and intermediates after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80%. Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment. Many of these products are problematic because of persistence (low biodegradability) and toxicity. The

effluent which is untreated is one of the major sources of consumed metal dyes, phenol, aromatic amines, several aromatic amines are known mutagens and carcinogens to human beings [5]. Dyes also affect internal organ like kidney, liver, gastrointestinal tract [6].

There are various microorganisms found in the contaminated environment, have potential to decolorize and even completely mineralize many dyes from the wastewater efficiently under certain environmental conditions. Have been reported by various researchers [7]. Several bacterial strains that can aerobically decolourize dyes have been isolated during the past few years. Many of these strains require organic carbon sources, as they cannot utilize dye as growth substrate. Biodegradation using microorganisms are gaining importance as it is cost effective, environmental friendly and produces less sludge [8].

Hence the present study was conducted to isolate the potential dye/stains (industrial as well as commonly used laboratory stains) degrading bacteria from industrial effluents and study their Dye degradation potential.

2 Materials and Methods

Effluent sample was collected in clean plastic can from the vicinity of Pulgam Textiles, Solapur, Maharashtra, India. Biological stains- Methylene

Blue, Malachite Green, Crystal Violet and Safranin were collected from the Microbiology Department of D. Y. Patil ACS College, Pimpri, Pune.

2.1 Physico-chemical property analysis:[9]

The collected effluent samples were analyzed to determine its physico-chemical parameters. The various parameters viz., Temperature, pH, Colour, Odour, Total dissolved solid (TDS), Total

suspended solids (TSS), Chemical oxygen demand (COD), Biological oxygen demand (BOD), Dissolved Oxygen (DO) were analysed in the laboratory by the standard protocols.

2.2 Isolation of dye degrading bacterial isolates from dye effluent:[10]

The bacterial isolates present in the textile dye effluent were isolated by enriching the sample with inorganic salts and Glucose as a carbon source along with 25ppm of Red and Blue dye each. The enrichment broth was incubated at $28 \pm 2^\circ \text{C}$ for 1 week at static conditions. The enriched broth was plated on sterile Minimal medium agar plates by Serial dilution (Pour plate) technique. In this

method, 1 ml of enriched broth was thoroughly mixed with 9 ml of sterile distilled water, and then it was serially diluted by following standard procedure up to concentration of 10^{-6} . Then, 1 ml of serially diluted broth from each concentration of samples were transferred to sterile petriplates and evenly distributed throughout the plates and sterile unsolidified Minimal Agar was poured and it was

allowed to solidify. The Minimal agar plates were incubated at $28 \pm 2^\circ\text{C}$ for 24 hours. After incubation, the bacterial colonies were isolated and purified from the plates. The well screened cultures were transferred to agar slant and stored at 4°C for

further study. All bacterial isolates were identified based on their colony characteristics, morphological characteristics and biochemical reactions.

2.3 Decolourization test:

2.3.1 Decolorization of Textile Dyes:[11]

The isolated bacteria were further tested to check their ability to decolorize the textile dyes. The bacterial isolates were grown in two different media-Medium A- supplemented with 1.0 ml of 10 % Glucose and medium B- supplemented with 1.0 ml of 10 % Casamino acids. Each medium was supplemented with increasing concentrations of the

dye (50 ppm, 100 ppm, 150 ppm and 200 ppm). The flasks were incubated at $28 \pm 2^\circ\text{C}$ for 24 hours under static conditions. After incubation the solution was centrifuged at 5000 rpm for 10 minutes. The optical density was taken at the appropriate wavelength (λ_{max}). Percentage decolourization was calculated by the formula-

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

2.3.2 Decolorization of Biological Stains:[12]

The decolourization capacity of isolated bacteria was also studied using biological stain solutions-Methylene Blue, Malachite Green, Safranin & Crystal Violet of different concentration. Each flask containing bacterial isolates along with distilled water was supplemented with increasing concentrations of the stain (50 ppm, 100 ppm, 150

ppm and 200 ppm). The flasks were incubated at $28 \pm 2^\circ\text{C}$ for 24 hours under static conditions. After incubation the solution was centrifuged at 5000 rpm for 10 minutes. The optical density was taken at the appropriate wavelength (λ_{max}). Percentage decolourization was calculated by the formula-

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

2.3.3 Bioassay for dye toxicity by Seed germination test-[13, 14]

In this experiment, the effect of four different concentrations of dyes were evaluated on germination of seeds of 3 plants: *Triticum* spp., *Vigna radiate* and *Brassica juncea*. The seeds were germinated in sterile Petri dishes. Three sets of thirty seeds each of *Triticum* spp., *Vigna radiate* 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 at room temperature ($28 \pm 2^\circ\text{C}$) for 6 days. Germination of seeds was daily recorded. At the end of the germination experiment, the shooting percentage and root length of seedlings were measured.

2.3.4 Seed germination test after treatment-[13, 14]

In another independent experiment, 100 ppm of the dye which was more decolorized by the bacteria isolated from the effluent sample was used to check the toxicity of the biologically treated dye on the germination of the seeds. The inoculations were made as follows: (a) 100 ppm of dye in plain water, (b) 100 ppm of dye in plain water & autoclaved, (c) 100 ppm of dye in plain water, inoculated, incubated at 28°C for 2 weeks, (d) centrifuged, 100 ppm of dye in plain water, inoculated, incubated at 28°C for 2 weeks, autoclaved.

The seeds were germinated in sterile Petri dishes, layered with sterile circular filter paper diameter-

10cm). 30 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 at room temperature (28±2 °C) for 6 days. Germination of seeds was daily recorded. At the end of the germination experiment, the shooting percentage and root length of seedlings were measured.

3 Results and Discussion

The dye effluents were collected from dying industry in Maharashtra, India. These industries discharge the brownish red colored effluents with dyes and toxic compounds into the open environment. The collected sample was analyzed to determine their physico – chemical characteristics. These characteristics were compared with the recommended level of NEQS as shown in Table-1 [15]. According to the results, the temperature of the effluent was 38.8°C, low as compared to the standards. The maximum pH range was recorded 5.5 which indicates acidic nature of the sample. The value of total dissolved solid was obtained 4440

mg/l, which indicated that the sample contained high concentration of dissolved solids which would affect the quality of freshwater and subsequently cause harm to aquatic life. The total suspended solid was recorded as 320 mg/l. The chemical oxygen demand of the sample was 763 mg/l which is much higher than the maximum recommended limit of NEQS. And the biological oxygen demand was recorded as 273 mg/l which was comparatively higher than the recommended value. Thus, the results showed that the textile effluent sample needed to be treated before discharging it into the environment.

Sr.No	Parameters	Dye Effluent Sample	NEQS*
1	Temperature	38.8	40
2	pH	5.5	6-9
3	Colour	Brownish red	Colorless
4	Odour	Unpleasant	Odourless
5	TS(mg/l)	4760	4500
6	TSS(mg/l)	320	-
7	TDS(mg/l)	4440	3800
8	DO(mg/l)	5.4	-
9	BOD(mg/l)	273	80-250
10	COD(mg/l)	763	156-400

NEQS- National Environmental Quality Standards

Table -1: Physico - chemical characterization of textile dye effluent samples

Identification of isolates obtained from effluent samples were done by morphological characterization, microscopically by Gram staining, and various biochemical tests recommended in the Bergey's Manual of

Determinative Bacteriology (Table 2) [16]. According to the tests, three organisms isolated may be *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp.

Sr.No	Colony & Morphological Characters	Isolate 1	Isolate 2	Isolate 3
1	Size	1-2mm	2mm	2.5mm
2	Shape	Irregular	Regular	Circular
3	Colour	Cream	Cream	Cream
4	Margin	Regular	Entire	Irregular
5	Elevation	Low convex	Low convex	Flat
6	Opacity	Opaque	Opaque	Opaque
7	Consistency	Butyrous	Mucoid	Butyrous
8	Gram character	Gram negative rods	Gram positive rods	Gram negative rods
9	Motility	Motile	Non-motile	Non-motile

2 (a) Colony & Morphological characteristics

Sr.No	Biochemical test	Isolate 1	Isolate 2	Isolate 3
1	Sugar fermentation			
	Glucose	Negative	Positive	Positive
	Lactose	Negative	Negative	Positive
	Maltose	Negative	Positive	Positive
	Sucrose	Negative	Positive	Positive
	Mannitol	Positive	Positive	Positive
	Arabinose	Negative	Positive	Positive
2	Catalase	Positive	Positive	Positive
3	Oxidase	Positive	Positive	Negative
4	Nitrate reduction test	Positive	Positive	Negative
5	Probable Genus level Identification	<i>Pseudomonas spp.</i>	<i>Bacillus spp.</i>	<i>Klebsiella spp.</i>

2 (b) Biochemical tests for the isolates(for sugar fermentation mention Acid/gas or only acid positive)

Table 2: Morphological, Biochemical and Physiological Characterization of the Microorganism
2 (a) Colony & Morphological characteristics 2 (b) Biochemical tests for the isolates

The 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 *Pseudomonas spp.*, *Bacillus spp.*, *Klebsiella spp.* all the three organisms could effectively decolorize the dye when grown in Medium B. The decolourization in Medium A was less in

comparison with Medium B 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 (Fig1). Highest percentage decolourization was shown by *Pseudomonas spp.* when grown in Medium B in Pigment Red 23 Dye as compared with Violet blue dye and other isolates (Table 3).

Sr .N o	Name of the dye	λ_{max} (nm)	Isolates	Media	Percentage Decolorization			
					50ppm	100ppm	150ppm	200ppm
1	Red dye	680	<i>Pseudomonas Spp</i>	Media A	79.4	75	69.2	57
				Media B	93.68	84.2	78.90	70.11
			<i>Klebsiella spp</i>	Media A	78	70.2	56	45.3
				Media B	90.42	87.42	76.53	56.40
			<i>Bacillus spp</i>	Media A	63.3	44.53	21.53	13.40
				Media B	84.2	78.4	68.9	56.3
2	Violet Blue dye	420	<i>Pseudomonas spp</i>	Media A	45.3	38.3	21.2	13.3
				Media B	78.1	69.13	58.53	45.99
			<i>Klebsiella spp</i>	Media A	35.3	23.7	18.3	12.3
				Media B	56.3	45.8	38.2	29.2
			<i>Bacillus spp</i>	Media A	68.7	68.53	56.2	45.2
				Media B	23.4	12.52	6.23	1.2

Table 3: Decolourization % of textile dye effluents by bacterial isolates

(b) Decolorisation of Textile Red Dyes by Isolates at different concentration in medium A & B

(b) Decolorisation of Textile Violet Blue Dyes by Isolates at different concentration in medium A & B

Fig 1: Decolorisation of Textile Dyes by Isolates at different concentration in medium A & B
(a) Red Dye (b) Violet Blue Dye

The dye decolourization capacity of *Pseudomonas spp* was studied using biological stain solutions- Methylene Blue, Malachite Green, Safranin& Crystal Violet (Table 4). It was found that

Pseudomonas spp was able to decolorize biological stains of 50ppm and 100ppm concentration only. And among the stains used Malachite green was decolorized more.

Sr.No	Stain	λ max(nm)	Percentage Decolorisation	
			50ppm	100ppm
1	Methylene Blue	570	75.86	60.13
2	Malachite Green	570	77.99	60.98
3	Safranin	450	40.50	49.35
4	Crystal Violet	520	69.5	40.50

Table 4: Percentage decolourization of Biological stains with *Pseudomonas spp* after 24 hours

The bioassay for dye toxicity in this study was based on measuring the effect of dye (Red dye) on seed germination, plant shooting and root elongation. The phytotoxicity of different soluble textile dyes was estimated by measuring the relative changes of the mentioned plant parameters using *Vigna radiate* (whole moong), *Triticum spp.* (Wheat) and *Brassica juncea* (Mustard seeds) seeds as test plants. The results show (Table 5) that high concentrations of dyes were more toxic to seed germination as compared with the lower concentrations. The results of this study suggest that while the exposure of seeds to low concentration of dye during germination were less toxic to seed germination as compared with the higher concentrations, the low concentration of the

dye could adversely affect the shooting percent significantly (Fig 2).

Sr.No	Seed Type	Dye Concentration	% Germination	Average Shoot length (cm)	Average Root length (cm)
1	<i>Triticum</i> 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	--	--
2	<i>Brassica juncea</i> (Mustard seeds)	Control	100%	5.88	4.95
		500 ppm	100%	4.98	4.23
		1000 ppm	100%	4.78	3.53
		5000ppm	60%	2.11	2.45
		10,000ppm	40%	1.11	1.45
3	<i>Vignaradiata</i> (Moong seeds)	Control	100%	5.00	4.92
		500	100%	4.53	4.00
		1000	100%	4.18	3.64
		5000	100%	No shooting	2.98
		1000	100%	No shooting	2.45

Table 5: Comparison between % germination, shoot and root length of different seeds at different dye concentrations

(a) Effect of textile dye on *Triticum* spp (Wheat) germination

(b) Effect of textile dye on *Vigna radiata*(Moong) seed germination

(c) Effect of textile dye on *Brassica juncea* (Mustard seeds) germination

Fig3: Effect of textile dye on (a)*Vignaradiata*(Moong), (b) *Triticumspp* (Wheat)and (c)*Brassica juncea* (Mustard seeds) germination

Since the decolorisation of Pigment Red 23 by *Pseudomonas spp* was seen more it was used for seed germination to check its toxicity after decolorisation. The effect of the decolorized dye was checked by checking the germination pattern of *Vigna radiate* (Moong), *Triticum spp* (Wheat) and *Brassica juncea* (Mustard) seeds (Fig 3). Different parameters were used, among which it was seen that when *Pseudomonas spp* was

autoclaved and applied on the seeds it effectively showed 100% germination in each type of seed used. The results obtained showed (Table 6) that decolourisation of the dye resulted in reduction in the toxicity of the dye. Hence when *Pseudomonas spp* was used to decolorize the textile dye Pigment Red 23, it not only decolorized it but also reduced its toxicity.

Sr.No	Seed Type	Parameters	Germination %	Average shootlength(cm)	Average rootlength (cm)
1	<i>Vigna radiate</i> (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
2	<i>Brassica juncea</i> (mustard seeds)	Water control	100	5.352	7.72
		Dye	100	4.46	6.28
		Autoclaved dye	100	4.3	4.14
		Inoculated/autoclaved	100	5.55	10.45
		Inoculated/centrifuged	100	4.877	5.65
3	<i>Triticum spp</i> (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 6: Comparison between % germination, shoot and root length of different seeds using decolorize dye after treatment

(a) Effect of treated dye sample on Vignaradiata(Moong seeds)

(b)Effect of treated dye sample on *Triticum spp* (Wheat)

(c) Effect of treated dye sample on *Brassica juncea* (Mustard seeds)

Fig 3: Effect of treated dye sample on (a) *Vigna radiata*(Moong seeds), (b) *Triticum spp* (Wheat), (c) *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

4 CONCLUSION:

Thus from the current study, it can be concluded that the physico-chemical properties of the textile

effluent used were comparatively more than the recommended NEQS standard, indicating that the

effluent needs to be treated before discharging it into the environment. The three isolates *Pseudomonas spp*, *Klebsiella spp* and *Bacillus spp* have the ability to degrade the textile effluent containing dye effectively and also degraded the biological satins upto 100ppm. From the bioassay for toxicity, it can be concluded that as the concentration of dye increases it results in

increased toxicity to seed germination as compared with lower concentrations. Toxicity of the degraded dye indicates that after decolourization the toxicity of the dye was reduced and 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.

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