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Decolorization and Biodegradation of Textile Azo Dye Disperse Red 78 by *Providencia rettgeri* Strain HSL1

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***Abstract*—**Removal of dyes from textile effluent with microorganisms, especially bacteria has recently gained attention. The present study was carried out to investigate the potential of a textile dye contaminated soil isolate *Providencia rettgeri* strain HSL1 (Genebank accession no. JX853768.1) for decolorization of model azo dye Disperse red 78. The bacterial strain has shown remarkably higher level of decolorization potential (98%) for selected dye Disperse red 78 (50 mg·L^{-1}) within 36 h at 30 ± 0.2 ^{o}C and pH 7.0. Reduction in the levels of BOD, COD and TOC from decolorized broth suggests the mineralization of dye. Induced activities of azo reductase and NADH-DCIP reductase were detected in cultures grown in the presence of dye. Germination (%) and growth efficiency of *Sorghum vulgare* and *Phaseolus mungo* seeds revealed the degradation of Disperse red 78 into less toxic products than control dye. Hence, *P. rettgeri* strain HSL1 can be applied for the treatment of textile dye contaminated sites.

***Keywords*—**Disperse red 78, *P. rettgeri* strain HSL1, decolorization, biodegradation, azo reductase, detoxification.

I. Introduction

THE application of synthetic dyes has increased in textile industries as they can be easily produced, offer a large variety of colors than natural dyes and are resistant to high temperatures. The aromatic structure of such dyes makes them resistant to the adverse effect of high temperature during the wet processing operations [1]. The utilization of synthetic dyes in textile industries is extensive, at around 100,000 tons per year, and 5–50 % of the dyes get eliminated into textile wastewaters [2]. Even at low concentrations, the presence of such dyes can cause waste streams to become highly colored. In addition, certain dyes and their biotransformation products have been shown to be eco-toxic and in some cases they are carcinogenic and mutagenic [3]. Among all textile dyestuff generated, the azo dyes constitute about 70 % and are used worldwide [4]. The azo dyes are considered as recalcitrant xenobiotic compounds due to the presence of a nitrogen double bond (–N=N–) and other groups such as sulphonic group which are difficult to biodegradation. Additionally, most of the synthetic azo dyes are mutagenic and carcinogenic to human beings [5]. Thus the treatment of such dyestuff containing wastewater becomes essential before discharged into water streams.

The current physical and/or chemical techniques to remove such dyestuff include coagulation, flocculation, adsorption, membrane filtration and irradiation [6]. Although these treatments are effective and achieve high levels of mineralization and decolorization, they have following drawbacks: high cost due to photocatalysis and advanced oxidation processes, potential production of highly toxic by-products, significant amounts of sludge get generated that requires a final destination like incineration or landfill disposal and in-applicability to a wide variety of dyes [7]. Thus, biodegradation seems to be an environmental/eco-friendly and less expensive alternative.

Biological degradation processes detoxifies the original dyestuff and also enables its conversion into the normal nutrient cycle in nature, reducing drastically the amount of sludge at the end of the process. Additionally, the microorganisms utilized in such treatment technologies can be grown using cheap and abundant carbon and nitrogen wastes derived from agro-industry. Several taxonomic groups of bacteria, fungi and algae have been reported for decolorization of azo dyes [8]. A fungal-bacterial consortium consisting of *Pseudomonas sp.* SUK1 and *Aspergillus ochraceus* NCIM 1146 have been reported to efficiently degrade and detoxify azo dye Navy blue HE2R, Rubine GFL and textile effluent [9],[10].

In this view, the present study was carried out to decolorize the azo dye Disperse red 78 using *P. rettgeri* strain HSL1 under various conditions. The optimization of degradation conditions for enhanced degradation and detoxification of dye was performed. Mineralization of dye was confirmed by COD and TOC analysis, while the activity of dye degrading enzymes was assayed spectrophotometrically. In addition, the impact of

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control dye and its degradation metabolites was evaluated by monitoring the toxicity to local crop plants.

II. Material and Methods

A. Chemicals and Azo Dye Used

Dehydrated microbiological medium nutrient broth (NB) was obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. 2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), methyl red, nicotinamide adenine dinucleotide (NADH) and dichlorophenol indophenols (DCIP) were purchased from Sigma-Aldrich, USA. All chemicals used were of analytical grade. Textile azo dye Disperse red 78 was generously gifted by Mahesh Textile Processors, Ichalkaranji, India (**Figure 1**).

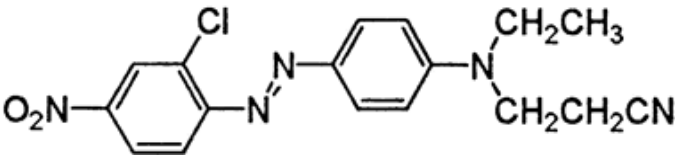

Figure 1 Structure of textile azo dye Disperse Red 78 (Molecular formula: $C_{17}H_{16}ClN_5O_2$; Molecular weight: 357.71)

B. Bacterial Strain and Culture Conditions

P. rettgeri strain HSL1 (Genebank accession no. JX853768.1) originally isolated from textile dye contaminated soils in our previous study was used. The stock culture of bacterial strain was maintained at 4 ^{o}C on nutrient medium slants containing 1% textile effluent to retain its dye decolorizing abilities. For decolorization assay, the enrichment was carried out by inoculating a loop of *P. rettgeri* strain HSL1 stock in NM and incubating it for 24 h at 30 ± 0.2 ^{o}C. Culture was grown at 30 ± 0.2 ^{o}C for all the subsequent assays.

C. Decolorization Experiments

The decolorization experiments were performed into 250 ml Erlenmeyer flask containing 100 ml of pre-enriched *P. rettgeri* strain HSL1. Effect of pH, incubation temperature and dye concentration on decolorization performance was also investigated. At defined time of intervals the aliquots of culture supernatant (3 ml) were withdrawn and suspended particles were removed by adding equal volume of methanol followed by centrifugation at 7500 g for 15 min. The resulted clear supernatant was analyzed for decolorization at maximum absorbance wavelength of 570 nm using UV-vis spectrophotometer (Hitahi U-2800; Hitachi, Tokyo, Japan). The control flask which is without dye or bacterial culture was also tested under same conditions. All decolorization experiments were performed in triplicate, and decolorization activity was expressed in terms of percent decolorization using the formula:

$$\text{Decolorization (\%)} = \frac{\text{Init. absorbance}_{(0\text{ h})} - \text{Observ'd absorbance after incubation}_{(t)}}{\text{Init. absorbance}_{(0\text{ h})}} \times 100$$

Measurement for reduction in chemical oxygen demand (COD) and biological oxygen demand (BOD) before and after the decolorization of Disperse red 78 by *P. rettgeri* strain HSL1 was carried out by employing the standard methods of APHA [11]. The total organic carbon (TOC) was estimated using the TOC analyzer (Hach DR 2700 spectrophotometer, Hach Co., USA) [12]. The removal ratio of TOC was calculated as follows:

$$\text{TOC removal ratio (\%)} = \frac{\text{Initial TOC}_{(0\text{ h})} - \text{Observed TOC}_{(t)}}{\text{Initial TOC}_{(0\text{ h})}} \times 100$$

Where, $TOC_{(0\text{ h})}$ and $TOC_{(t)}$ are the initial TOC value at 0 h and the TOC value after particular reaction time *t*, respectively.

D. Estimation of Enzyme Activity

Extraction of extracellular enzymes was carried out by centrifugation of decolorized broth at 7500 g for 15 min in cold condition, and supernatant was used for assessing enzyme activities [13]. The remaining cell debris were suspended in 50 mM potassium phosphate buffer (pH 7.4), homogenized and sonicated giving 7 strokes of 30 s each for 2 min interval based on 50 amplitude output at 4 ± 1 ^{o}C (Sonics-vibracell ultrasonic processor). These sonicated cells were further centrifuged at 7500 g for 15 min at 4 ± 1 ^{o}C, and supernatant was used as the source of intracellular enzymes.

The activity of laccase was determined by measuring the oxidation of 2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at 420 nm [14]. Azoreductase activity was measured by assessing methyl red reduction at 430 nm [15]; while NADH–DCIP reductase activity was assed as reported previously [16]. All enzyme assays were run in triplicate, average rates were calculated and one unit of enzyme activity was defined as a change in absorbance unit min^{-1} mg of $protein^{-1}$. The protein content was determined by using the method of Lowry, et al. [17] with bovine serum albumin as the standard.

E. Phytotoxicity Studies

The toxicity of dye Disperse red 78 and its degraded metabolites was studied through growth experiments using common agricultural plants *Sorghum vulgare* (monocot) and *Phaseolus mungo* (dicot). The phytotoxicity tests were carried out by the methods of Lade et al. [10]. Briefly, seeds were allowed to germinate by daily watering 10 ml each of dye Disperse red 78 (50 mg L^{-1}), its degradation metabolites (50 mg L^{-1}) and distilled water as control. Length of shoot, root and seed germination (%) was recorded after 13 days. The percent of germination was calculated using the formula:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sowed}} \times 100$$

F. Statistical Analysis

Data were evaluated by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test, through the ASSISTAT 7.5 software (assistat.com).

III. Results and Discussion

A. Decolorization of Disperse red 78

Decolorization potential of *P. rettgeri* strain HSL1 was tested against commonly used textile azo dye Disperse red 78 at various environmental conditions. Complete decolorization of dye was observed in 36 h at 30 ± 0.2 °C temperature in static incubation condition (**Figure 2**). No considerable decolorization was observed in shaking condition at 120 rpm (data not shown). Increased biomass occurred on dye containing broth in shaking incubation conditions but no decolorization took place as the azo dyes would be difficult to breakdown under these conditions [18].

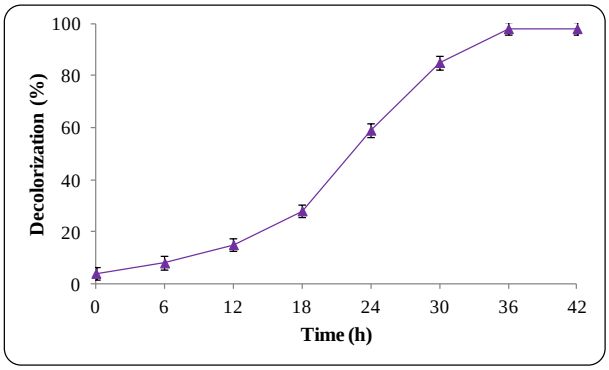

Figure 2 Decolorization of Disperse red 78 by *P. rettgeri* strain HSL1

The enhanced decolorization rate in static condition might be due to the involvement of enzyme azoreductase which functions well in anaerobic conditions. Azoreductase is the key enzyme responsible for the reductive cleavage of azo dyes, and the presence of oxygen inhibits its azo bond reduction activity. Similar findings were reported in previous study, where *Pseudomonas sp.* SUK1 exhibited higher decolorization rate of reactive dye Red BLI under microaerophilic condition whereas shaking culture showed only the growth but no decolorization [19]. *Providencia sp.* SDS isolated from dye contaminated sludge was also shown to decolorize the textile dye Red HE3B and effluent in consortium with *Ps. aeuroginosa* strain BCH in microaerophilic condition [20].

B. Optimization of Physicochemical Conditions

The effect of environmental parameters greatly influences the decolorization ability of microorganisms. In view of this, the effect of broth pH, incubation temperature and dye concentration on decolorization of dye Disperse red 78 by *P. rettgeri* strain HSL1 was evaluated. Result of the pH study shown that bacterial strain able to decolorize the dye at broad range of pH, however, the optimum pH was found to be 7.0 (**Figure 3a**). The pH of the medium is known to be responsible for transport of dye molecules across the cell membrane, which was considered as the rate limiting step for the decolorization [21]. The enhanced decolorization of dye Disperse red 78 was found at 30 ± 0.2 °C temperature (**Figure 3b**), and further increase or decrease in incubation temperature resulted into marginal reduction in the decolorization performance. Maximum decolorization of dye was found at the concentration of 50 mg L^{-1} within 36 h by *P. rettgeri* strain HSL1 (**Figure 3c**).

The analysis of dye decolorized broth for change in physicochemical parameters suggests the significant reduction in value of BOD (75 %), COD (86 %) and TOC (54 %). These observations confirmed the mineralization of dye by *P. rettgeri* strain HSL1, however, higher concentration seemed to increase decolorization time.

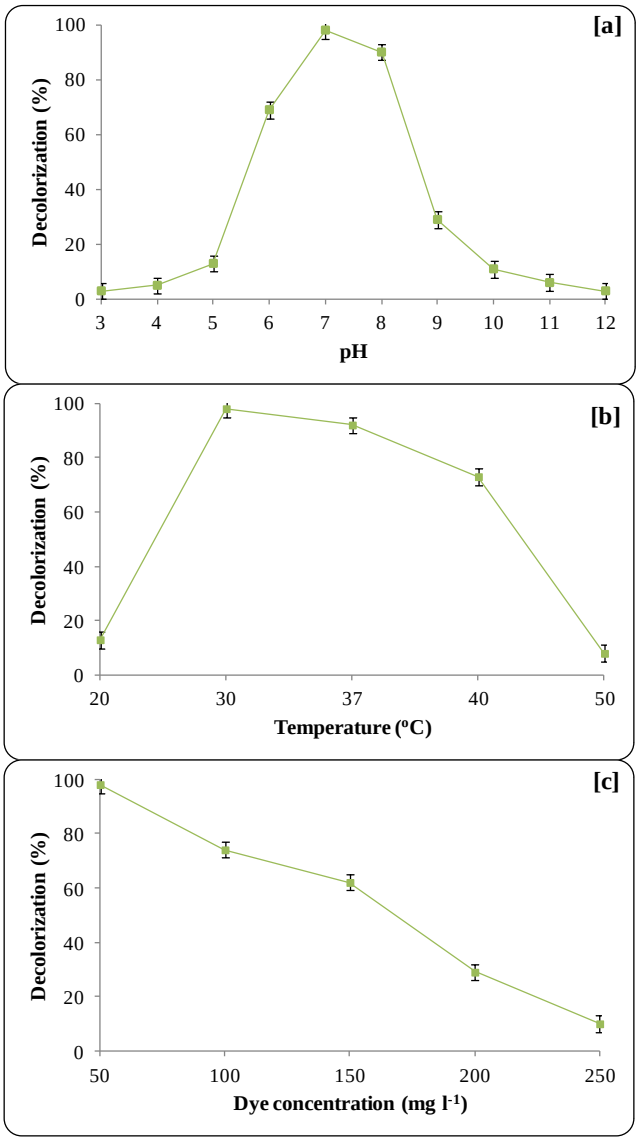

Figure 3 Optimization of degradation conditions; Effect of pH [a], temperature [b], and dye concentration [c] on decolorization of Disperse red 78 by *P. rettgeri* strain HSL1

C. Enzyme Analysis

The enzyme activities of laccase, azoreductase and NADH-DCIP reductase were determined after complete decolorization of dye Disperse red 78 by *P. rettgeri* strain HSL1. The activity assay for laccase enzyme revealed the presence of enzyme in both control and test broth but no induction was observed (**TABLE I**). However, significant induction in the activities of azo reductase (146 %) and NADH-DCIP reductase (86 %) from decolorized broth was observed which indicates its active involvement in degradation process. These enzymes are known to play important roles in

degradation of dyes and therefore their induced activity pattern confirms the biotransformation of dye Disperse red 78. Induction of this enzyme also depends on the structure of the dye and metabolites formed. The role of oxidoreductive enzymes in the decolorization of reactive azo dye Red HE3B have been characterized in *Providencia sp.* SDS [20].

TABLE I ENZYME ACTIVITIES DURING DECOLORIZATION OF DISPERSE RED 78 BY *P. RETTGERI* STRAIN HSL1

Enzymes	Control cells	After decolorization
Laccase[1]	0.280 ± 0.03	0.290 ± 0.04
Azo reductase[2]	0.156 ± 0.01	0.385 ± 0.02*
NADH-DCIP reductase[3]	15 ± 1.04	28 ± 2.06*

[1]µM of ABTS oxidized min^{-1} ml of $enzyme^{-1}$
[2]µM of Methyl red reduced min^{-1} ml of $enzyme^{-1}$
[3]µM of DCIP reduced min^{-1} ml of $enzyme^{-1}$
Values are mean of three experiments, ± SEM, significantly different from control cells at *P < 0.001 by one-way analysis of variance (ANOVA) with Tukey Kramer comparison test.

D. Phytotoxicity Studies

Use of untreated and treated dye containing water for agricultural purposes has direct impact on soil fertility as well as indirectly affects human health through the consumption of such crops. Thus, it is of concern to assess the phytotoxicity of the dye Disperse red 78 before and after degradation by *P. rettgeri* strain HSL1. The results of the toxicity study showed that there was an inhibition of germination for both the seeds *S. vulgare* and *P. mungo* by 60 % treated with solutions containing 50 mg L^{-1} of dye (**TABLE II**). However, complete germination was observed in both the seeds irrigated with dye degradation metabolites. Disperse red 78 affected significantly the elongation of shoot and root than its degraded metabolites by *P. rettgeri* strain HSL1. This indicates the less toxic nature of degradation metabolites to the plants. Parshetti, et al. observed the reduced germination of *T. aestivum* with malachite green treatment as compared to its degradation product and distilled water [22].

TABLE II PHYTOTOXICITY STUDIES OF THE DISPERSE RED 78 AND ITS METABOLITES OBTAINED AFTER DEGRADATION BY *P. RETTGERI* STRAIN HSL1

Samples	*Sorghum vulgare*			*Phaseolus mungo*		
	Germination (%)	Shoot length (cm)	Root length (cm)	Germination (%)	Shoot length (cm)	Root length (cm)
Distilled water	100	10.5 ± 0.3	4.2 ± 0.2	100	11.2 ± 0.3	4.8 ± 0.2
Disperse red 78	40	6.4 ± 0.3*	2.6 ± 0.2*	40	6.1 ± 0.2*	2.3 ± 0.1*
Degradation metabolites	100	10.3 ± 0.4	3.9 ± 0.3	100	11.1 ± 0.2	4.5 ± 0.3

Values are mean of three experiments, ± SEM. Seeds germinated textile effluent are significantly different from control (distilled water) at *P < 0.001 by one-way analysis of variance (ANOVA) with Tukey-Kramer comparison test.

IV. CONCLUDING REMARKS

The above results showed that *P. rettgeri* strain HSL1 could decolorize and degrade 50 mg L^{-1} of textile azo dye Disperse red 78 in 36 h at 30 ± 0.2 °C. Reduction in the levels of COD and TOC values confirmed the mineralization of treated dye. This is likely due to the induced enzymatic activities of azo reductase and NADH-DCIP reductase which reduced the dye molecule. The effectiveness of degradation was confirmed by phytotoxicity study, which indicated that *P. rettgeri* strain HSL1 has transformed the Disperse red 78 into non-toxic metabolites. These observations suggest that the *P. rettgeri* strain HSL1 could effectively be used as an alternative to physicochemical methods of textile wastewater treatment.

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