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Biodegradation and Detoxification of Reactive Red 250 by *Pseudomonas aeruginosa* CR-25

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Abstract

The major sources generating a large amount of colored effluents are textile dyeing and printing industries. Untreated effluents from dyestuff production and dyeing mills may be highly colored and thus particularly objectionable and offensive if discharged into water. Bioremediation addresses the limitations of these conventional techniques by bringing about the actual destruction of many organic contaminants at a reduced cost. The bacterium *Pseudomonas aeruginosa* CR-25 was identified by 16S rRNA gene sequence analysis (GenBank: EU109736).

CR-25 isolated from textile effluent decolorized and biotransformed Reactive Red 250 (RR250) to aromatic amines benzidine and 4-chloro aniline. Further incubation under aerobic conditions led to the complete degradation of these intermediates. The decolorization activity of RR250 was analyzed under various nutritional and environmental parameters. The toxicity of cell-free culture (obtained after decolorization) was carried out on the germination of seeds of *Arachishypogia, Cicerarientum, Triticumvulgare and Phaseolusmungo*. Seeds irrigated with cell-free culture under static culture condition demonstrated toxic effects and obtained after sequential static-shaking culture condition reduced toxic effects. Decolorization and degradation potential of CR-25 can be exploited for bioremediation of polluted sites.

Keywords: Reactive Red 250; Decolorization; *Pseudomonas aeruginosa* CR-25; Aromatic amines

Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life. The textile industry is one of them, which extensively uses synthetic chemicals as dyes. Wastewater from textile industries poses a threat to the environment, as a large amount of chemically different dyes are used. A significant proportion of these dyes enter the environment *via* wastewater. Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide. Pollution due to textile industry effluent has increased during recent years. The textile finishing generates a large amount of wastewater containing dyes and represents one of the largest causes of water pollution as 10%-15% of dyes are lost in the effluent during the dying process. The traditional textile finishing industry consumes about 100 liters of water to process about 1 kg of textile material. The new closed-loop technologies, such as the reuse of microbial or enzymatical treatment of dyeing effluents, could help to reduce this enormous water pollution [1].

Azo dyes have been used increasingly in industries because of their case and cost- effectiveness in synthesis compared to natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic include a broad spectrum of different chemical structures, such as aromatic amines (C_6H_5-NH_2), which are suspected carcinogens, phenyl (C_6H_5-CH_2) and naphthyl (NO_2OH).

Azo bonds present in these compounds are resistant to breakdown, with the potential for persistence and accumulation in the environment.

However, they can be degraded by bacteria under aerobic and anaerobic conditions. Several physicochemical techniques have been proposed for the treatment of colored textile effluents.

These include adsorption on different materials, oxidation and precipitation by Fenton's reagent, bleaching with chloride or ozone photodegradation or membrane filtration. All these physical or chemical methods very expensive and result in the production of a large amount of sludge, which creates a secondary level of land pollution. Therefore, economical and safe removal of the polluting dyes is still an important issue [2].

Bioremediation through microorganisms has been identified as a cost-effective and environment-friendly alternative for disposal of textile effluent. It has been reported that complete mineralization of dyes is possible only if an anaerobic reduction is followed by aerobic oxidation of the amines formed in the reductive steps.

In the present study, decolorization and biodegradation of Reactive Red 250, a water-soluble, benzidine based azo dye was carried out under static, shaking and sequential static-shaking culture conditions. Evaluation of toxic effects of dye treated under different environmental conditions was carried out [3].

Materials and Methods

Chemicals

All the chemicals used were of analytical grade and media from Hi-Media, India. The dyes were procured from various dye manufacturing units of Ankleshwar and Ahmedabad. A stock solution (1000 mg/ml^{-1}) of a Reactive Red 250 dye was prepared in double-distilled water. Required concentrations were prepared by diluting the stock solution [4].

Medium

Mineral Salt Medium (MSM) consisting (g.l^{-1}): (K_2HPO_4 (2.0); KH_2PO_4 (1.0); $MgSO_4$ $7H_2O$ (0.50); NaCl (0.5); $FeSO_4$ $7H_2O$ (0.02); NH_4Cl (1.0); $CaCl_2$ $2H_2O$ (0.1)) was prepared. The pH of the medium was adjusted to 7. This medium was used for the primary screening of bacterial populations present in the textile effluents.

Peptone, Yeast extract, Sodium chloride (PYS) medium consisting of (g): peptone (5.0); yeast extract (5.0); NaCl (3.0) was prepared. The medium was supplemented with Reactive Red 250 (100 mg/ml). The pH of the medium was adjusted to 7. This medium was used for the screening of dye decolorizing bacterial populations [5].

Identification of microorganism: 16S rRNA sequence analysis

Pseudomonas aeruginosa CR-25 used for this study was identified by 16S rRNA. Amplification of 1494 bp out of 1500 bp of 16s rRNA gene was carried out using consensus primers and high fidelity PCR Polymerase. It was cloned into a plasmid, which were sequenced bidirectional using forward, reverse and internal primers.

Experimental setup for the decolorization and biodegradation studies

Different concentrations of Reactive Red 250 dye (mg/ml) 100, 200, 400, 600, 800 and 1000 were added to PYS medium inoculated with an actively growing culture of *P. aeruginosa* CR-25 which is having ODλ600 nm of 1.0 (c.a. 1.161 × 1011 cells ml^{-1}). These flasks were incubated at 37°C under sequential static (48 h) and shaking (48 h) culture conditions till decolorization was achieved. The percent decolorization was determined spectrophotometrically by measuring absorbance at λ530 nm using a UV-Visible spectrophotometer. The biodegradation was analyzed by monitoring an increase in biomass, change in the intracellular protein, HPLC and phytotoxicity studies [6].

Effect of different carbon and nitrogen

Reactive Red 250 (100 mg/ml) containing MSM were supplemented with 0%-1% various carbon sources (Glucose, Starch, Maltose, Galactose, Inositol, Arabinose, Sorbitol, Mannitol, Fructose, Sucrose, Rhamnose, Mannose and Xylose) and nitrogen sources (Ammonium nitrate, Ammonium chloride, Ammonium sulfate, Potassium nitrate, Sodium nitrate, Peptone, Tryptone, Yeast extract, Beef extract, Casein hydrolysate). The carbon sources were sterilized separately (10 psi, 10 min) and added to the medium. The medium was inoculated with 1% *P. aeruginosa* CR-25 culture containing ca. 1.161 × 1011 cells.ml^{-1} [7].

Effect of pH

PYS medium containing Reactive Red 250 was prepared in different buffer solutions. The buffers selected for media preparation were 0.1 M citrate buffer (pH 4 and 5), 0.1 M sodium phosphate buffer (pH 6 and 7) and 0.1 M Tris buffer (pH 8 and 9). Samples were harvested at an interval of 48 h and analyzed for decolorization [8].

Effect of temperature

PYS medium was supplemented with Reactive Red 250 (100 mg. l^{-1}). The flasks were inoculated with *P. aeruginosa* CR-25 (ca.1.161 × 1011 cells.ml^{-1}) and incubated at 10, 20, 37, 50°C and 60°C temperature under static culture condition. Appropriate controls were also included. Samples were harvested at an interval of 48 h and analyzed for decolorization [9].

Effects of the age of inoculum

Culture of *P. aeruginosa* CR-25 having different ages like 6 h, 12 h, 18 h, 24 h and 36 h was used as inoculum. 100 ml of PYS medium with Reactive Red 250 (100 mg/ml) were prepared. In a set of five flasks, each flask was inoculated with 1% inoculum (ca.1.161 × 1011 cells.ml^{-1}) and incubated at 37°C. Inoculated and un-inoculated controls were also included. The flasks were harvested at an interval of 48 h and analyzed for decolorization [10].

Effect of static and shaking environment on decolorization

PYS medium was supplemented with Reactive Red 250 (100 mg/ml^{-1}). The flasks were inoculated with *P. aeruginosa* CR-25 (ca.1.161 × 1011 cells.ml^{-1}) and incubated at 37°C temperature under static and shaking culture conditions. Appropriate controls were also included. Samples were harvested after 48 h and analyzed for decolorization [11].

Decolorization assay

Decolorization of Reactive Red 250 was analyzed spectrophotometrically by measuring A530nm n-butanol extract of the culture medium. The un-inoculated flasks containing dye was used as a control. Decolorization activity (%) was calculated as: Decolorization activity (%)=((A-B)/A) × 100. Where A=Initial absorbance and B=Observed absorbance. In the case of other textile dyes, the culture medium was centrifuged (10,000 rpm, 15 min). The supernatant was analyzed spectrophotometrically at the λ_{max} of the respective dye. Inoculated (without dye) and uninoculated controls were used as a reference [12].

Estimation of bacterial growth

The culture medium was centrifuged (10,000 rpm, 15 min). The pelleted biomass was boiled in 1M NaOH for 25 min and the protein content was estimated. Measure in intracellular protein was correlated to determine bacterial growth. Glucose utilization during dye degradation was estimated by the phenol-sulphuric acid method. All the experiments were carried out in triplicate and the data presented here are the average of three [13].

Analytical methods

High-Performance Liquid Chromatography (HPLC) analysis: Cultures were centrifuged and the supernatant was extracted three

times with diethyl ether. The products from the air-dried extracts were solubilized in methanol and filtered through 0.2 µm filter and analyzed by HPLC. Linear gradient of the mobile phase employed was Methanol: (0.57 ml.min^{-1}) Ammonium di-hydrogen phosphate+(0.78) Disodium hydrogen phosphate (15:85) at 0 minute and 80:20 at 45 minutes [14].

Seed germination assay

Effects of decolorized and degraded dye products on germination of seeds of *Arachis hypogia L., Cicer arientum L., Triticum vulgare L.* and *Phaseolus mungo L.* were evaluated. The experiment was conducted in two phases.

In the first phase, Reactive Red 250 (400 mg/ml) was decolorized under static culture condition by *P. aeruginosa* CR-25. Biomass was separated by centrifugation (10,000 rpm, 15 min) and the supernatant was collected and labeled as "Static Treatment" (ST).

In the second phase, the decolorized sample was further treated under shaking culture conditions (100 rpm, 24 h, 37°C). Biomass was separated as described above and the supernatant was obtained and labeled as "Sequential Static-Shaking Treatment (SSST)". "Dye Control" (DC), was prepared using the same concentration of Reactive Red 250. The tap water was used as "Water Control" (WC) and "Medium Control" (MC) was prepared to dissolve the components of the MSM medium except for dye [15].

Seed germination assay was performed in 15 cm sterile petri dishes layered with sterile filter paper. Seeds were surface-sterilized according to and transferred to the surface of the paper in the petri dishes. Ten seeds per plate were used in germination assays. Seeds were treated with ST, SSST, DC, WC and MC samples. Every other day germinating seeds were treated with 5 ml of each sample. Each treatment was replicated three times in complete randomized distribution. Germination of seeds treated with water was used as a control. All dishes were kept at room temperature for five days and observations were recorded daily. Seeds were considered germinated when the radical and hypocotyls appeared together [16].

Results and Discussion

Identification of microorganism

Dye decolorizing strain CR-25 was identified to be *Pseudomonas aeruginosa* based on the 16S rRNA gene sequence and exhibited 100% similarity with the strain *Pseudomonas aeruginosa* (AF23778). Information about other closely related bacterial species and strains is evident from the alignment view. The 16S rRNA gene sequence is submitted in NCBI under accession number EU109736 [17].

Effect of dye concentrations

The decolorization of various concentrations of Reactive Red 250 during incubation of five days under static culture conditions in the PYS medium. Pseudomonas aeruginosa CR-25 decolorized Reactive Red 250 up to 1000 mg/ml^{-1}. Concentrations of more than 1000 ppm were found to be inhibitory (Figures 1-3)(Table 1).

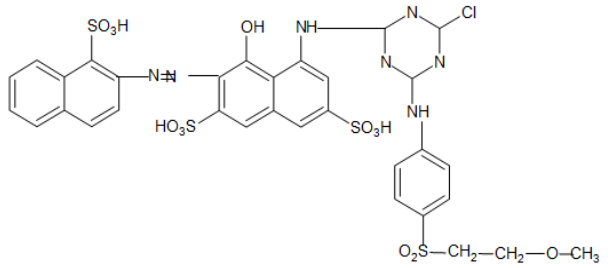

Figure 1: Structure of the dye Reactive Red 250.

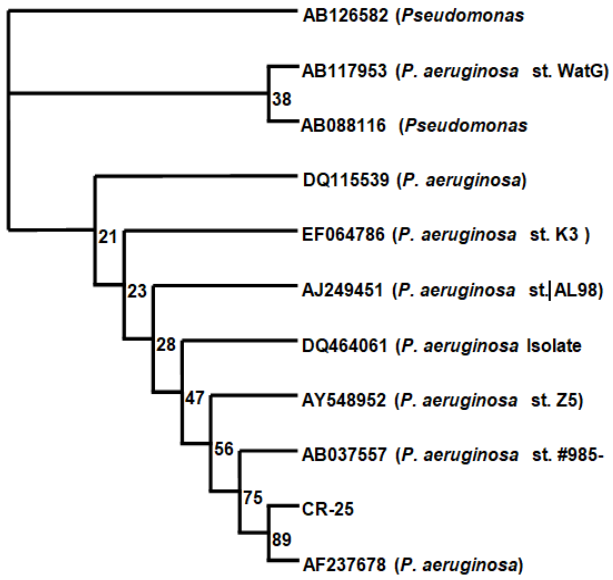

Figure 2: Phylogenetic tree of *Pseudomonas aeruginosa* CR-25.

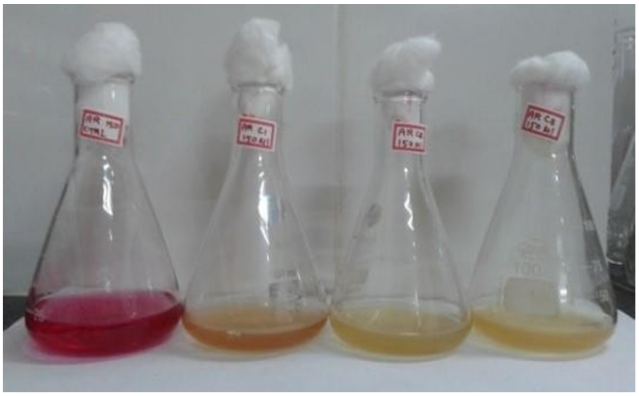

Figure 3: Flasks illustrating decolorization of Reactive Red 250 by *P. aeruginosa* CR-25 under static culture condition at 37°C. From left to right: Flask with dye and culture incubated for time 0 hour, 24 hours(1 day), 72 hours (3 days) and 120 hours (5 days).

Organism	Dye (mg/ml)	Decolorization (%)				
		24 h	48 h	72 h	96 h	120 h
Pseudomonas aeruginosa CR-25	100	88 ± 1.1	96 ± 1.5	98 ± 1.0	98 ± 1.0	98 ± 0.9
	200	86 ± 1.3	89 ± 1.1	91 ± 1.7	96 ± 1.0	96 ± 1.1
	400	77 ± 1.5	80 ± 1.0	86 ± 1.9	90 ± 1.1	90 ± 1.0
	600	72 ± 1.8	78 ± 1.5	81 ± 2.0	84 ± 1.7	86 ± 1.2
	800	68 ± 0.9	73 ± 2.1	75 ± 2.5	77 ± 1.4	78 ± 1.5
	1000	66 ± 2.3	70 ± 1.2	73 ± 2.1	74 ± 1.1	74 ± 1.2

Table 1: Decolorization of Reactive Red 250 at various concentration by *Pseudomonas aeruginosa* CR-25.

Effect of different carbon and nitrogen

P. aeruginosa CR-25 did not use dyes as the sole source of carbon and nitrogen. In fact, in addition organisms required carbon and nitrogen sources (as co-substrates) for the metabolism of the dye. Various carbon sources were tested for the decolorization of Reactive Red 250 (100 mg/ml). 0.6% glucose concentration was found to be optimal as an initial source of carbon for maximum decolorization of Reactive Red 250. The finding obtained indicates decolorization of azo dye by *P. aeruginosa* CR-25 is dependent on the metabolism of carbohydrates. The degradation of Navitan Fast Blue S5R by *Pseudomonas aeruginosa* supports this finding. The co-metabolism of sulphonated orange-I by *Pseudomonas sp*., where the organism was not able to utilize the sulphonated dye as the sole substrate. Further, decolorization of textile dyes Orange II, AO8 and AR88 by *Sphingomonas sp*. Strain 1CX was possible only in the presence of carbohydrates (Figure 4)(Table 2) [18].

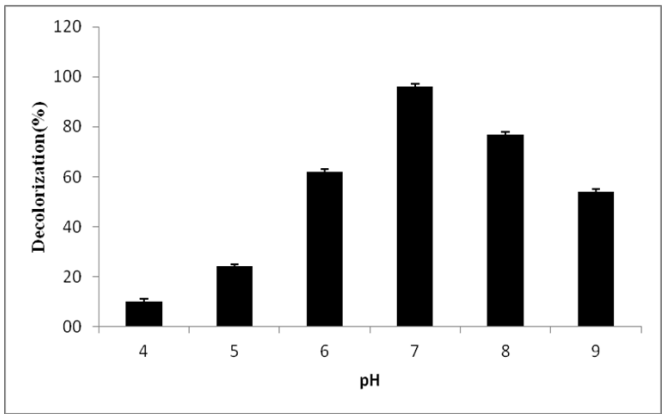

Figure 4: Effect of initial pH on decolorization of Reactive Red 250 by *P. aeruginosa* CR-25 under static culture condition at 37° C.

Carbon source	After 48 h incubation		
	Decolorization (%)	Growth (mg/ml)	Decolorization Index (% decolorization to Biomass)
Glucose	96 ± 0.2	720 ± 0.23	0.13
Starch	47 ± 0.5	618 ± 0.54	0.07
Maltose	44 ± 0.1	594 ± 0.69	0.07
Galactose	54 ± 0.6	594 ± 0.12	0.09
Inositol	48 ± 0.5	578 ± 0.41	0.08
Arabinose	33 ± 0.7	574 ± 0.36	0.05
Sorbitol	53 ± 1.4	574 ± 0.65	0.09
Mannitol	60 ± 0.9	514 ± 0.18	0.12
Fructose	70 ± 0.2	498 ± 0.36	0.14
Sucrose	42 ± 0.6	584 ± 0.56	0.07
Rhamnose	41 ± 0.4	404 ± 0.59	0.1
Mannose	33 ± 0.2	574 ± 0.46	0.06
Xylose	26 ± 0.9	554 ± 0.78	0.05

Table 2: Effects of different carbon sources (6 g) on the decolorization of reactive red 250 by *P. aeruginosa* CR-25 (Dye concentration 100 mg/ml).

The effects of various inorganic and organic nitrogen sources on dye decolorization of Reactive Red 250 were studied. Among the inorganic nitrogen sources, ammonium salts were the preferred nitrogen sources for decolorization. In the presence of ammonium chloride, 87% ± 0.1% decolorization was obtained with 684 mg ± 0.25 mg of protein.l^{-1}. Decolorization obtained in the presence of potassium nitrate, sodium nitrate and casein hydrolysate was 33% ± 0.2%, 29% ± 0.8% and 15% ± 0.3%, respectively. The minimum concentration of ammonium salts required by *P. aeruginosa* CR-25 for decolorization of Reactive Red 250 was found to be 0.1%. The related findings showed maximum decolorization of Navitan Fast Blue S5R by *Pseudomonas aeruginosa* in the presence of ammonium salts [19]. Related findings of ammonium salts supporting the decolorization of Orange II, AO8 and AR88 by *Sphingomonas sp*. strain 1CX (Table 3).

After 48 h Incubation under static condition		
Nitrogen Source	**Decolorization (%)**	**Growth (mg Protein)**
Ammonium nitrate	84 ± 0.5	604 ± 0.12
Ammonium chloride	87 ± 0.1	684 ± 0.25
Ammonium sulphate	84 ± 0.6	624 ± 0.65
Potassium nitrate	33 ± 0.2	364 ± 0.45
Sodium nitrate	29 ± 0.8	284 ± 0.65
Peptone	95 ± 0.5	702 ± 0.27
Tryptone	89 ± 0.2	667 ± 0.36
Yeast extract	88 ± 0.4	625 ± 0.31
Beef extract	78 ± 0.8	598 ± 0.54
Casein hydrolysate	15 ± 0.3	374 ± 0.69

Table 3: Effects of different nitrogen sources (1 g) on the decolorization of Reactive Red 250 by *P. aeruginosa* CR-25 (Dye concentration 100 mg/ml-1)

Among organic nitrogen sources, in the presence of peptone, 95% ± 0.5% decolorization was achieved which was followed by tryptone (89% ± 0.2%), yeast extract (88% ± 0.4%), beef extract (78% ± 0.8%) and casein hydrolysate (15% ± 0.3%). Kothari (2002) also reported similar findings on the effects of organic nitrogen sources like peptone and yeast extract on decolorization of Reactive Red H5BL by *Pseudomonas sp*.

Effect of pH

Ionic strength (pH) of the medium is of prime importance for almost all physiological processes. Optimal degradation or synthesis occurs at a specific pH value. Decolorization and biodegradation are also pH-dependant processes [20].

Bacterial cultures generally exhibit maximum decolorization at pH value near 7. The decolorization of Reactive Red 250 by *P. aeruginosa* CR-25 was observed between pH values 6 and 9, but the maximum decolorization was achieved at pH 7. Both, *E. coli* and *P. luteola*, were reported for best decolorization at a pH 7 with constant decolorization rate up to pH 9.5 and *K. pneumoniae* RS-13 completely degraded methyl red in the pH range of 6-8. A pH value between 6 and 9 was optimum for the decolorization of triphenylmethanes and azo dyes by *Pseudomonas sp*. Moreover, it has been reported that generally, azo dye reduction by bacterial cultures to more basic aromatic amines leads to a rise in pH of the medium by about 0.8 to 1.0 values.

Effect of temperature

Microbial growth is temperature specific and the temperature is an important physical parameter that affects cell metabolism directly or indirectly. Those processes, which are catalyzed by an enzyme, are affected by incubation temperature. Variation in temperature decreases or increases the enzyme activity. Dye decolorization and degradation are an enzyme-catalyzed reaction and the rate of which can be reflected in the amount of dye decolorized [21].

The decolorizing dye activity of *P. aeruginosa* CR-25 was found to increase with an increase in incubation temperature from 10°C to 37°C with maximum activity attained at 370°C (Figure 5).

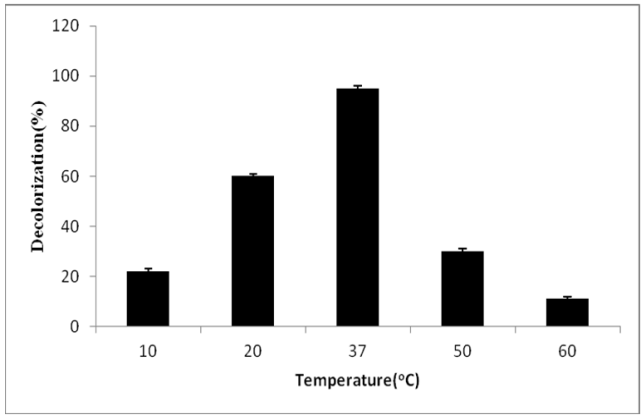

Figure 5: Effect of temperature on decolorization of Reactive Red 250 by *P. aeruginosa* CR-25 under static culture condition at pH 7.

Further increase in temperature resulted in a reduction in the decolorization activity of *Pseudomonas aeruginosa* CR-25. The decline in decolorizing activity at higher temperatures can be attributed to the loss of cell viability or to the denaturation of azo reductase enzyme.

Klebiella pneumoniae RS-13 decolorized Methyl red in the temperature range of from 23°C to 37°C, whereas, at 45° C, decolorization was completely inhibited *Pseudomonas sp.* reportedly decolorized Malachite green, Fast green, Brilliant green, Congo red and Methylene blue optimally at 37°C [22].

Effects of the age of inoculum

The decolorization of Reactive Red 250 by varying age culture of *P. aeruginosa* CR-25. The maximum decolorization of Reactive Red 250 was achieved when 18 h old culture *P. aeruginosa* CR-25 was used as inoculum (Figure 6).

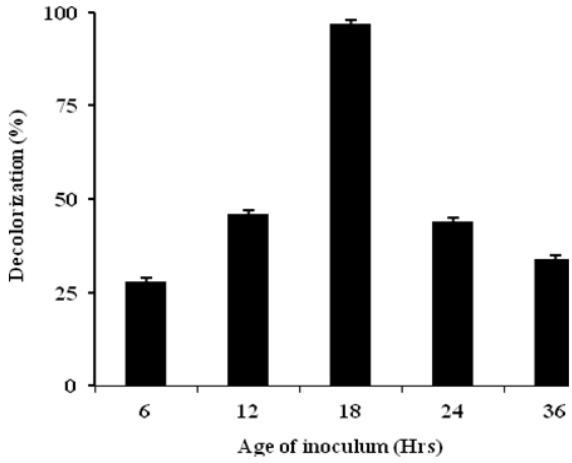

Figure 6: Effect of inoculum of varying age of *P. aeruginosa* CR-25 on decolorization of Reactive Red 250 under static culture condition at 37°C.

Decolorization, biomass and glucose consumption

The relationship between bacterial decolorization of Reactive Red 250, change in biomass (monitored in the form of protein mg/ml^{-1}) and glucose consumption by *P. aeruginosa* CR-25. Biomass production proportionates glucose utilization while dye decolorization begins at the late exponential growth phase. The reducing equivalents from glucose metabolism might have been involved in the dye degradation. Similar results were reported during the decolorization of Navitan Fast Blue S5R by *Pseudomonas aeruginosa* (Figure 7) [23].

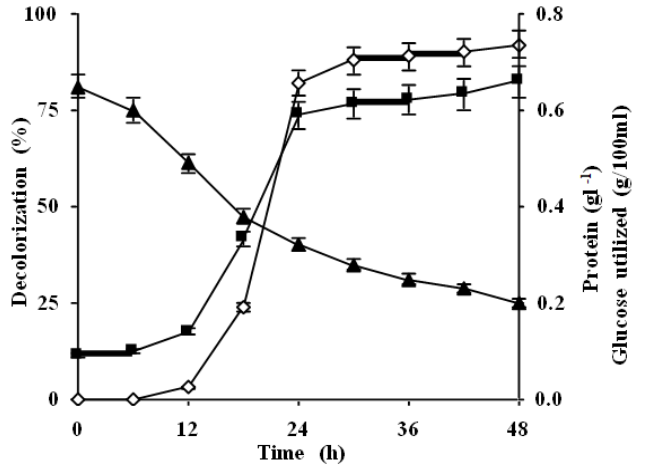

Figure 7: Change in biomass of *P. aeruginosa* CR-25 during decolorization of Reactive Red 250 (100 mg/ml). Note: Glucose-▲- (g/100 ml); Protein-■- (g.l^{-1}) and decolorization -◊- (%).

Effect of static and shaking environment on decolorization

The effects of the static and shaking environment on the decolorization of Reactive Red 250 were carried out. The complete decolorization of dye Reactive Red 250 was observed under static culture conditions. Under the shaking culture condition, decolorization was achieved. A similar pattern was observed during the decolorization of all other dyes. The decolorization of Reactive Red H5BL and Purple H3R achieved under static culture condition was 91% ± 0.2% and 89% ± 1.0% while under shaking culture condition, it was 38% ± 0.5% and 37% ± 0.4% (Figures 8 and 9)(Table 4).

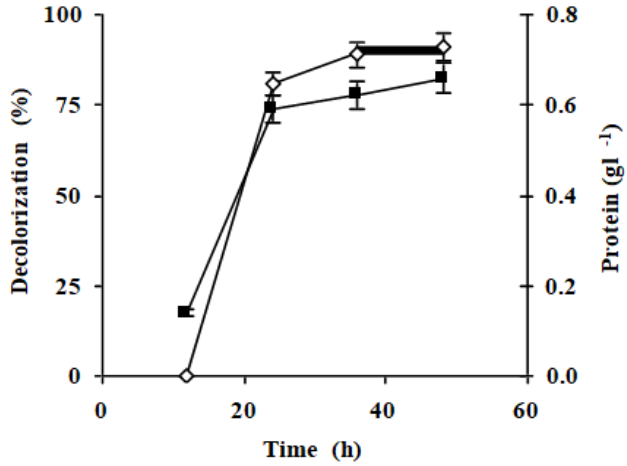

Figure 8: Time course of decolorization of Reactive Red 250 (100 mg/ml) under static culture of *P. aeruginosa* CR-25 at 37°C. Note: Protein -■- (g.l^{-1}) and decolorization -◊- (%).

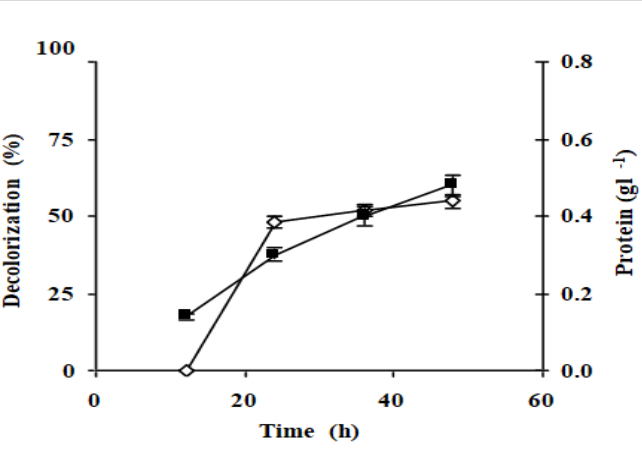

Figure 9: Time course of decolorization of Reactive Red 250 (100 mg/ml) under shaking culture of *P. aeruginosa* CR-25 at 37°C. Note: Protein -■- (g.l^{-1}) and decolorization -◊- (%).

Textile dyes (100 mg/ml)	λmax (nm)	Dye decolorized (%) after 120 hrs	
		Static culture	Shaking culture
Reactive Red 250	530	98 ± 1.0	55 ± 0.3
Reactive Red H5BL	512	91 ± 0.2	38 ± 0.5
Reactive Violet H5RL	560	82 ± 0.9	36 ± 0.1
Reactive Violet Navy Blue H2GL	596	58 ± 1.1	28 ± 0.9
Reactive Violet Brown HGRL	482	88 ± 0.6	38 ± 0.2
Reactive Red H8B	527	87 ± 0.8	34 ± 1.2
Reactive Violet M5B	542	76 ± 0.2	30 ± 0.8
Purple H3R	565	89 ± 1.0	37 ± 0.4
Reactive Orange 3R	500	82 ± 0.7	32 ± 1.0
Reactive Blue-81	664	38 ± 0.4	12 ± 0.1
Acid Red -249	543	59 ± 0.1	22 ± 0.3

Table 4: Decolorization of various textile azo dyes by *P. aeruginosa* CR-25.

HPLC analysis of breakdown products

To investigate the breakdown products formed during decolorization of Reactive Red 250 (at 300 mg/ml concentrations under static culture condition), HPLC analysis of the decolorized dye sample was performed. The result obtained reveals the presence of two aromatic amines, namely benzidine (retention time 16.423) and 4-chloroaniline (retention time 21.691). The decolorized sample, which contained benzidine and 4-chloroaniline, was treated further under the shaking culture condition for 24 h. HPLC analysis of extracted products was performed. The results obtained show the reduction in the peak area of benzidine and 4-chloroaniline. The reduction in peak area under shaking culture condition indicates the disappearance of benzidine and 4-chloroaniline and hence dye Reactive Red 250 undergo degradation by sequentially anaerobic (static) aerobic (shaking) treatment (Figures 10 and 11) [24].

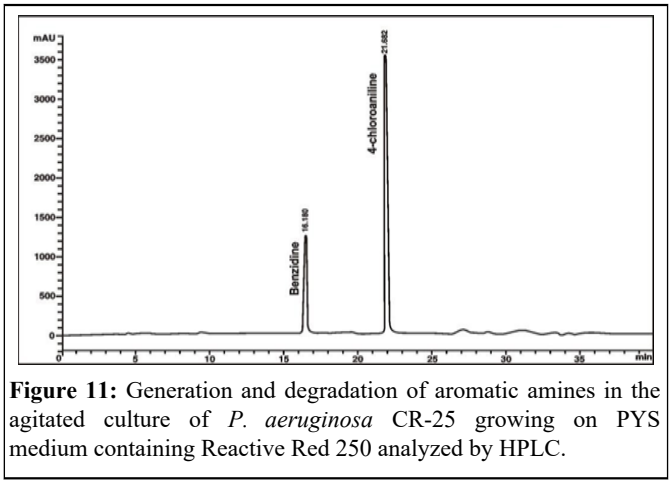

Figure 11: Generation and degradation of aromatic amines in the agitated culture of *P. aeruginosa* CR-25 growing on PYS medium containing Reactive Red 250 analyzed by HPLC.

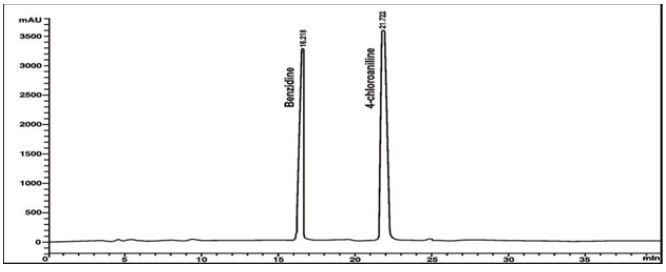

Figure 10: Generation and degradation of aromatic amines in the static culture of *P. aeruginosa* CR-25 growing on PYS medium containing Reactive Red 250 analyzed by HPLC.

Seed germination

The effects of pure, decolorized and degraded dyes on germination of *Triticum vulgare* (Wheat) and *Phaseolus mungo* (Mung). 13% germination was observed when seeds of wheat were irrigated with sample ST. Seeds irrigated with DC show 86% germination. ST and DC irrigated seeds of Mung, shows 30% and 80% germination, respectively. Seeds of Wheat and Mung treated with MC and WC show 100% germination. The bio-toxicity effects of some of the textile dyes on germination and early seedling growth of four plants: Clover, wheat, lettuce and tomato. In 1996, the toxicity of Polycyclic Aromatic Hydrocarbons (PAHs), Anthracene (ANT), Benzo(a)Pyrene (BAP) and fluoranthene on the duckweed *Lemna gibba L.* and *Brassica napus L.* (oilseed rape) seeds (Figures 12-15).

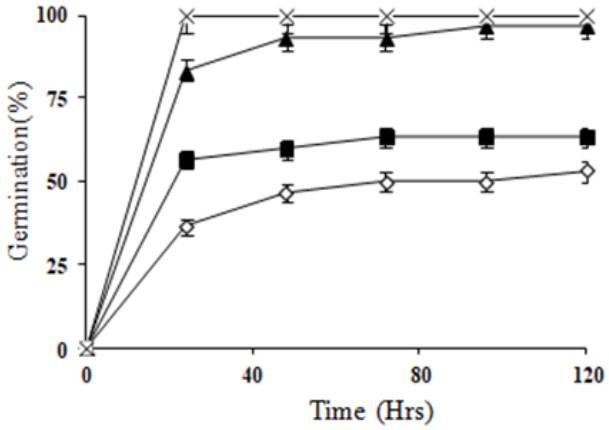

Figure 12: Effect of decolorized and degraded dye products on germination of seeds of *Arachis hypogia L*. Note: Medium control (▲), Dye control (■), Treated sample (◊) and Water control (x).

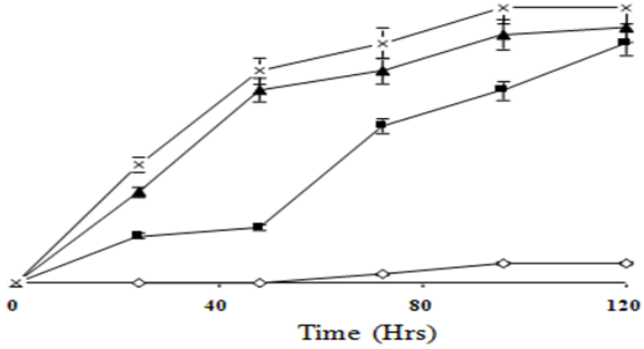

Figure 13: Effect of decolorized and degraded dye products on germination of seeds of *Cicer arientum L*. Note: Medium control (▲), Dye control (■), Treated sample (◊) and Water control (x).

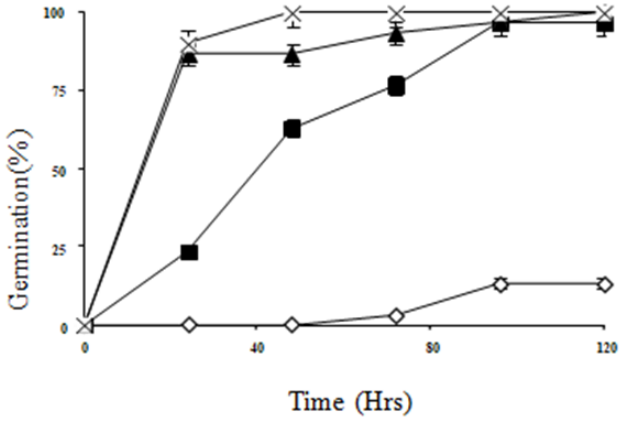

Figure 14: Effect of decolorized and degraded dye products on germination of seeds of *Tritum vulger L*. Note: Medium control (▲), Dye control (■), Treated sample (◊) and Water control (x).

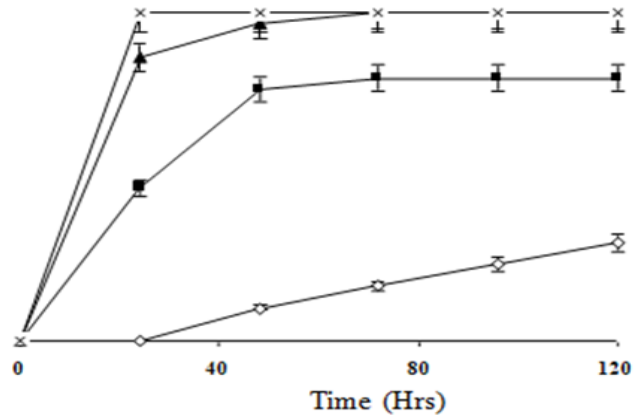

Figure 15: Effect of decolorized and degraded dye products on germination of seeds of *Phaseolus mungo L*. Note: Medium control (), Dye control (■), Treated sample (◊) and Water control (x).

Inhibition of seed germination, when treated with ST samples, isdue to toxic effects of aromatic amines benzidine and 4-chloroaniline (accumulated during decolorization of Reactive Red 250 under static culture treatment). This is in agreement with the postulation of that azo dyes may be toxic only after reduction and cleavage of the azo linkage, producing aromatic amines [25].

Conclusion

Seeds treated with SSST samples show a significant increase in germination, which shows the degradation of benzidine and 4-chloroaniline generated during of Reactive Red 250 under static culture condition. A similar study of degradation of aromatic amines under aerobic condition. The outstanding degradative ability of *P. aruginosa* CR-25 in decolorizing a variety of dyes seems to have the potential for biological treatment of thousands of simple to very complex dye-containing effluent in the textile dyeing and printing industry.

P. aeruginosa CR-25, a bacterium isolated from textile dye wastewater, achieved a complete decolorization and biodegradation of benzidine and 4-chloroanilin, toxic intermediates of Reactive Red 250. Phytotoxicity (seed germination assay) demonstrated the detoxification of the dye after degradation of the toxic intermediates under agitating (aerobic) culture condition.

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