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Research Article

Effect of different environmental conditions for the bacterial decolourization of reactive orange – 16

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Abstract

In this present study, the effect of different environmental conditions for the bacterial decolourization of Reactive Orange – 16 was investigated. Six different bacterial isolates were isolated and identified from the textile dye effluent. The isolated bacterial isolates were identified and characterized as *Bacillus odyssey*, *Bacillus thuringiensis*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus aureus*. The effect of different environmental conditions for the decolourization of Reactive Orange – 16 was studied. The dye decolourization was maximum at pH 7 and the optimum temperature for the decolourization was 40°C. Among the various carbon and nitrogen sources tested, sucrose and peptone showed maximum decolourization percentage. The decolourization of reactive azo dyes was assessed in different conditions *viz*., Static condition and Shaking condition. The decolourization in shaking condition was more effective when compared to the static conditions.

Keywords: Textile dye, Reactive Orange – 16, Bacteria, Decolourization and Optimization.

Introduction

Dyes make the world more beautiful through coloured 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, of which azo dyes, characterized by an azo-bond (R_1–N=N–R_2), represent about 70% by weight (Hao *et al*., 2000). Azo dyes are the most common synthetic colourants released to the environment via textile, pharmaceutical and chemical industries (Saranraj and Sivasakthivelan, 2013). The discharge of azo dyes in water bodies is problematic not only for aesthetic reasons, but also because azo dyes and their cleavage products (aromatic amines) are carcinogenic (Weisburger, 2002; Saranraj, 2013).

India's dye industry produces every type of dyes and pigments. Production of dye stuff and pigments in India is close to 80,000 tones. 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 (Saranraj *et al*., 2010; Sriram *et al*., 2013; Saranraj *et al*., 2014).

Out of several methods that are used in the treatment of textile effluents to achieve decolourization, including physiochemical methods like filteration, specific coagulation, use of activated carbon and chemical flocculation some of the methods are effective but quite expensive. Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluent. Bioremediation is a pollution control technology that uses biological

systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. This natural process, bioremediation, includes bioengineering the capabilities of intrinsic microorganisms, to clean up the environment is an effective alternative to conventional remediation methods (Vidali, 2009).

In the textile industry different structures of synthetic dyes are often used during fiber processing, and therefore the effluents produced are markedly variable in chemical composition, including organics, nutrients, sulphur compounds, salts and different toxic substances In biological treatment processes, various physicochemical operational parameters, such as the level of agitation, oxygen, temperature, pH, dye structure, dye concentration, supplementation of different carbon and nitrogen sources, electron donor and redox mediator, directly influence the bacterial decolorization performance of azo dyes. Thus, to make the process more efficient, faster and practically applicable, prior determination of the effect of each factor on the bacterial decolorization of azo dyes is essential.

Materials and Methods

Collection of Textile dye effluent

The dye house effluent was collected from a dyeing unit in Theco Silks, Thirubhuvanam region, Kumbakonam district, Tamil Nadu, India. It was refrigerated at 4°C and used without any preliminary treatment.

Dyes used

The Reactive Orange - 16 was used in this present research. The dye samples were commercially graded and supplied by the dealers of "SIGMA Aldrich, USA".

Isolation of bacterial isolates from Textile dye effluent

The bacterial isolates present in the textile dye effluent were isolated by Serial dilution **(**Pour plate) technique. In this method, 1 ml of sample was thoroughly mixed with 99 ml of sterile distilled water, and then it was serially diluted by following standard procedure upto concentration of 10^{-6}. Then, 1 ml of serially diluted samples from each concentration of samples were transferred to sterile petriplates and evenly distributed throughout the plates and sterile unsolidified Nutrient agar was poured and it was allowed to solidify. The Nutrient agar plates were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were isolated from the plates.

Maintenance of bacterial isolates

Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar slants and stored at 4°C.

Identification of bacteria isolated from Textile dye effluent

Identification of the bacterial isolates was carried out by the routine bacteriological methods i.e.,

a. By the colony morphology
b. Preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Catalase and Oxidase.
c. Plating on selective medias.
d. By performing biochemical tests.

Effect of different environmental conditions for the decolourization of Reactive Orange – 16

Effect of pH

Colonies of an overnight growth were suspended in normal saline to obtain an optical density of 0.6 at 610 nm wavelength. One milliliter of the cell suspension was inoculated in 250 ml Erlenmeyer flasks containing Nutrient broth and Reactive Orange - 16 (500 mg/L). The pH of the medium was adjusted to 5, 6, **7**, 8 and 9 with hydrochloric acid and sodium hydroxide. The cultures were incubated at 30°C for 4 days in a rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = \frac{\text{Initial OD - Final OD}}{\text{Initial OD}} \times 100$$

Effect of Temperature

One milliliter of the bacterial cell suspension was inoculated in 250 ml Erlenmeyer flasks containing Nutrient Broth and Reactive Orange - 16 (500 mg/L). The cultures were incubated at different temperature *viz*., 20°C, 30°C, 40°C, 50°C and 60°C for 4 days in a rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated.

Effect of various carbon sources

The effect of various carbon sources *viz*., Sucrose, Glucose, Lactose, Starch, Maltose was analyzed in this present study. The carbon sources (1:100) were added in the Nutrient broth containing Reactive Orange - 16 (500 mg/ L) and incubated at 30°C for 4 days in a rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated.

Effect of various nitrogen sources

The effect of various nitrogen sources *viz*., Yeast extract, Ammonium chloride, Ammonium sulphate and Peptone was analyzed in this present study. The nitrogen sources (1:100) were added in the Nutrient broth containing Reactive Orange - 16 (500 mg/L) and incubated at 30°C for 4 days in a rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated.

Effect of different conditions

The Reactive dye decolourization was assessed in different conditions *viz*., Static condition and Shaking condition. One milliliter of the bacterial cell suspension was inoculated in 250 ml Erlenmeyer flasks containing Nutrient broth and Reactive Orange - 16 (500 mg/L). The cultures were incubated at 37°C for 4 days in a static condition and rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated.

Results and Discussion

In the present study, six different bacterial isolates were isolated and identified from the textile dye effluent. The isolated bacterial isolates were identified and characterized as *Bacillus odyssey, Bacillus thuringiensis, Bacillus subtilis, Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus*. All the bacterial isolates except *Escherichia coli* and *Proteus mirabilis* showed Gram positive reaction. The characteristics of the bacterial strains isolated from textile dye effluent were compared with MTCC Reference strains. Khera *et al*. (2005) have reported isolation of organisms adapted to high dye concentration from sites near textile industries complex. The selected isolate is a sporulating Gram positive motile rod, occurring singly, grew as rough colony on nutrient agar. On the basis of conventional biochemical tests, it was identified as *Bacillus cereus* or *Bacillus thuringiensis*. Staining of the parasporal body showed its presence, which indicated the identity of the isolate as *Bacillus thuringiensis* (Saranraj and Stella, 2012; Saranraj and Stella, 2014; Saranraj and Sujitha, 2014; Jayanthi *et al*., 2014).

Saranraj *et al*. (2010) isolated five bacterial species *viz*., *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae*. Sadeeshkumar *et al*. (2011) isolated and identified three different bacterial isolates *viz*., *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* from textile dye effluent. Giek Far Chan *et al*. (2012) isolated and investigated the dye decolourization ability of a novel bacterial consortium, which consists of *Citrobacter freundii, Enterococcus casseliflavus* and *Enterobacter cloacae*. Sriram *et al*. (2013) isolated three different bacterial isolates *viz*., *Bacillus* sp., *Escherichia coli* and *Pseudomonas fluorescens* from textile dye effluent contaminated soil sample and used for the degradation study (Saranraj *et al*., 2010; Saranraj and Stella, 2012; Jayanthi *et al*., 2013; Saranraj and Sujitha, 3013). Recently, Saranraj *et al*. (2014) isolated and identified six different bacterial isolates *viz*., *Bacillus odyssey, Bacillus thuringiensis, Bacillus subtilis, Bacillus cereus, Alcaligenes* sp., and *Nocardiopsis alba* from the textile dye effluent sample.

The decolourization of Reactive Orange – 16 was investigated at different pH and the results were furnished in Table – 1. Five different pH *viz*., pH – 5,

pH – 6, pH – 7, pH – 8 and pH – 9 were tested in the present decolourization study. Among the six bacterial isolates tested, maximum decolourization of Reactive orange - 16 was observed in *Bacillus odyssey* at pH – 7 (70.66%) followed by *Bacillus thuringiensis* (66.97%), *Bacillus subtilis* (64.20%), *Escherichia coli* (60.93%) and *Proteus mirabilis* (53.05%). The bacterial isolate *Staphylococcus aureus* showed minimum decolourization of Reactive Orange – 16 (49.28%). Next to pH – 7, maximum bacterial decolourization of Reactive Orange – 16 was observed at pH –6, pH –5, pH – 8 and pH – 9.

Asad *et al*. (2007) found that pH between 6.0 and 8.0 was optimum for decolourization of triphenylmethane and azo dyes by *Bacillus* sp. Dafale *et al*. (2008) found that, the specific decolourization rate increased with increasing pH from 5 to 7, which remained approximately the same for pH 7 – 8. This seems to indicate that neutral and slightly basic pH values would be more favorable for decolourization process of Remazole Black B by a bacterial consortium containing *Pseudomonas aeruginosa*. In contrast to the present results pH 7 was the optimum pH for the decolourization of reactive red 195 by *Enterobacter* sp. and the decolourization percentage decreased as pH increased (Kalyani *et al*., 2008).

The effect of decolourization of Reactive Orange – 16 at different temperature was investigated in the present study and the results were furnished in Table – 2. Five different temperatures *viz*., 20°C, 30°C, 40°C, 50°C and 60°C were tested in the present decolourization study. Among the six bacterial isolates tested, maximum decolourization of Reactive orange - 16 was observed in *Bacillus odyssey* at 40°C (63.78%) followed by *Bacillus thuringiensis* (57.66%), *Bacillus subtilis* (56.58%), *Escherichia coli* (51.71%), *Proteus mirabilis* (43.75%) and *Staphylococcus aureus* showed minimum decolourization of Reactive Orange – 16 (39.98%). Next to 40°C, maximum bacterial decolourization of Reactive Orange – 16 was observed at 30°C, 20°C, 50°C and 60°C.

The optimum temperature for decolourization was found to be about 30°C. It was observed that *Klebsiella pneumoniae* and *Bacillus liquefaciens* showed no decolourization of Methyl Red at 45°C (Wong and Yuen, 1998). *Klebsiella pneumoniae* and *Bacillus liquefaciens* are mesophiles and the temperature tested in the study (20, 25, 37, 45 and 50°C) did not have significant effect on growth and N,N-dimethyl-p-phenylenediamine (DMPD) degradation by these bacteria under varied temperature.

Dafale *et al*. (2008) found that, 37°C was the optimal temperature for decolourization of remazol black-B (RB-B) by a bacterial consortium containing *Pseudomonas aeruginosa*. In contrast to the present results, Hu *et al*. (1994) incubated *Pseudomonas luteola* at 28°C to obtain maximum decolourization power of textile wastewater. Hefang *et al*. (2004) investigated the effect of temperature on the decolourization of azo dye Direct fast scarlet 4BS by microbial consortium.

The increase in decolourization percentage after addition of carbon sources is attributed to the fact that the dyes are deficient in carbon content and biodegradation without any extra carbon sources is difficult (Padmavathy *et al*., 2003). The decrease in decolourization percent after addition of some carbon sources and the ability of some carbon sources to induce growth without increase in decolourization may attributed to that, the sugars may inhibit the decolourization of azo dyes because its effect as catabolite repression (Chang *et al*., 2001).

The effect of different carbon sources on decolourization of Reactive Orange – 16 was investigated and the results were furnished in Table – 3. Five different carbon sources *viz*., starch, glucose, sucrose, lactose and maltose were tested in the present decolourization study. Among the six bacterial isolates tested, maximum decolourization of Reactive orange - 16 was observed by *Bacillus odyssey* in the medium supplemented with sucrose (79.81%) followed by *Bacillus thuringiensis* (73.97%), *Bacillus subtilis* (73.22%), *Escherichia coli* (63.34%) and *Proteus mirabilis* (61.61%). The bacterial isolate *Staphylococcus aureus* showed minimum decolourization of Reactive Orange – 16 (59.14%). Next to sucrose, maximum bacterial decolourization of Reactive Orange – 16 was observed in the medium supplemented with glucose, starch, lactose and maltose.

Presence of starch as the best co-metabolite in decolourization of azo dyes was supported by many studies Padmavathy *et al*. (2003) found that starch was the best carbon source in azo dye biodegradation from

Table - 1: Bacterial decolourization of Reactive Orange – 16 at different Ph

Initial OD of Reactive Orange – 16 at 480 nm = 1.193

S. No	Bacterial isolates	Final OD and% Decolourization				
		pH 5	pH 6	pH 7	pH 8	pH 9
1.	*Bacillus odyssey*	0.421 (64.71%)	0.399 (66.55%)	0.350 (70.66%)	0.497 (58.34%)	0.540 (54.73%)
2.	*Bacillus thuringiensis*	0.466 (60.93%)	0.433 (63.70%)	0.394 (66.97%)	0.541 (54.65%)	0.584 (51.04%)
3.	*Bacillus subtilis*	0.498 (58.25%)	0.477 (60.01%)	0.427 (64.20%)	0.585 (50.96%)	0.623 (47.77%)
4.	*Escherichia coli*	0.534 (55.23%)	0.506 (57.58%)	0.466 (60.93%)	0.626 (47.52%)	0.666 (44.17%)
5.	*Proteus mirabilis*	0.601 (49.62%)	0.584 (51.04%)	0.560 (53.05%)	0.651 (45.43%)	0.702 (41.15%)
6.	*Staphylococcus aureus*	0.652 (45.34%)	0.630 (47.19%)	0.605 (49.28%)	0.692 (41.99%)	0.745 (37.55)

Table - 2: Bacterial decolourization of Reactive Orange – 16 at different temperatures

Initial OD of Reactive Orange – 16 at 480 nm = 1.193

S. No	Bacterial isolates	Final OD and% Decolourization				
		20°C	30°C	40°C	50°C	60°C
1.	*Bacillus odyssey*	0.480 (59.76%)	0.461 (61.35%)	0.432 (63.78%)	0.598 (49.87%)	0.651 (45.43%)
2.	*Bacillus thuringiensis*	0.576 (51.71%)	0.545 (54.31%)	0.505 (57.66%)	0.652 (45.34%)	0.695 (41.74%)
3.	*Bacillus subtilis*	0.609 (48.95%)	0.589 (50.62%)	0.518 (56.58%)	0.696 (41.65%)	0.734 (38.47%)
4.	*Escherichia coli*	0.645 (45.93%)	0.617 (48.28%)	0.576 (51.71%)	0.735 (38.39%)	0.775 (35.03%)
5.	*Proteus mirabilis*	0.712 (38.03%)	0.695 (41.74%)	0.671 (43.75%)	0.762 (36.12%)	0.813 (31.85%)
6.	*Staphylococcus aureus*	0.763 (36.04%)	0.741 (37.88%)	0.716 (39.98%)	0.803 (32.69%)	0.856 (28.24%)

Table - 3: Bacterial decolourization of Reactive Orange – 16 using various carbon sources

Initial OD of Reactive Orange – 16 at 480 nm = 1.214

S. No	Bacterial isolates	Final OD and% Decolourization				
		Starch	Glucose	Sucrose	Lactose	Maltose
1.	*Bacillus odyssey*	0.312 (74.29%)	0.287 (76.35%)	0.245 (79.81%)	0.378 (68.86%)	0.435 (64.16%)
2.	*Bacillus thuringiensis*	0.385 (68.28%)	0.365 (69.93%)	0.316 (73.97%)	0.474 (60.95%)	0.513 (57.74%)
3.	*Bacillus subtilis*	0.394 (67.54%)	0.354 (70.84%)	0.325 (73.22%)	0.434 (64.25%)	0.473 (61.03%)
4.	*Escherichia coli*	0.502 (58.64%)	0.470 (61.28%)	0.445 (63.34%)	0.542 (55.35%)	0.598 (50.74%)
5.	*Proteus mirabilis*	0.534 (56.01%)	0.506 (58.31%)	0.466 (61.61%)	0.626 (48.43%)	0.666 (45.14%)
6.	*Staphylococcus aureus*	0.541 (52.96%)	0.520 (57.16%)	0.496 (59.14%)	0.579 (52.30%)	0.633 (47.85%)

Table - 4: Bacterial decolourization of Reactive Orange – 16 using various nitrogen sources

Initial OD of Reactive Orange – 16 at 480 nm = 1.456

S. No	Bacterial isolates	Final OD and% Decolourization			
		Yeast extract	Ammonium chloride	Peptone	Ammonium sulphate
1.	*Bacillus odyssey*	0.421 (71.08%)	0.375 (74.24%)	0. 354 (75.68%)	0.467 (67.92%)
2.	*Bacillus thuringiensis*	0.447 (69.29%)	0.416 (71.42%)	0.393 (73.00%)	0.534 (63.32%)
3.	*Bacillus subtilis*	0.538 (63.04%)	0.493 (66.14%)	0.461 (68.33%)	0.557 (61.74%)
4.	*Escherichia coli*	0.687 (52.81%)	0.616 (57.69%)	0.546 (62.50%)	0.745 (48.83%)
5.	*Proteus mirabilis*	0.687 (52.81%)	0.637 (56.25%)	0.593 (59.27%)	0.766 (47.39%)
6.	*Staphylococcus aureus*	0.714 (50.96%)	0.698 (52.06%)	0.669 (54.05%)	0.797 (45.26%)

Table - 5: Decolourization of Reactive Orange – 16 under different conditions

Initial OD of Reactive Orange – 16 at 480 nm = 1.193

S.No	Bacterial isolates	% Decolourization			
		Static condition		Shaking condition	
		Final OD	% Decolourization	Final OD	% Decolourization
1.	*Bacillus odyssey*	0.350	70.66%	0.323	72.92%
2.	*Bacillus thuringiensis*	0.394	66.97%	0.372	68.81%
3.	*Bacillus subtilis*	0.427	64.20%	0.403	66.21%
4.	*Escherichia coli*	0.466	60.93%	0.441	63.03%
5.	*Proteus mirabilis*	0.560	53.05%	0.532	55.40%
6.	*Staphylococcus aureus*	0.605	49.28%	0.580	51.38%

synthetic waste water under aerobic co-metabolite conditions also Georgiou *et al*. (2005) suggested the use of potato – starch industrial wastes to increase the decolourization of textile waste water in large scale. Also starch was added by Olukanni *et al*. (2006) in studying the textile effluent biodegradation potentialities of textile effluent - adapted and non-adapted bacteria. In contrast to the present study, glucose was used as a carbon source in decolourization of reactive azo dyes.

Effects of some other carbon sources on bacterial decolourization performance have been studied in former researches. Lactate, peptone, succinate, yeast extract, and formate were proved to enhance decolourization, while sucrose, and dextrin resulted in lower decolourization activities (Xu *et al*., 2006). A screening test for the ability of this isolates to utilize azo dyes as a sole carbon source was established to select the most potent organisms and exclude that decolourization may occur due to adsorption only. This technique was used by Asad *et al*. (2007) where the ability of halophilic and halotolerant bacterial isolates to utilize Remazole black - B as sole carbon source was used to select the most effective isolates. Ayed *et al*. (2010) used glucose in decolourization of Remazol Black B by halotolerant and halophilic isolates.

The effect of different nitrogen sources on decolourization of Reactive Orange – 16 was assessed and the results were furnished in Table – 4. Five different nitrogen sources *viz*., yeast extract, ammonium chloride, peptone and ammonium sulphate were tested in the present decolourization study. Among the six bacterial isolates tested, maximum decolourization of Reactive orange - 16 was observed by *Bacillus odyssey* in the medium supplemented with peptone (75.68%) followed by *Bacillus thuringiensis* (73.00 %), *Bacillus subtilis* (68.33%), *Escherichia coli* (62.50%), *Proteus mirabilis* (59.27 %) and *Staphylococcus aureus* (54.05 %) showed minimum decolourization of Reactive Orange – 16. Next to peptone, maximum bacterial decolourization of Reactive Orange – 16 was observed in the medium supplemented with ammonium chloride, yeast extract and ammonium sulphate.

Nigam *et al*. (1996) reported that bacterial consortium PDW did not show decolourization when yeast extract was omitted from the medium. Growth of *Pseudomonas luteola* was directly related to the concentration of yeast extract and when the concentration of yeast extract was reduced growth and colour removal decreased (Hu, 1998). Different concentrations of yeast extract along with glucose were tested and it was found that medium containing 0.05% yeast extract showed maximum decolourization (94%) whereas a further increase in concentration of yeast extract showed a decrease in decolourization. Nigam *et al*. (1996) have also reported maximum decolourization of azo dyes in presence of yeast

extract (5 g/L) in PDW consortium. Peptone, as a nitrogen source, other than yeast extract was used in the medium with BHM and glucose and it exhibited good decolourizing (90%) ability. The color removal percentage of most dyes increased sharply after addition of yeast extract and this is in accordance with other reports (Asad *et al*., 2007).

The decolourization of Reactive Orange – 16 by bacterial isolates and bacterial consortium under static and shaking condition was investigated and the results were furnished in Table – 5. Among the six bacterial isolates tested, maximum decolourization of Reactive orange - 16 was observed by *Bacillus odyssey* (72.92%) under shaking condition followed by *Bacillus thuringiensis* (68.81%), *Bacillus subtilis* (66.21%), *Escherichia coli* (63.03%) and *Proteus mirabilis* (55.40%). The bacterial isolate *Staphylococcus aureus* showed minimum decolourization of Reactive Orange – 16 (51.38%). The decolourization of Reactive Orange – 16 was maximum in shaking condition when compared to the static condition. Various groups have reported that bacterial degradation is best under aerobic and shaking conditions (Chen *et al*., 2003). In order to test is this was also true for our isolates, a study was done under static and shaking conditions. The degradation of the dye also appeared to be dependent on the shaking culture of the culture.

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