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Effect of Physical Factors for the Bacterial Decolourization of Reactive Textile Dyes

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Abstract: Dyes are recalcitrant by design and not readily amendable to common treatment methods, imposing a challenge for closed water systems.In the present study, the effect of physical factors (pH and Temperature) was studied for the bacterial decolourization of Reactive Textile Dyes. 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.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*, *Bacillus cereus*, *Alcaligenes* sp. and *Nocardiopsis alba*.The decolourization of Reactive dyes was investigated at different pH *viz*., 5, 6, 7, 8 and 9 were tested in the present decolourization study. Maximum decolourization was observed at pH – 7. Next to pH – 7, maximum bacterial decolourization of Reactive dyes were observed at pH – 6, 5, and 9. The effect of decolourization of Reactive dyes at five different temperatures *viz*., 20, 30, 40, 50 and 60 °C were tested in the present decolourization study. Maximum decolourization of Reactive dyes was observed at 40°C. Next to 40°C, maximum bacterial decolourization of Reactive dyes were observed at 30 °C, 20 °C, 50 °C and 60 °C.

Key words: Reactive Textile Dyes · Bacteria · pH · Temperature and Decolourization.

INTRODUCTION

Azo dyes are largest group of dyes. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries [1]. Azo dyes are characterized by the presence of one or more azo groups – N = N -, which are responsible for their colouration and when such a bond is broken the compound loses its colour. They are the largest and most versatile class of dye, but have structural properties that are not easily degradable under natural conditions and are not typically removed from water by conventional waste water system. Azo dyes are designed to resist chemical and microbial attacks and to be stable in light and during washing. Many are carcinogenic and may trigger allergic reactions in man. It is estimated that over 10% of the dye used in textile processing does not bind to the fibres and is therefore released to the environment. Some of these compounds cause serious threat because of their carcinogenic potential or cytotoxicity [2].

The medium pH is an important factor with regard to decolourization. The pH has a major effect on the efficiency of dye decolourization and the optimal pH for color removal is often between 6.0 and 10.0 [3]. The rate of color removal is higher at the optimum pH, and tends to decrease rapidly at strongly acid or strongly alkaline pH. It is thought that the effects of pH may be related to the transport of dye molecules across the cell membrane, which is considered as the rate limiting step for the decolourization[4]. In our laboratories similar results were

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observed with many microbial strains. Biological reduction of the azo bond can result in an increase in the pH due to the formation of aromatic amine metabolites, which are more basic than the original azo compound [5].

Generally, altering the pH within a range of 7.0 – 9.5 has very little effect on the dye reduction process. However, Chang *et al*. [6] found that the dye reduction rate increased nearly 2.5-fold as the pH was raised from 5.0 to 7.0, while the rate became insensitive to pH in the range of 7.0 – 9.5. Jadhav *et al*. [7] showed that with the decolourization of Brilliant Blue G by isolates -GB (Combination of *Galactomycesgeotrichum* and *Bacillus* sp.) the decolourization was not pH dependent and complete decolourization was observed in the pH range from 5 to 9. The effective decolourization of Reactive Red 190 by isolated *Citrobacter* sp. in strongly acidic (At pH 4) and strongly alkaline (At pH 12) conditions has also been reported [8]. This pH tolerance of decolorizing bacteria is quite important, as it makes them suitable for practical bio-treatment of dyeing mill effluents [9].

In microorganisms the environmental temperature directly establishes temperature, as the microbial cell responds to temperature changes by adaptation *via* biochemical or enzymatic mechanisms. Consequently, temperature is a factor of paramount importance for all processes associated with microbial vitality, including the remediation of water and soil. Some studies dealing with the activation energy of microbial decolourization of azo dyes has been undertaken [10] in which narrow temperature ranges were determined as being necessary for the decolourization of azo dyes by extremely complex consortia of microorganisms inhabiting active sludge. In addition it has also been reported that in microbial physiology temperature changes leadto a sudden alteration of the activation energy [11]. Moreover, the effects of temperature on the growth rate, biomass yield and reaction mechanism have also been reported [12]. It was observed that the decolourization rate of azo dyes increases up to the optimal temperature, and afterwards there is a marginal reduction in the decolourization activity. This decline at higher temperatures can be attributed to the loss of cell viability or the denaturation of an azo reductase enzyme [13, 14]. However, it has been shown that with certain whole bacterial cell preparations the azoreductase enzyme is relatively thermostable and can remain active up to temperatures of 60°C over short periods of time [15]. In the present study, the effect of physical factors (pH and Temperature) was studied for the bacterial decolourization of Reactive Textile Dyes.

MATERIALS AND METHODS

Dyes Used: Reactive azo dyes were used in this study. The dye samples were commercially graded and supplied by the dealers of "SIGMA Aldrich, U.S.A". Reactive azo dyes used in this research were,

- Reactive Orange – 16 (ëm = 480 nm)
- Reactive Black – B (ëm = 600 nm)
- Reactive Yellow – MR (ëm = 600 nm)

Bacterial Isolates Selected for the Present Research: 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*, *Bacillus cereus*, *Alcaligenes* sp. and *Nocardiopsis alba*.

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.

Effect of pH on Decolourization of Reactive Textile Dyes: 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 azo dyes (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 by following the formula of Dafale *et al*. [16].

% Decolourization = Initial OD – Final OD/Initial OD × 100

Effect of Temperature on Decolourization of Reactive Textile Dyes: One milliliter of the bacterial cell suspension was inoculated in 250 ml Erlenmeyer flasks containing Nutrient Broth and Reactive azo dyes (500 mg/l). The cultures were incubated at different temperatures *viz*., 20, 30, 40, 50 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 by following the formula of Dafale *et al*. [16].

% Decolourization = Initial OD – Final OD/Initial OD × 100

RESULTS AND DISCUSSION

Bacterial Decolourization of Reactive Dyes at Different pH: ThepH is among the other most important factors for any microbial activity. Each microorganism possesses a pH range for its growth and activity of metabolite production with optimal value in between the range. The pH of culture medium plays a critical role for the optimal physiological performance of microbial cells and the transport of various nutrient components across the cell membrane. Thus, the pH of the decolourization medium has a marked effect on the cell growth and enzyme production.

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 %), *Bacillus cereus* (60.93 %) and *Alcaligenes* sp. (45.43 %). The bacterial isolate *Nocardiopsis alba*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.

The effect of decolourization of Reactive Black – B at different pH was evaluated and the results were given in Table – 2. 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, *Bacillus odyssey* showed maximum decolourization of Reactive Black – B at pH – 7 (67.21 %) followed by *Bacillus thuringiensis* (62.51 %), *Bacillus subtilis* (61.58 %), *Bacillus cereus* (60.01 %)and *Alcaligenes* sp. (56.72 %). The bacterial isolate *Nocardiopsis alba*showed minimum decolourization of Reactive Black – B (53.99 %). Next to pH – 7, maximum bacterial decolourization of Reactive Black – B was observed at pH –6, pH –5, pH – 8 and pH – 9.

The decolourization of Reactive Yellow – MR at different pH was determined in the present study and the results were showed in Table – 3. 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 Yellow – MR was observed in *Bacillus odyssey* at pH – 7 (65.40%) followed by *Bacillus thuringiensis* (64.27 %), *Bacillus subtilis* (58.02 %), *Bacillus cereus* (45.43 %) and *Alcaligenes* sp. (47.38 %). The bacterial isolate *Nocardiopsis alba*showed minimum decolourization of Reactive Yellow – MR (42.48 %). Next to pH – 7, maximum bacterial decolourization of Reactive Yellow – MR was observed at pH –6, pH –5, pH – 8 and pH – 9.

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

		Final OD and% Decolourization				
S. No	Bacterial isolates	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.	*Bacillus cereus*	0.534 (55.23%)	0.506 (57.58%)	0.466 (60.93%)	0.626 (47.52%)	0.666 (44.17%)
5.	*Alcaligenes* sp.	0.601 (49.62%)	0.584 (51.04%)	0.560 (53.05%)	0.651 (45.43%)	0.702 (41.15%)
6.	*Nocardiopsis alba*	0.652 (45.34%)	0.630 (47.19%)	0.605 (49.28%)	0.692 (41.99%)	0.745 (37.55 %)

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

Table 2: Bacterial decolourization of Reactive Black – B at different pH

		Final OD and% Decolourization				
S. No	Bacterial isolates	pH 5	pH 6	pH 7	pH 8	pH 9
1.	*Bacillus odyssey*	0.541 (57.66%)	0.488 (61.80%)	0.419 (67.21%)	0.590 (53.83%)	0.651 (49.06%)
2.	*Bacillus thuringiensis*	0.585 (54.22%)	0.529 (58.66%)	0.479 (62.51%)	0.633 (50.46%)	0.692 (45.85%)
3.	*Bacillus subtilis*	0.629 (50.78%)	0.568 (55.55%)	0.491 (61.58%)	0.675 (47.18%)	0.733 (42.64%)
4.	*Bacillus cereus*	0.671 (47.49%)	0.604 (52.73%)	0.511 (60.01%)	0.716 (43.97%)	0.775 (39.35%)
5.	*Alcaligenes* sp.	0.712 (44.28%)	0.650 (49.13%)	0.553 (56.72%)	0.749 (41.39%)	0.805 (37.01%)
6.	*Nocardiopsis alba*	0.755 (40.92%)	0.695 (45.61%)	0.588 (53.99%)	0.792 (38.02%)	0.853 (33.25%)

Initial OD of Reactive Black – B at 600 nm = 1.278

Table 3: Bacterial decolourization of Reactive Yellow – MR at different pH

S.No	Bacterial isolates	Final OD and% Decolourization				
		pH 5	pH 6	pH 7	pH 8	pH 9
1.	*Bacillus odyssey*	0.567 (48.66%)	0.498 (57.25%)	0.403 (65.40%)	0.632 (45.75%)	0.687 (41.03%)
2.	*Bacillus thuringiensis*	0.606 (47.98%)	0.551 (52.70%)	0.415 (64.27%)	0.675 (42.06%)	0.721 (38.10%)
3.	*Bacillus subtilis*	0.650 (44.20%)	0.592 (49.18%)	0.489 (58.02%)	0.716 (38.54%)	0.765 (34.33%)
4.	*Bacillus cereus*	0.792 (32.01%)	0.633 (45.66%)	0.551 (52.70%)	0.758 (34.93%)	0.802 (31.15%)
5.	*Alcaligenes* sp.	0.839 (27.98%)	0.675 (42.06%)	0.613 (47.38%)	0.800 (31.33%)	0.845 (27.46%)
6.	*Nocardiopsis alba*	0.878 (24.63%)	0.620 (46.78%)	0.670 (42.48%)	0.841 (27.81%)	0.888 (23.77%)

Initial OD of Reactive Yellow – MR at 600 nm = 1.165

Table 4: Bacterial decolourization of Reactive Orange – 16 at different temperatures

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.	*Bacillus cereus*	0.645 (45.93%)	0.617 (48.28%)	0.576 (51.71%)	0.735 (38.39%)	0.775 (35.03%)
5.	*Alcaligenes* sp.	0.712 (35.03%)	0.695 (41.74%)	0.671 (43.75%)	0.762 (36.12%)	0.813 (31.85%)
6.	*Nocardiopsis alba*	0.763 (36.04%)	0.741 (37.88%)	0.716(39.98%)	0.803 (32.69%)	0.856 (28.24%)

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

Mali *et al*. [17] found that pH between 6.0 and 8.0 was optimum for decolourization of triphenylmethane and azo dyes by *Bacillus* sp. Asad *et al*. [18] found that decolourization rate of Remazol Black B. increased as pH increased. Dafale *et al*. [19] 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 [20].

Bacterial Decolourization of Reactive Dyes at Different Temperature: The effect of decolourization of Reactive Orange – 16 at different temperature was investigated in the present study and the results were furnished in Table – 4. Five different temperatures *viz*., 20, 30, 40, 50 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%), *Bacillus cereus* (51.71%) and *Alcaligenes* sp. (43.75%). The bacterial isolate *Nocardiopsis alba*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 effect of decolourization of Reactive Black – B at different temperature was evaluated and the results were given in Table – 5. 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 Black – B was observed in *Bacillus odyssey* at 40°C (59.31%) followed by *Bacillus thuringiensis* (54.61%), *Bacillus subtilis* (52.89%), *Bacillus cereus* (51.33%)and *Alcaligenes* sp. (48.04%). The bacterial isolate *Nocardiopsisalba*showed minimum decolourization of Reactive Black – B (47.10%). Next to 40°C, maximum bacterial decolourization of Reactive Black – B was observed at 30°C, 20°C, 50°C and 60°C.

The decolourization of Reactive Yellow – MR was studied at different temperature and the results were showed in Table – 6. 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 Yellow – MR was observed in *Bacillus odyssey* at 40°C (55.87 %) followed by *Bacillus thuringiensis* (54.84 %), *Bacillus subtilis* (49.35%), *Bacillus cereus* (43.17%) and *Alcaligenes* sp. (37.85%). The bacterial isolate *Nocardiopsis alba*showed minimum decolourization of

Table 5: Bacterial decolourization of Reactive Black – B at different temperatures

S. No	Bacterial isolates	Final OD and% Decolourization 20°C	30°C	40°C	50°C	60°C
1.	*Bacillus odyssey*	0.651 (49.0%)	0.598 (53.30%)	0.520 (59.31%)	0.701 (45.14%)	0.762 (40.37%)
2.	*Bacillus thuringiensis*	0.696 (45.53%)	0.630 (50.70%)	0.580 (54.61%)	0.744 (41.78%)	0.803 (37.16%)
3.	*Bacillus subtilis*	0.730 (42.87%)	0.679 (46.87%)	0.602 (52.89%)	0.786 (38.49%)	0.843 (34.03%)
4.	*Bacillus cereus*	0.782 (38.81%)	0.715 (44.05%)	0.622 (51.33%)	0.827 (35.28%)	0.886 (30.67%)
5.	*Alcaligenes* sp.	0.823 (35.60%)	0.761 (40.45%)	0.664 (48.04%)	0.850 (33.48%)	0.916 (28.32%)
6.	*Nocardiopsis alba*	0.865 (32.31%)	0.806 (36.93%)	0.676 (47.10%)	0.903 (29.34%)	0.964 (24.56%)

Initial OD of Reactive Black – B at 600 nm = 1.278

Table 6: Bacterial decolourization of Reactive Yellow – MR at different temperatures

S.No	Bacterial isolates	Final OD and% Decolourization 20°C	30°C	40°C	50°C	60°C
1.	*Bacillus odyssey*	0.678 (41.80%)	0.609 (47.72%)	0.514 (55.87%)	0.743 (36.22 %)	0.798 (31.50%)
2.	*Bacillus thuringiensis*	0.717 (38.45%)	0.662 (43.17%)	0.526 (54.84%)	0.786 (32.53%)	0.832 (28.58%)
3.	*Bacillus subtilis*	0.761 (34.67%)	0.703 (39.65%)	0.590 (49.35%)	0.827 (29.01%)	0.876 (24.80%)
4.	*Bacillus cereus*	0.803 (31.10%)	0.740 (36.48%)	0.662 (43.17%)	0.869 (25.40%)	0.913 (21.63%)
5.	*Alcaligenes* sp.	0.830 (28.75%)	0.786 (32.53%)	0.724 (37.85%)	0.911 (21.80%)	0.956 (17.93%)
6.	*Nocardiopsis alba*	0.967 (16.99%)	0.830 (28.75%)	0.782 (32.87%)	0.952 (28.28%)	0.990 (15.02%)

Initial OD of Reactive Yellow – MR at 600 nm = 1.165

Reactive Yellow – MR (32.87%). Next to 40°C, maximum bacterial decolourization of Reactive Yellow – MR 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 *Alcaligenes liquefaciens* showed no decolourization of Methyl Red at 45 °C [21]. *Klebsiella pneumoniae* and *Alcaligenes liquefaciens* are mesophilic and the temperature tested in the study (20 C, 25 °C, 37 °C, 45 °C 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*. [22] found that, 37 °C was the optimaltemperature for decolourization of Remazol Black - B(RB-B) by a bacterial consortium containing *Pseudomonas aeruginosa*. In contrast to the present results, Hu [23] incubated *Pseudomonasluteola* at 28 °C to obtain maximum decolourization power of textile wastewater. Hefang*et al*. [24] investigated the effect of temperature on the decolourization of azo dye Direct fast scarlet 4BS by microbial consortium.

CONCLUSIONS

The conclusions of the present research work were

- 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%), *Bacillus cereus* (60.93%), *Alcaligenes* sp. (45.43%) and *Nocardiopsis alba*(49.28%). *Bacillus odyssey* showed maximum decolourization of Reactive Black – B at pH – 7 (67.21%) followed by *Bacillus thuringiensis* (62.51%), *Bacillus subtilis* (61.58%), *Bacillus cereus* (60.01%), *Alcaligenes* sp. (56.72%) and *Nocardiopsis alba*(53.99%) Maximum decolourization of Reactive Yellow – MR was observed in *Bacillus odyssey* at pH – 7 (65.40%) followed by *Bacillus thuringiensis* (64.27%), *Bacillus subtilis* (58.02%), *Bacillus cereus* (45.43%), *Alcaligenes* sp. (47.38%) and *Nocardiopsis alba*(42.48%).
- 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 %), *Bacillus cereus* (51.71 %), *Alcaligenes* sp. (43.75 %) and *Nocardiopsis alba* (39.98 %). Maximum decolourization of Reactive Black – B was observed in *Bacillus odyssey* at 40 °C (59.31 %) followed by *Bacillus thuringiensis* (54.61 %), *Bacillus subtilis* (52.89 %), *Bacillus cereus* (51.33 %), *Alcaligenes* sp. (48.04%) and *Nocardiopsis alba* (47.10 %). Maximum decolourization of Reactive Yellow – MR was observed in *Bacillus odyssey* at 40 °C (55.87 %) followed by *Bacillus thuringiensis* (54.84 %), *Bacillus subtilis* (49.35 %), *Bacillus cereus* (43.17 %), *Alcaligenes* sp. (37.85 %) and *Nocardiopsis alba* (32.87 %).

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