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Studies on the Effect of Carbon and Nitrogen Sources for the Decolourization of Reactive Textile Dyes by Bacterial Isolates

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Abstract: Colour removal, in particular, has recently become a major scientific interest. Microbial decolourization and degradation is an environmentally friendly and cost-competitive alternative to chemical decomposition processes.In the present study, the effect of carbon and nitrogen sources on bacterial decolourization of Reactive Textile Dyes was investigated. 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 effect of different carbon sources *viz*., starch, glucose, sucrose, lactose and maltose on decolourization of Reactive dyes were investigated in the present study. Next to sucrose, maximum bacterial decolourization of reactive dyes was observed in the medium supplemented with glucose, starch, lactose and maltose.The effect of different nitrogen sources *viz*., yeast extract, ammonium chloride, peptone and ammonium sulphate on decolourization of Reactive dyes were analyzed in the present research. Next to peptone, maximum bacterial decolourization of Reactive dyes were observed in the medium supplemented with ammonium chloride, yeast extract and ammonium sulphate.

Key words: Textile Dyes · Bacteria · Carbon Sources · Nitrogen Sources and Decolourization.

INTRODUCTION

Dyeing of textile request water and generates a substantial quality of effluents containing mineral salts and dyes at high concentration,an estimated 700000 tons of dyes are produced annually worldwide of which 60 – 70% are azo dyes. Chronic effects of dyestuffs, especially of azo dyes, have been studied for several decades. Azo dyes in purified form are mutagenic or carcinogenic, except for some azo dyes, leads to formation of aromatic amines and several aromatic amines are known mutagens and carcinogens to human beings [1].

Azo dyes are deficient in carbon sources and the biodegradation of dyes without any supplement of carbon or nitrogen sources is very difficult[2]. Azo dye decolourization by mixed as well as pure cultures generally requires complex organic sources, such as yeast extract, peptone, or a combination of complex organic sources and carbohydrates[3, 4]. During decolourization of azo dyes *via* reduction of azo bonds, it was reported that reducing equivalents from various carbon sources are transferred to the dye. It was also observed that in anaerobic consortia, acidogenic bacteria convert the soluble substrates, such as carbohydrates, to volatile organic acids or alcohols, such as acetic acid and

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methanol, which act as competitive substrates for methanogenic, sulfate reducing and acetogenic bacteria [5, 6].

Some studies performed the azo dye decolourization in the presence of additional carbon and nitrogen sources. In these, the addition of carbon sources seemed to be less effective in promoting decolourization, probably due to the preference of the cells in assimilating the extra carbon sources over using the dye compound as the carbon source [7]. In contrast, addition of the organic nitrogen sources, such as peptone, beef extract, urea, yeast extract and so on, can regenerate NADH, which acts as an electron donor for the reduction of azo dyes by microorganisms and thus effective decolourization was observed [8]. To make the process economically feasible and practically applicable, some investigators have used lignocellulosic agricultural waste as an additional supplement for effective decolourization.In the present study, the effect of carbon and nitrogen sources on bacterial decolourization of Reactive Textile Dyes was investigated.

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 (λm = 480 nm)
- Reactive Black – B (λm = 600 nm)
- Reactive Yellow – MR (λm = 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 Various Carbon Sources on Decolourization of Reactive Textile Dyes: The effect of various carbon sources *viz*., sucrose, glucose, lactose, starch and maltose was carried out in this present study. The Carbon sources (1:100) were added in Nutrient Broth containing Reactive azo dyes (500mg/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 by following the formula of Dafale*etal*. [9].

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

Effect of Various Nitrogen Sources on Decolourization of Reactive Textile Dyes: The effect of various nitrogen sources *viz*., yeast extract, ammonium chloride, ammonium sulphate and peptone was investigated in the present study. The Nitrogen sources (1:100) were added in the Nutrient Broth containing Reactive azo dyes (500mg/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 by following the formula of Dafale *et al*. [9].

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

RESULTS AND DISCUSSION

Effect of Different Carbon Sources on Bacterial Decolourization of Reactive Dyes: 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 [10]. 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 might be attributed to that, the sugars may inhibit the decolourization of azo dyes because its effect as catabolite repression [11].

The effect of different carbon sources on decolourization of Reactive Orange – 16 was investigated and the results were furnished in Table – 1. 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 %), *Bacillus cereus* (63.34 %)and *Alcaligenes* sp. (61.61 %). The bacterial isolate *Nocardiopsis alba* showed minimum

Table 1: Bacterial decolourization of Reactive Orange – 16 using various carbon sources

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.	*Bacillus cereus*	0.502 (58.64%)	0.470 (61.28%)	0.445 (63.34%)	0.542 (55.35%)	0.598 (50.74%)
5.	*Alcaligenes* sp.	0.534 (56.01%)	0.506 (58.31%)	0.466 (61.61%)	0.626 (48.43%)	0.666 (45.14%)
6.	*Nocardiopsis alba*	0.541 (52.96%)	0.520 (57.16%)	0.496 (59.14%)	0.579 (52.30%)	0.633 (47.85%)

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

Table 2: Bacterial decolourization of Reactive Black – B using various carbon sources

S.No	Bacterial isolates	Final OD and% Decolourization				
		Starch	Glucose	Sucrose	Lactose	Maltose
1.	*Bacillus odyssey*	0.438 (69.41%)	0.385 (73.11%)	0.320 (77.65%)	0.488 (65.92%)	0.540 (62.29%)
2.	*Bacillus thuringiensis*	0.483 (66.27%)	0.418 (70.81%)	0.365 (74.51%)	0.530 (62.98%)	0.590 (58.79%)
3.	*Bacillus subtilis*	0.525 (63.33%)	0.465 (67.52%)	0.380 (73.46%)	0.572 (60.05%)	0.621 (56.63%)
4.	*Bacillus cereus*	0.568 (60.33%)	0.502 (64.94%)	0.410 (71.36%)	0.615 (57.05%)	0.670 (53.21%)
5.	*Alcaligenes* sp.	0.609 (57.47%)	0.548 (61.73%)	0.451 (68.50%)	0.647 (54.81%)	0.700 (51.11%)
6.	*Nocardiopsis alba*	0.653 (54.39%)	0.585 (59.14%)	0.480 (66.48%)	0.688 (51.95%)	0.749 (47.69%)

Initial OD of Reactive Black – B at 600 nm = 1.432

Table 3: Bacterial decolourization of Reactive Yellow – MR using various carbon sources

S.No	Bacterial isolates	Final OD and% Decolourization				
		Starch	Glucose	Sucrose	Lactose	Maltose
1.	*Bacillus odyssey*	0.416 (69.72%)	0.355 (74.16%)	0.335(75.61%)	0.492 (64.19%)	0.534 (61.13%)
2.	*Bacillus thuringiensis*	0.458 (66.66%)	0.402 (70.74%)	0.342 (75.10%)	0.525 (61.79%)	0.580 (57.78%)
3.	*Bacillus subtilis*	0.501 (63.53%)	0.448 (67.39%)	0.382 (72.19%)	0.569 (58.58%)	0.615 (55.24%)
4.	*Bacillus cereus*	0.545 (60.33%)	0.480 (65.06 %)	0.398 (71.10%)	0.608 (55.74%)	0.662 (51.81%)
5.	*Alcaligenes* sp.	0.609 (55.67%)	0.532 (61.28%)	0.463 (66.30%)	0.650 (52.69%)	0.695 (49.41%)
6.	*Nocardiopsis alba*	0.715 (47.96%)	0.670 (51.23%)	0.522 (62.00%)	0.748 (45.56%)	0.798 (41.92%)

Initial OD of Reactive Yellow – MR at 600 nm = 1.374

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.

The effect of different carbon sources on decolourization of Reactive Black - B was evaluated and the results were given in Table – 2. Five different carbon sources *viz*., starch, glucose, sucrose, lactose and maltose were tested in the present study. Among the six bacterial isolates tested, maximum decolourization of Reactive Black – B was observed by *Bacillus odyssey* in the medium supplemented with sucrose (77.65 %) followed by *Bacillus thuringiensis* (74.51 %)*, Bacillus subtilis* (73.46 %)*, Bacillus cereus* (71.36 %)and *Alcaligenes* sp. (68.50%). The bacterial isolate *Nocardiopsis alba*showed minimum decolourization of Reactive Black – B (66.48 %). Next to sucrose, maximum bacterial decolourization of Reactive Black – B was observed in the medium supplemented with glucose, starch, lactose and maltose.

The effect of different carbon sources on decolourization of Reactive Yellow - MR was studied and the results were showed in Table – 3. Five different carbon sources *viz*., starch, glucose, sucrose, lactose and maltose were tested in the present study. Among the six bacterial isolates tested, maximum decolourization of Reactive Yellow - MR was observed by *Bacillus odyssey* in the medium supplemented with sucrose (75.61 %) followed by *Bacillus thuringiensis* (75.10 %)*, Bacillus subtilis* (72.19 %)*, Bacillus cereus* (71.10 %)and *Alcaligenes* sp. (66.30%). The bacterial isolate *Nocardiopsis alba*showed minimum decolourization of Reactive Yellow - MR (62.00 %). Next to sucrose, maximum bacterial decolourization of Reactive Yellow - MR was observed in the medium supplemented with glucose, starch, lactose and maltose.

Mitsunori and Masayuki [12] showed that, the ability of *Pseudomonas aeruginosa* strain DP-4 to degrade 2,4-dichlorophenol (DCP) was achieved by adding 1 mg//l

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

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.	*Bacillus cereus*	0.687 (52.81 %)	0.616 (57.69 %)	0.546 (62.50 %)	0.745 (48.83 %)
5.	*Alcaligenes* sp.	0.687 (52.81 %)	0.637 (56.25 %)	0.593 (59.27 %)	0.766 (47.39 %)
6.	*Nocardiopsis alba*	0.714 (50.96 %)	0.698 (52.06 %)	0.669 (54.05 %)	0.797 (45.26 %)

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

Table 5: Bacterial decolourization of Reactive Black – B using various nitrogen sources

S. No	Bacterial isolates	Final OD and% Decolourization			
		Yeast extract	Ammonium chloride	Peptone	Ammonium sulphate
1.	*Bacillus odyssey*	0.533 (64.77%)	0.458 (69.72%)	0.422 (72.10%)	0.589 (61.07%)
2.	*Bacillus thuringiensis*	0.589 (61.07%)	0.511 (66.22%)	0.456 (69.86%)	0.613 (59.48%)
3.	*Bacillus subtilis*	0.645 (57.36%)	0.556 (63.25%)	0.496 (67.21%)	0.697 (53.93%)
4.	*Bacillus cereus*	0.686 (54.65%)	0.620 (59.02%)	0.560 (62.98%)	0.751 (50.36%)
5.	*Alcaligenes* sp.	0.716 (52.67%)	0.684 (54.79%)	0.615 (59.35%)	0.784 (48.18%)
6.	*Nocardiopsis alba*	0.883 (41.63%)	0.811 (46.39%)	0.780 (48.44%)	0.898 (40.64%)

Initial OD of Reactive Black – B at 600 nm = 1.513

glucose in the culture medium. Moreover, other investigators supported the importance of the supplementation of some nutrients to enhance the biodegradation of some toxic compounds. Presence of starch as the best co-metabolite in decolourization of azo dyes was supported by many studies. Padmavathy *et al*. [13] found that starch was the best carbon source in azo dye biodegradation from synthetic waste water under aerobic co-metabolite conditions. Also Georgiou *et al*. [14] suggested the use of potato – starch industrial wastes to increase the decolourization of textile waste water in large scale. Starch was also added by Olukanni *et al*. [15] 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 by *Pseudomonas luteola* [16].

The 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 [17]. 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*. [18] where the ability of halophilic and halotolerant bacterial isolates to utilize Remazol black - B as sole carbon source was used to select the most effective isolates. Ayed*etal*. [19] used glucose in decolourization of remazol black B by halotolerant and halophilic isolates.

Effect of Different Nitrogen Sources on Bacterial Decolourization of Reactive Dyes: 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 %), *Bacillus cereus* (62.50 %) and *Alcaligenes* sp. (59.27 %). The bacterial isolate *Nocardiopsis alba* showed minimum decolourization of Reactive Orange – 16 (54.05 %). Next to peptone, maximum bacterial decolourization of Reactive Orange – 16 was observed in the medium supplemented with ammonium chloride, yeast extract and ammonium sulphate.

The effect of different nitrogen sources on decolourization of Reactive Black – B was reported and the results were given in Table - 5. 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

Table 6: Bacterial decolourization of Reactive Yellow – MR using various nitrogen sources

S.No	Bacterial isolates	Final OD and% Decolourization			
		Yeast extract	Ammonium chloride	Peptone	Ammonium sulphate
1.	*Bacillus odyssey*	0.526 (63.64%)	0.494 (65.86%)	0.460 (68.21%)	0.599 (34.96%)
2.	*Bacillus thuringiensis*	0.496 (54.94%)	0.591 (59.15%)	0.530 (63.37%)	0.652 (39.87%)
3.	*Bacillus subtilis*	0.553 (51.90%)	0.645 (55.42%)	0.590 (59.22%)	0.696 (43.19%)
4.	*Bacillus cereus*	0.628 (45.61%)	0.740 (48.85%)	0.687 (52.52%)	0.787 (31.02%)
5.	*Alcaligenes* sp.	0.941 (34.96%)	0.870 (39.87%)	0.822 (43.19%)	0.998 (31.02%)
6.	*Nocardiopsis alba*	0.763 (32.55%)	0.901 (37.73%)	0.823 (43.12%)	0.976 (29.55%)

Initial OD of Reactive Yellow – MR at 600 nm = 1.447

tested, maximum decolourization of Reactive Black – B was observed by *Bacillus odyssey* in the medium supplemented with peptone (72.10 %) followed by *Bacillus thuringiensis* (69.86 %), *Bacillus subtilis* (67.21 %), *Bacillus cereus* (62.98 %) and *Alcaligenes* sp. (59.35 %). The bacterial isolate *Nocardiopsis alba* showed minimum decolourization of Reactive Black – B (48.44 %). Next to peptone, maximum bacterial decolourization of Reactive Black – B was observed in the medium supplemented with ammonium chloride, yeast extract and ammonium sulphate.

The effect of different nitrogen sources on decolourization of Reactive Yellow – MR was determined and the results were listed in Table - 6. 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 Yellow – MR was observed by *Bacillus odyssey* in the medium supplemented with peptone (68.21 %) followed by *Bacillus thuringiensis* (63.37 %), *Bacillus subtilis* (59.22 %), *Bacillus cereus* (52.52 %) and *Alcaligenes* sp. (43.19 %). The bacterial isolate *Nocardiopsis alba* showed minimum decolourization of Reactive Black – B (42.12 %). Next to peptone, maximum bacterial decolourization of Reactive Yellow – MR was observed in the medium supplemented with ammonium chloride, yeast extract and ammonium sulphate.

Nigam *et al*. [20] reported that isolates 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 were decreased [21]. 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*. [22] 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 [23-27].

Concerning the effect of the addition of different nitrogen sources for the purpose of decolourization of the azo dyes, it was found that organic nitrogen source peptone was the best inducer for the decolourization of the two dyes by the two strains. In contrast to the case of carbon source, peptone was the best nitrogen source for growth in addition to decolourization. Presence of peptone as the best nitrogen source was proved by many azo dyes bioremediation studies. Chen *et al*. [28] found that peptone gave the best color removal percentage for azo dye Red RBN by *Proteus mirabilis* and the substitution of inorganic nitrogen (NH_4Cl) for peptone gave poor cell growth and low color removal. Hu [29] used medium containing peptone as a nitrogen source in decolourization of reactive azo dyes by *Pseudomonas luteola*. In contrast to these results inorganic nitrogen sources (NH_4Cl) was used in anaerobic treatment of azo dye Acid Orange 7 under fedbatch and continuous conditions [30].

CONCLUSIONS

The conclusions of the present research work were:

- 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 %), *Bacillus cereus* (63.34 %), *Alcaligenes* sp. (61.61 %) and *Nocardiopsis alba* (59.14 %). Maximum decolourization of Reactive Black – B was observed by *Bacillus odyssey* in the medium supplemented with sucrose (77.65 %) followed by *Bacillus thuringiensis* (74.51 %), *Bacillus subtilis* (73.46 %),

Bacillus cereus (71.36 %), *Alcaligenes* sp. (68.50 %) and *Nocardiopsis alba* (66.48 %). Maximum decolourization of Reactive Yellow - MR was observed by *Bacillus odyssey* in the medium supplemented with sucrose (75.61 %) followed by *Bacillus thuringiensis* (75.10 %), *Bacillus subtilis* (72.19 %), *Bacillus cereus* (71.10 %), *Alcaligenes* sp. (66.30 %) and *Nocardiopsis alba* (62.00 %).

- 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 %), *Bacillus cereus* (62.50 %), *Alcaligenes* sp. (59.27 %) and *Nocardiopsis alba* (54.05 %). Maximum decolourization of Reactive Black – B was observed by *Bacillus odyssey* in the medium supplemented with peptone (72.10 %) followed by *Bacillus thuringiensis* (69.86 %), *Bacillus subtilis* (67.21 %), *Bacillus cereus* (62.98 %), *Alcaligenes* sp. (59.35 %) and *Nocardiopsis alba* (48.44 %). Maximum decolourization of Reactive Yellow – MR was observed by *Bacillus odyssey* in the medium supplemented with peptone (68.21 %) followed by *Bacillus thuringiensis* (63.37 %), *Bacillus subtilis* (59.22 %), *Bacillus cereus* (52.52 %), *Alcaligenes* sp. (43.19 %) and *Nocardiopsis alba* (42.12 %).

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