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Full Length Article

Accelerated Biodecolorization of Reactive Dyes with Added Nitrogen and Carbon Sources

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ABSTRCAT

Synthetic dyes being xenobiotics and recalcitrant in nature are under high consideration for environmental chemists. They are resistant to be decomposed by conventional treatment technologies. So recently biodegradation has been accepted as an efficient and attractive approach to decompose these complex molecules. The aim of present research work is directed as finding the effect of added nutrient sources both for carbon and nitrogen on decolorization of two commonly used reactive azo dye: Reactive Black B and Reactive Orange 16. The results clearly showed that additional nutrient sources are effective in increasing dye decolorization rate only at lower levels i.e., 2 $mgmL^{-1}$. At higher levels they decrease color removal upto 50%. Moreover color removal extent is greatly affected by type of microbial consortia and chemical structure of dye. © 2010 Friends Science Publishers

Key Words: Decolorization; Reactive black B; Reactive orange 16; Biodegradation; Azo dyes

INTRODUCTION

Azo dyes are commonly used in several industries including textile, dyeing, printing and cosmetic industries. Due to their complex structure, they are highly persistent in natural environments, which may lead to acute toxicity of ecosystems. Worldwide over, 10,000 different dyes and pigments are currently in use (Revenkar & Lele, 2007). Presence of dyes in wastewater causes an unpleasant appearance by imparting the color on one hand and on the other hand its breakdown products are highly toxic, carcinogenic and mutagenic as well (Conneely *et al*., 1999; Xu *et al*., 2005). There are a number of physical (Anastasios *et al*., 2005; Theodora *et al*., 2006) and chemical treatments (Pak & Chang, 1999; Kabita *et al*., 2001) for color removal from textile wastewater.

Microorganisms can play a very significant role in decomposition and ultimate mineralization of these xenobiotics i.e., dyes (Lie *et al*., 1996; Khalid, 2008). Environmental biotechnology is based on ability of microorganism (both bacterial & fungal) to decompose larger chemical compounds, which are xenobiotics. Many researchers have studied in detail and have isolated several microbial strains having potential to decolorize a large number of dyes belonging to different classes have been isolated (Sushama *et al*., 2009). Biodegradation of reactive azo dyes present in textile wastewater is a complicated procedure due to versatility in structure of dyes. A number of environmental factors including both dye and non-dye related parameters are reported in literature, which directly or indirectly influences the process. These factors include temperature, dissolved oxygen, redox mediators, type of microorganisms, amount of nutrients, type and chemical structure of dye under study (Wong & Yuen, 1996; Keharia *et al*., 2007; Seesuriyachan *et al*., 2007)

Many research reports are available, which explain successful decolorization of dyes by using purified microbial cultures. But these findings do not find much application in practical treatment system due to complexity and heterogeneity of chemical compounds present in textile wastewater. The present project included the use of such microbial populations, which are native part of these effluent channels and can tolerate the hostile conditions. Moreover it included decolorization of two commonly used reactive azo dyes i.e., Reactive Black-B (RBB) and Reactive Orange 16 (RO16) in the presence of additional carbon and nitrogen nutrients to study their effect on decolorization of selected azo dyes.

MATERIALS AND METHODS

All chemicals used in the study were of analytical reagent grade. Dyes used were of commercial grade being provided by a local textile industry. The media used for isolation and growth of microbial strains (and for decolorization of dyes under study) was minimal salt media

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of following composition gL^{-1}: Na_2HPO_4 (3.6), $NH_4)_2SO_4$ (1.0), K_2HPO_4 (1.6), $MgSO_4$ (1.0), Fe(NH_4) citrate (0.01), $CaCl_2.2H_2O$ (0.10) and 10 mL of trace elements solution per litre. Solution having trace elements was of following composition:$ZnSO_4.7HH_2O$ (10.0),$MnCl_2.4H_2O$ (3.0), $CoCl_2.6H_2O$ (1.0), $NiCl_2.6H_2O$ (2.0), $NO_2MoO_4.2H_2O$ (3.0), H_3BO_3 (3.0) and $CuCl_2.2H_2O$ (1.0) mg L^{-1}. The pH of media was maintained at 7.0±0.05. The MSM was supplemented with 0.1% (w/v) of yeast extract and glucose (sterilized stock solutions were added to sterilized MSM).

Optimization of decolorization conditions: In preliminary study decolorization experiments were run under various culture conditions using traditional stepwise protocol i.e., varying a factor at a time and keeping others constant (Kalme *et al.*, 2007) for the optimization of dye concentration, pH, temperature and incubation periods under aerobic conditions (not part of this Paper). In the next step, different additional carbon (glucose, sewage sludge, starch) at 2-10 g L^{-1} and nitrogen sources (ammonium sulfate, urea, ammonium nitrate) at 2-10 g L^{-1} were added to study their effect on dye decolorization rate under previously optimized conditions.

Decolorization assay via UV-Vis spectroscopy: Samples withdrawn from serum vials obtained after centrifugation were run through spectrophotometer (Model Hawlete Pachard 8452A) to check absorbance at λmax of selected dyes at 597 nm for Reactive Black B and 494 nm for Reactive Orange 16. The uninoculated dye free medium was used as blank. All assays of dye decolorization were performed in triplicate, while the decolorization efficiency was expressed as:

$$\text{Decolorization efficiency \%} = \text{I-F/F x 100}$$

Where

I = Absorbance of media prior to incubation

F = Absorbance of decolorized media.

Amount of dyes present in liquid culture media before and after biodecolorization by the tested bacterial and fungal strain was determined from the respective concentration versus absorbance calibration curves obtained for respective dyes. Standard error and deviation was calculated for obtained data.

RESULTS

From a series of isolation and purification experiments three microbial strains were obtained showing maximum decolorization potential of RBB and RO 16 (data not shown). These were named as B1, B2 (bacterial strains) and F1 (fungal strain). Optimization of reaction conditions for highest color removal with these strains was done (data not shown here) and under the optimized conditions effect of added carbon and nitrogen sources (Fig. 1-12) was studied.

In the case of Reactive Black-B, maximum decolorization achieved by bacterial strain B1 was 74%, with B_2 87% and with fungal strain (F_1) 90% at optimized

Fig. 1: Effect of glucose on % decolorization of Reactive Black-B

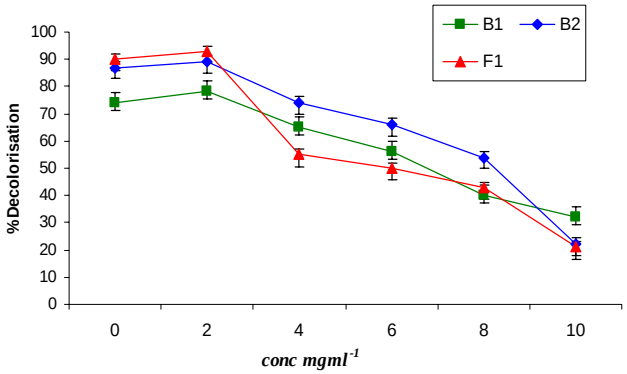


Fig. 2: Effect of sewage sludge on the % decolorization of Reactive Black-B

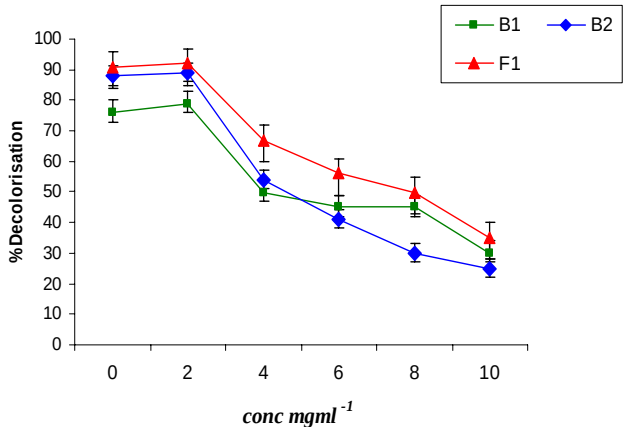
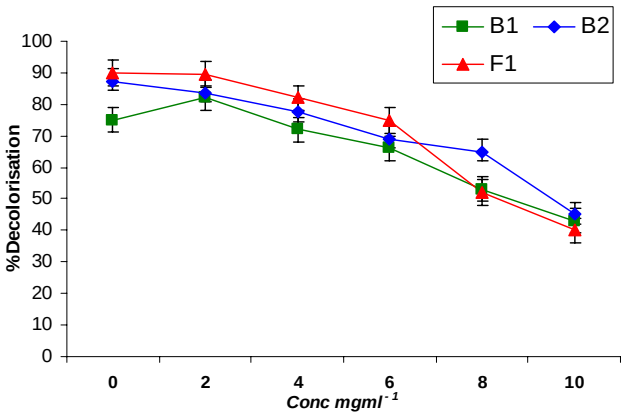


Fig. 3: Effect of starch on % decolorization of Reactive Black-B



conditions. By adding 2 mg mL^{-1} of glucose as additional carbon source all three strains showed increase in their decolorization potential reaching to a level of 78.2% (B_1), 89% (B_2) and 93% for F_1.Similarly with sewage sludge a positive effect on decolorization potential was observed but only at 2 mg mL^{-1}. Further addition of sewage sludge,

Fig. 4: Effect of glucose on % decolorization of Reactive Orange 16

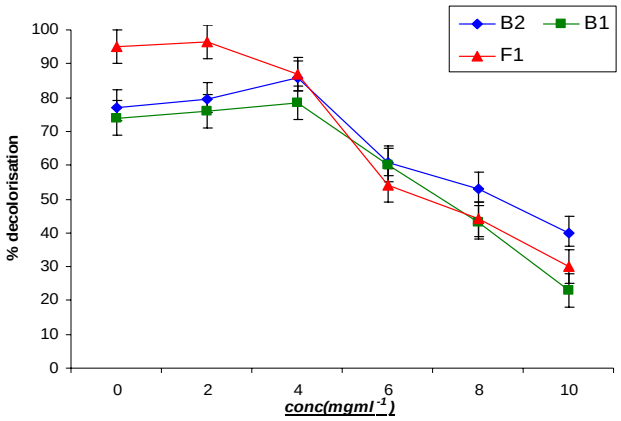


Fig. 5: Effect of starch on % decolorization of Reactive Orange 16

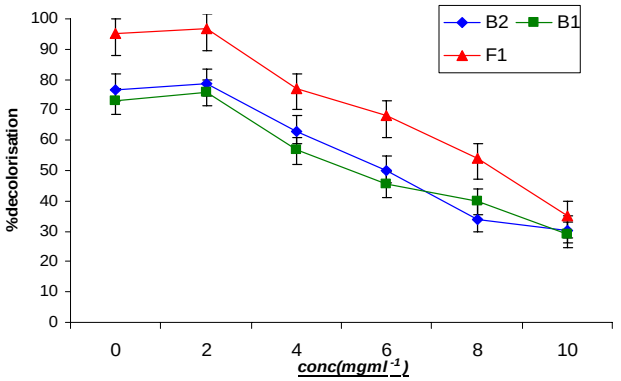
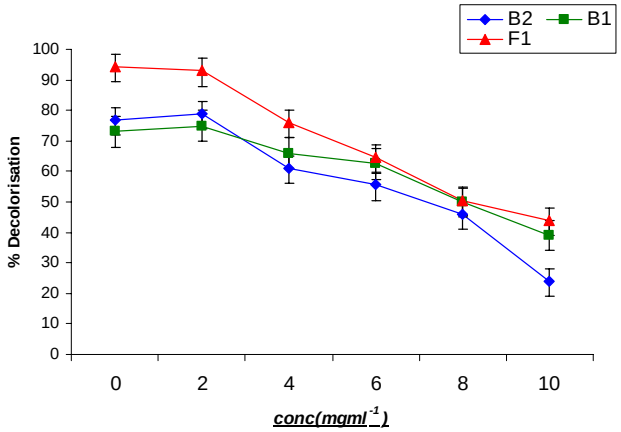


Fig. 6: Effect of sewage sludge on %% decolorization of Reactive Orange 16



glucose or starch lead to a sharp decrease in % decolorization of Reactive Black-B (Fig. 1-3). In the case of Reactive Orange 16 glucose increased the decolorization extent up to 4 mg mL^{-1} addition for bacterial strains (B_1 78.4% & B_2 85.9%), while for fungal strain a negligible increase was observed (only at 2 mg mL^{-1} addition). For

Fig. 7: Effect of urea on % decolorization of Reactive Black-B

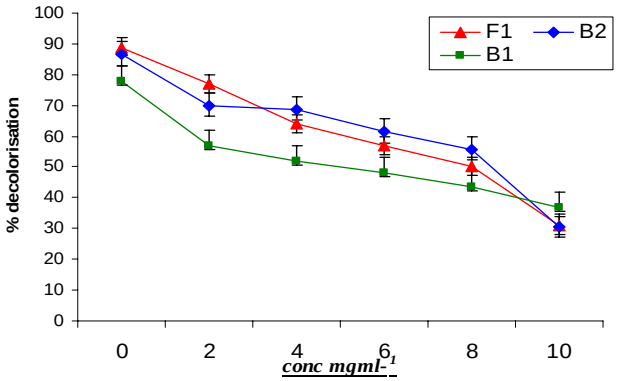
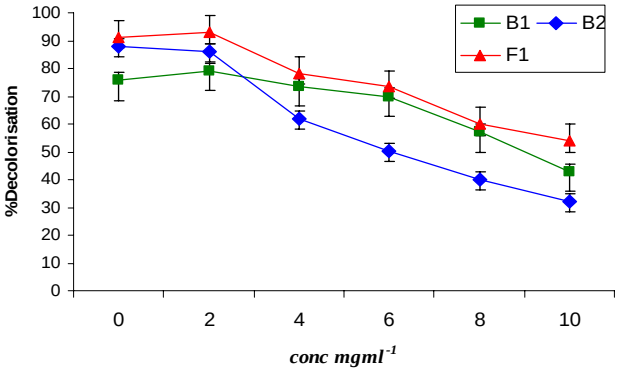


Fig. 8: Effect of Ammonium Nitrate on % decolorization of Reactive Black-B



starch maximum decolorization of Reactive Orange 16 was obtained at 2 mg mL^{-1} of starch i.e., 86% for B_1, 78.9% for B_2 and 94% for F_1. Further increase up to 10 mg mL^{-1} lead to decrease in color removal extent. Sewage sludge could not show any significant positive effect on %decolorization of Reactive Orange 16 at any level of addition. It could be summarized that none of added carbon source could show a positive correlation with any dye decolorization rate at higher levels of addition. On the other hand they caused a sharp decrease in decolorization ability of all the three strains with dyes under study (almost less than 25%).

The data on decolorization of selected reactive azo dyes (RBB & RO16) with addition of extra nitrogen sources have been presented in (Fig. 7-12). No remarkable increasing effect of urea on decolorization of Reactive Black B could be seen for any microbial strain. A prominent, inhibitory effect was recorded at 10mg mL^{-1} addition when only 30.6%, 37% and 40% dye was decolorized by B2, B1 and F1 respectively. Remarkable difference among behaviors of strains was observed with ammonium nitrate, as bacterial strain B_2 showed continuous decrease in color removal ability by addition of ammonium nitrate (Fig. 8) at all levels. While B1 and F1 gave a little increased dye decolorization at lower level but at higher levels of addition the decolorization ability was decreased.

Fig. 9: Effect of Ammunium sulphate on % decolorization of Reactive Black-B

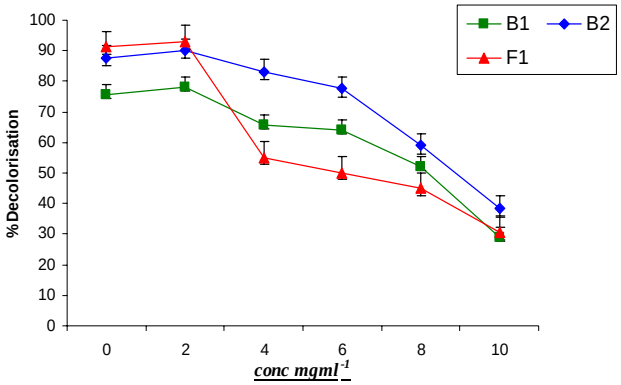


Fig. 10: Effect of urea on % decolorization of Reactive orange 16

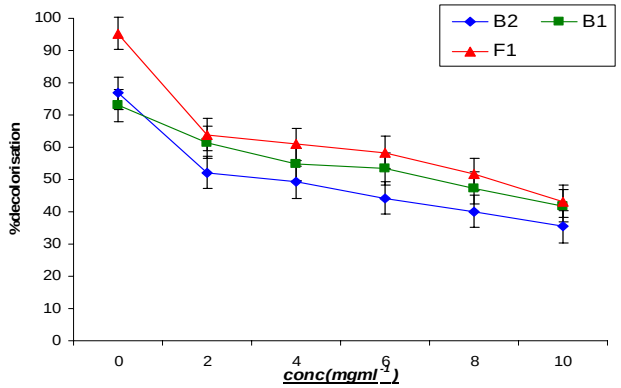
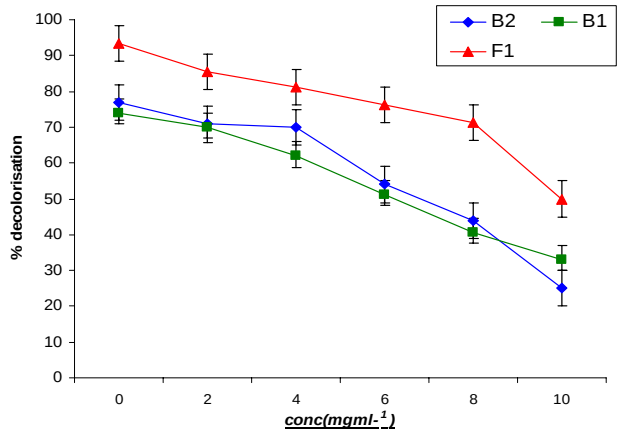


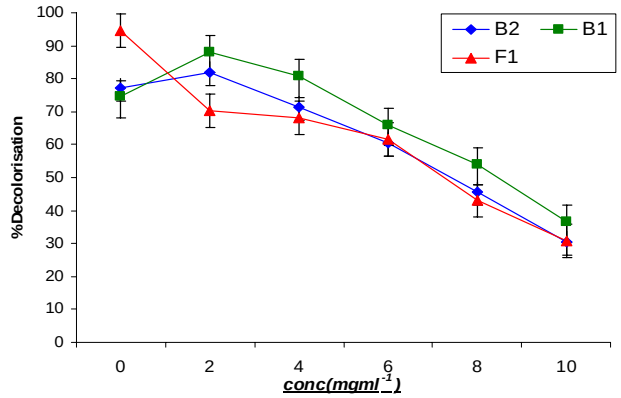
Fig. 11: Effect of Ammunium nitrate on % decolorization of Reactive orange 16



In the case of ammonium sulphate (Fig. 9) a negative correlation was recorded among dye decolorization and added amount. Fungal strain showed lower tolerance and 60% decrease in color removal rate of Reactive Black B at 10 mg mL^{-1} addition of ammonium sulphate.

In the case of Reactive Orange 16 both urea and ammonium nitrate could not enhance color removal rate

Fig. 12: Effect of Ammunium Sulphate on % decolorization of Reactive orange 16



even at lower concentration. However among the two, urea caused more sharp decrease in decolorization potential of all microbial strains. Comparison among the strains revealed that bacterial stain B_2 and fungal strain F_1 were more sensitive (Fig. 10 & 11). Ammonium sulphate showed an increasing trend in color removal at 2 mg mL^{-1} addition with B_1 and B_2. However F_1 behaved negatively and a 20% decrease in decolorization ability was recorded at 2 mg mL^{-1} addition of ammonium sulphate (Fig. 12).

DISCUSSION

It is clear from the results presented above that all the three newly isolated microbial strains were able to grow on chosen additional carbon sources although their effect on decolorization potential was not remarkably positive. With additional carbon sources other than dyes as nutrient, microbial growth was not inhibited as microbial counts were higher in liquid media (data not shown), which is in harmony to the findings Kirchman (1990) and Laird *et al.* (1990). Decrease in decolorization potential may be as a result of accumulation of simple carbon compound acting as a co metabolic substrate in media. Similar trend has been reported by others (Swamy & Ramsay, 1999; Adosinda *et al.*, 2001; Xu *et al.*, 2006). Contrary to these results, a number of researchers have reported an enhanced transformations of dye by using glucose, acetic acid, ethanol and found there role in enzyme production (Panswad & Luangdilk, 2000; Tan *et al.*, 2000; Wesenberg *et al.*, 2003). Variability among the earlier reported studies and foregoing research might be due to difference in microbial characteristic and enzymes responsible for color removal. It can be concluded that in start metabolism of additional carbon source like glucose or starch might caused an increased production of nucleotides (Khehra *et al.*, 2005) leading to increase in decolorization efficiency. However at higher concentrations the same carbon sources on metabolism produced organic acids, which in turn decreased the pH of media. As each microbial strain and its enzyme are highly specific to pH so decrease in decolorization extent of dyes

occurred with a change in pH. Behavior of each strain was different for both dyes understudy due to the different chemical structure and various carbon sources as well.

As far as nitrogen sources are concerned none of them could be proved as an enhancing factor for decolorization rate of dyes under study. A strong inhibitory effect was recorded at higher levels for all additional nitrogen sources under investigation. These results are in harmony with those of Zhang *et al.* (1999), Tatarko and Bumpus (1998) and Sanghi *et al.* (2006), who also reported inhibition of dye decolorization with supplemental nitrogen. The facts responsible for these effects are different for three nitrogen compounds understudy. The addition of inorganic nutrients like nitrogen does not always enhance degradation of organic compounds, because there are many other factors which many decrease microbial activity (Steffensen & Alexander, 1995). Urea when dissolved in liquid culture causes a shift of pH more towards acidic side, which decreased the color removal, growth as well as enzyme activity of strains. Presence of nitrate in culture media might slow down process of color removal (Carliell *et al.,* 1995; Panswad & Luangdilik, 2000), because it serves as electron acceptor which could interfere the first step in dye decolorization. Overall the presence of these simple inorganic/organic nitrogen sources act as attractive substrates and microorganism preferably metabolize them rather than amines or complex azo dyes present in media.

CONCLUSION

The newly isolated unidentified bacterial and fungal strains had excellent potential for degradation and decolorization of reactive azo dye, Reactive Black-B, Reactive Orange 16. Decolorization potential of these strains can not be significantly increased by additional nutrient (C & N) supplements.

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