

Optimization of Environmental Parameters on Decolorization of Remazol Black B Using Mixed Culture

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Abstract In the present study, an attempt was made to examine the potential of mixed culture (contains *Pseudomonas putida*, *Psueomonas stutzeri*, *Pseudomonas aeruginosa*, *Pseudomonas mendocina*) for decolorization of Remazol Black B dye in batch reactors. The effect of pH, temperature, inoculum, initial concentration of dye and initial concentration of glucose was studied with an aim to determine the optimal conditions required for maximum decolorization and degradation. The culture exhibited maximum decolorization ability at pH between 7-8 and at 30°C. A 10% (v/v) inoculum and 1% (w/v) glucose concentration were found to be the optimum for decolorization. A maximum of 98% decolorization was observed at 25 ppm initial concentration of dye after 18 hours of incubation period. At higher dye concentration of 300 ppm, the removal in colour was found to be 75% in 48 hours of incubation period. The results show that the enriched mixed culture from activated sludge has an excellent potential in removal of Remazol Black B dye from wastewater under aerobic conditions.

Keywords: Remazol Black B, pH, temperature, decolorization

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1. Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life (Shah et al., 2013). The textile industry is one of them, which extensively use synthetic chemicals as dyes. Waste waters from textile industries pose a threat to the environment, as large amount of chemically different dyes are used (Shah et al., 2013). Reactive dyes, including many structurally different dyes, are extensively used in the textile industry because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimal energy consumption. The three most common groups are azo, anthraquinone and phthalocyanine (Shah et al., 2013). Various kinds of physico-chemical methods are in use for the treatment of wastewater contaminated with dye. These methods are not environment friendly and cost-effective and hence become commercially unattractive (Karimniae et al., 2007). Many microorganisms belonging to the different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolourize azo dyes (Asad et al., 2007). A lot of information is available on removal and degradation of Remazol Black B using pure strains of bacteria, fungi, algae and yeast such as *Shewanella strain J18 143*, *Rhizopus arrhizus*, *Phlebia tremellosa*, *Trametes versicolor*, *Bjerkandera* sp. BOS55, *P. chrysosporium*, *Chlorella vulgaris*, *Candida tropicalis*,

Saccharomyces cerevisiae, *Kluyveromyces marxianus* IMB3, *P. mirabilis*, *P. luteola*, *Pseudomonas* sp. and *K. rosea* (Aksu et al., 2000., Kirby et al., 2000., Swamy et al., 1999., Meehan et al., 2000). The removal and degradation of Remazol Black B dye by various physicochemical methods have also been studied (Robinson et al., 2002., Hepel & Hazelton., 2005., Vinodgopal et al., 1998). However, maintaining the purity of single cultures in the large scale application and their inability to degrade all different dyes present in the actual effluent are the drawbacks for their commercial application (Pearce et al., 2006, Andre et al., 2007). Therefore, the use of mixed culture seems to have more potential for large scale application at field level. The syntrophic interactions present in the mixed communities lead to complete mineralization of azo dyes (Chang et al., 2004). In the present study, an attempt has been made for the degradation and decolorization of an azo dye (Remazol Black B) using aerobic mixed culture in batch reactors. Effect of various process parameters like pH, temperature, inoculum concentration, glucose concentration and initial concentration of dye was studied.

2. Materials & Methods

2.1. Dye Solution

Remazol black B (RBB) used in the present study is acidic and soluble in water. A stock solution of 1000 ppm

was initially prepared and the solutions of the desired concentrations for various experiments were obtained by successive dilution.

2.2. Inoculum Source and Acclimatization

Activated sludge collected from common effluent treatment plant of Ankleshwar, Gujarat, India was used as the parent source of inoculum in the present study. For enrichment of the culture, the heterogenous population was first grown aerobically in a medium containing 1% (w/v) glucose (COD= 1200 -1400mg/L) as the carbon and energy source and Remazol black B dye. During acclimatization period, the amount of glucose was regularly checked and maintained at 1%. The composition of the synthetic medium used in the present study was as follows: glucose, 10.000 g/L; Yeast extract, 0.34 gL⁻¹; NH₄Cl, 0.84 gL⁻¹; KH₂PO₄, 0.134 gL⁻¹; K₂HPO₄, 0.234 gL⁻¹; MgCl₂.6H₂O, 0.084 gL⁻¹. The culture was gradually exposed to increasing concentrations of RBB dye in order to acclimatize the microbial culture. Successive transfers of culture (180×10^5) into fresh glucose medium containing higher concentrations of RBB upto 300 ppm was done at 37°C in rotary condition. This acclimatized microbial culture was used for all experiments.

2.3. Batch Experiments

The experiments were performed in batch mode in 500 ml Elynmers flasks. A working volume of 200 ml was employed throughout the study. The glucose media and dye (concentration according to the requirement i.e. 10 ppm, 20 ppm and 50 ppm) were added to the flasks. The flasks were incubated with 10 % (v/v) acclimatized inoculum. After adding glucose media, inoculum and required concentration of dye, the flasks were kept in an orbital shaker at 160 rpm and 30°C. The pH of the solution was adjusted to 5, 6, 7, 8 and 9 with 1 N hydrochloric acid or sodium hydroxide. In addition, control flasks containing only dye and media and without inoculum were also kept to see the abiotic decolorization, if any. All the experiments were performed in duplicate.

2.4. Analytical Methods

At different time intervals, the samples were withdrawn from the flasks and centrifuged at 6000 rpm for 10 min. to precipitate suspended biomass. The concentration of dye in the supernatant was determined by reading absorbance at 595nm. This absorbance was compared with standard curve plotted using different concentrations of the dye. The measurement of absorbance and centrifugation were done by using Shimadzu UV- 1800 spectrophotometer and Remi model centrifuge, respectively.

3. Results and Discussion

3.1. pH Effect

Effect of pH (5-9) on decolorization with time of Remazol Black B at 100 ppm initial concentration of dye with 10 % inoculum is shown in Figure 1. The figure clearly shows that the percentage removal of dye increased with increase in time irrespective of pH. The maximum removal (96 %) of dye was found at pH 7 and 8

after 30 hours of incubation period. Further increase in pH beyond 8 and decrease in pH below 7 resulted in decreased percentage removal of dye. The optimum pH was found to be between 7 and 8 for maximum removal of dye. The pH has a major effect on the efficiency of dye decolorization, and the optimal pH for color removal is often between 6.0 and 10.0 for most of the dyes (Chen et al., 1999). The pH tolerance of decolorizing bacteria is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions and at high temperatures (Aksu Z., 2003).

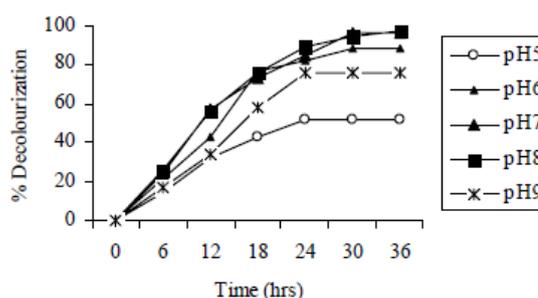


Figure 1. Effect of pH on decolorization with time

3.2. Inoculum Concentration Effect

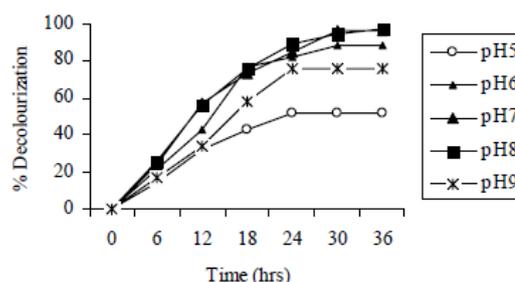


Figure 2. Effect of inoculum concentration on decolorization with time

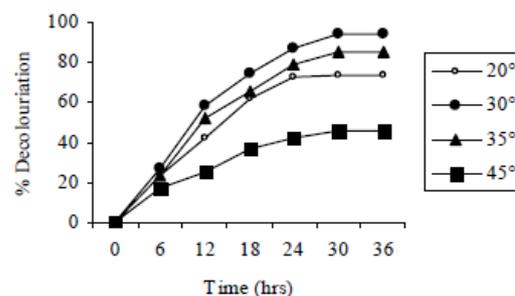


Figure 3. Effect of temperature on decolorization with time

Figure 2 shows the effect of inoculum concentration (5-20 %) with time on decolorization of dye at 100 ppm initial concentration. It is clear from the figure that percentage removal of dye increased with an increase in time for all concentrations of inoculum. The percentage removal of dye rapidly increased till 30 hrs then became constant at all concentrations of inoculum. After 36 hours the percentage removal of dye was found to be 71, 90, 94 and 96% at inoculum concentrations 5, 10, 15 and 20% respectively. There was no significant difference in percentage removal at 10, 15 and 20% inoculum concentrations and hence 10 % Effect of Temperature Figure 3 shows decolorization of dye with time at different temperatures (20, 30, 35 and 45°C) at 100 ppm

initial dye concentration and 10 % inoculum. It is clear from the figure that percentage removal of dye increased with an increase in temperature from 20 to 300 C. The percentage removal of dye decreased with further increase in temperature up to 450C. Decolorizing activity was significantly suppressed at 45^o C, this might be due to the loss of cell viability or deactivation of the enzymes responsible for decolorization at 45^oC (Cetin et al., 2006). Effect of glucose concentration Figure 4 shows the effect of glucose concentration (0.10 –2%) on decolorization with time performed at 100 ppm initial concentration of dye, 10 % inoculum concentration and at 300 C in an orbital shaker at 180 rpm. The figure clearly shows that maximum removal of dye (96%) was achieved after 30 hours of incubation period using 1% glucose media. Further increase in glucose concentration up to 2% resulted in decreased removal up to 57%. However, the lower concentration of glucose also inhibited the decolorizing activity of mixed culture. The reason for low decolorization at 0.10 % and 0.25 % might be that low glucose concentrations could not meet the growth requirements of the microbes. When the glucose concentration was 2%, the bacteria could utilize glucose preferentially, thus resulting in lower decolorization extent. In the present study it was found that 1 % glucose concentration is optimum for decolorization of Reamzol Black B dye. Effect of initial concentration of dye Figure 5 shows the effect of initial concentration of dye ranging from 25–300 ppm of dye at pH 7, 10 % inoculum concentration and at 30^oC. It is clear from the figure that percentage removal of dye increased with an increase in time irrespective of initial dye concentration. Further, percentage removal of dye decreased with an increase in dye concentration. Percentage removal of dye was found to be 98, 94, 90, 88, 85, 81 and 75 at 25, 50, 100, 150, 200, 250 and 300 ppm initial concentrations of dye, respectively.

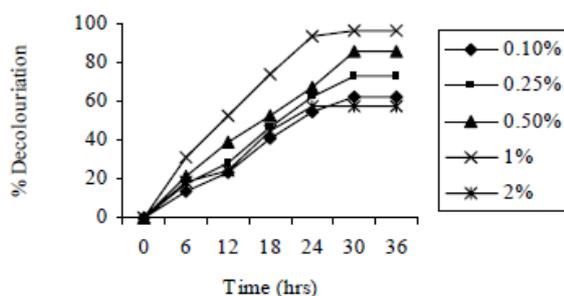


Figure 4. Effect of glucose concentration on decolorization with time

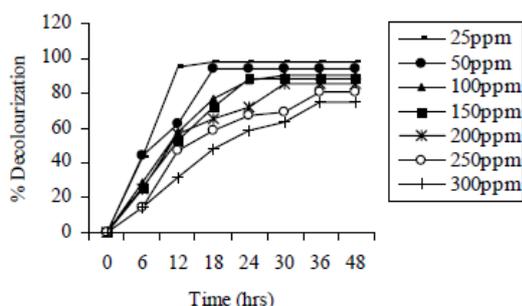


Figure 5. Effect of initial concentration of dye on decolorization with time

4. Conclusion

The present study reveals that enriched aerobic mixed culture can be used successfully for decolorizing Remazol Black B dye. The culture exhibited maximum decolorization ability at pH between 7-8 and 30^o C. Moreover, 10% (v/v) inoculum and 1 % glucose concentrations were found to be optimum for decolorization. At 25 ppm initial dye concentration a maximum of 98% removal of color was observed after 18 hours. At higher dye concentration of 300 ppm, the removal in color was found to be 75 after 48 hours of incubation period. On the basis of the results of the present study suitable strategy can be developed for the treatment of waste water contaminated with dye.

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