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# Effect of Process Parameters on Aerobic Decolourization of Reactive Azo Dye using Mixed Culture

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***Abstract*****—**In the present study, an attempt was made to examine the potential of aerobic mixed culture for decolourization 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 decolourization and degradation. The culture exhibited maximum decolourization 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 decolourization. A maximum of 98% decolourization 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 good potential in removal of Remazol Black B dye from wastewater under aerobic conditions.

***Keywords*****—**Aerobic conditions, Decolourization, Mixed culture, Remazol Black B.

## 1. INTRODUCTION

SYNTHETIC dyes are colouring agents mainly used in textile industries which generate a huge amount of wastewater in the process of dyeing. It is estimated that these industries discharge around 280,000 tonnes of dyes worldwide every year into the environment. A very small amount of dye in water (10-50 mg $L^{-1}$) affects the aesthetic value, transparency of water and gas solubility of water bodies [1]. The effluents from these industries are complex, contain a wide variety of dyes and other products such as dispersants, acids, bases, salts, detergents, humectants, oxidants, etc. Discharge of these coloured effluents into rivers and lakes results into reduced dissolved oxygen concentration, thus creating anoxic conditions that are lethal to resident organisms. Many reports indicate that textile dyes and effluents have toxic effect on the germination rates and biomass concentration of several plant species which play many important ecological functions such as providing the habitat for wildlife, protecting soil from erosion and providing bulk of organic matter that is significant to soil fertility [2-3]. The toxicity of effluent is because of the presence of dye or its degraded products which are mutagenic or carcinogenic [4]. Therefore, the treatment of industrial effluents contaminated with dye becomes necessary prior to their final discharge to the environment.

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 [5].Many microorganisms belonging to the different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolourize azo dyes [6]. Pure fungal cultures have been used to develop bioprocesses for the mineralization of azo dyes, but the long growth cycle and moderate decolourization rate limit the performance of fungal decolourization system [7].

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* [8 -12]. The removal and degradation of Remazol Black B dye by various physicochemical methods have also been studied [13-15]. 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 [16-17]. 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 [18].

In the present study, an attempt has been made for the degradation and decolourization 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.

## II. MATERIALS AND METHODS

### *2.1. Preparation of Dye solution*

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Remazol black B (RBB) used in the present study is acidic and soluble in water. The chemical structure of the above dye is shown in Figure. 1. A stock solution of 1000 ppm was initially prepared and the solutions of the desired concentrations for various experiments were obtained by successive dilution.

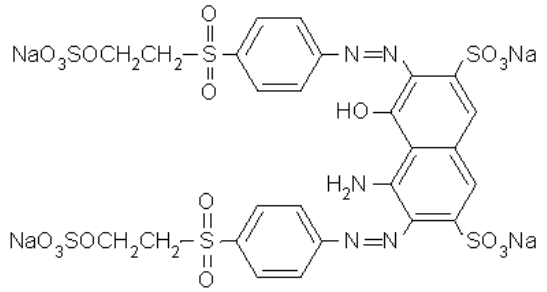


Fig. 1 Structure of Remazol Black B

*2.2 Source of inoculum and Acclimatization*

Activated sludge collected from an effluent treatment plant of a textile industry 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= 1100 - 1250mg/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 $gL^{-1}$; $NH_4Cl$, 0.84 $gL^{-1}$ ;$KH_2PO_4$ ,0.134 $gL^{-1}$ ; $K_2HPO_4$ ,0.234 $gL^{-1}$; $MgCl_2.6H_2O$, 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 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 Bach 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 180 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 decolourization, 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 5000 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 systronic UV- VIS spectrophotometer 117 and Hitech model centrifuge, respectively.

## III. RESULTS AND DISCUSSION

*3.1 Effect of pH*

Effect of pH (5-9) on decolourization with time of Remazol Black B at 100 ppm initial concentration of dye with 10 % inoculum is shown in figure 2. 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 [19]. The pH tolerance of decolourizing bacteria is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions and at high temperatures [20].

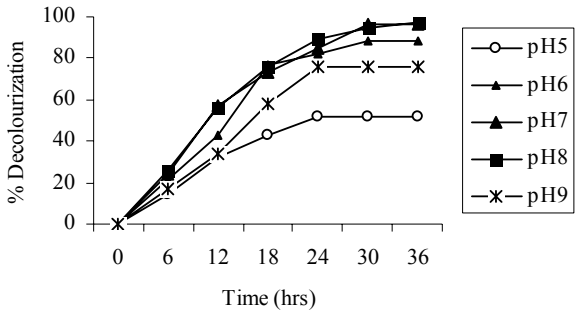


Fig. 2 Effect of pH on decolourization with time

*3.2Effect of inoculum concentration*

Figure 3 shows the effect of inoculum concentration (5-20 %) with time on decolourization 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 %

inoculum concentration was selected for further experiments.

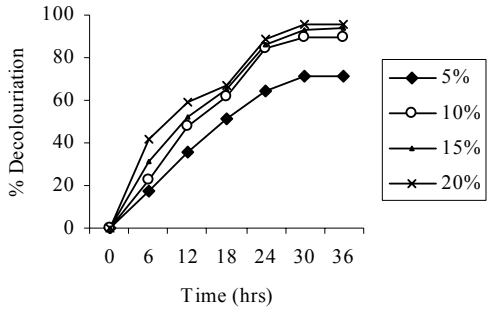


Fig. 3 Effect of inoculum concentration on decolourization with time

### *3.3 Effect of Temperature*

Figure 4 shows decolourization of dye with time at different temperatures (20, 30, 35 and $45^0$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 $30^0$ C. The percentage removal of dye decreased with further increase in temperature upto $45^0$C. Decolourizing activity was significantly suppressed at $45^0$ C, this might be due to the loss of cell viability or deactivation of the enzymes responsible for decolourization at $45^0$C [21].

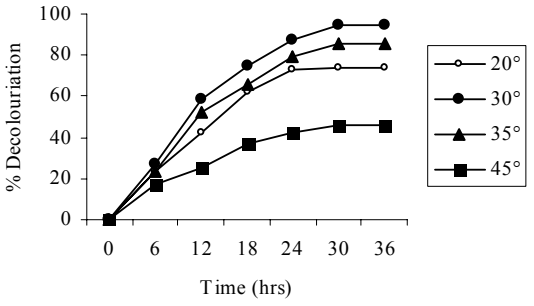


Fig. 4 Effect of temperature on decolourization with time

### *3.4 Effect of glucose concentration*

Figure 5 shows the effect of glucose concentration (0.10 – 2%) on decolourization with time performed at 100 ppm initial concentration of dye, 10 % inoculum concentration and at $30^0$ 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 upto 2% resulted in decreased removal upto 57%. However, the lower concentration of glucose also inhibited the decolourizing activity of mixed culture. The reason for low decolourization 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 decolourization of Reamzol Black B dye.

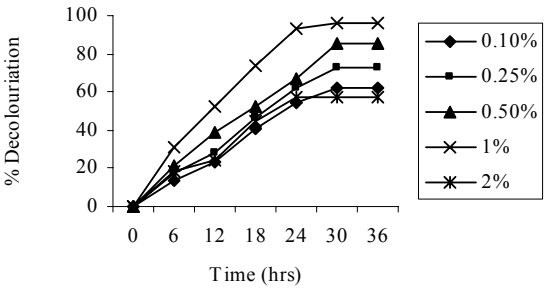


Fig. 5 Effect of glucose concentration on decolourization with time

### *3.5 Effect of initial concentration of dye*

Figure 6 shows the effect of initial concentration of dye ranging from 25–300 ppm of dye at pH 7, 10 % inoculum concentration and at $30^0$C. 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.

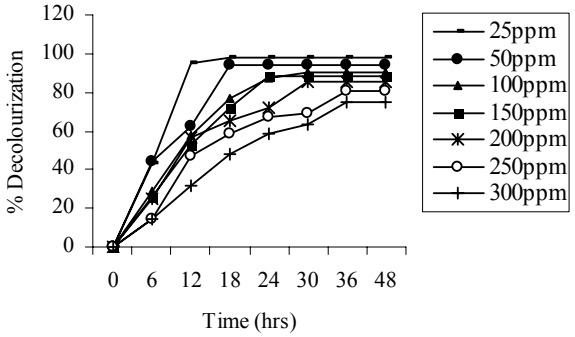


Fig. 6 Effect of initial concentration of dye on decolourization with time

## IV. CONCLUSION

The present study reveals that enriched aerobic mixed culture can be used successfully for decolourizing Remazol Black B dye. The culture exhibited maximum decolourization ability at pH between 7-8 and 30° C .Moreover, 10% (v/v) inoculum and 1 % glucose concentrations were found to be optimum for decolourization. At 25 ppm initial dye concentration a maximum of 98% removal of colour was observed after 18 hours. At higher dye concentration of 300 ppm, the removal in colour 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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