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Original Research Paper

A Comparative Study of Biodegradation of Textile Azo Dyes by *Escherichia coli* and *Pseudomonas putida*

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ABSTRACT

Azo dyes are synthetic in origin having complex aromatic structure and widely used in textile, paper, food, cosmetics and pharmaceutical industries. Most of the azo dyes used in textile industry are xenobiotic compounds and recalcitrant to conventional degradation process. These dyes cause serious environmental problems because of their carcinogenic nature and reduced penetration of light in aquatic environment. In the present study, the efficiency of the two bacterial cultures on degradation of three different toxic azo dyes (acid, direct and reactive azo dyes) were analysed. The bacterial strains used in this study are *Escherichia coli* and *Pseudomonas putida*. To increase the degradation potential, the organisms were acclimatized to the dye environment for sufficient period by gradually decreasing the nutrient broth concentration and increasing the dye concentration so that it can effectively degrade dye rich textile effluent. The effect of concentration of dye, pH, temperature and agitation was studied to determine the optimal conditions for effective decolourisation and degradation. FT-IR and UV spectral analysis were performed to confirm the biodecolourisation.

INTRODUCTION

Textile industry is one of the major important polluting industries in last few decades. More than 60% of the world production of dyes is consumed by textile industries. Nearly 10-20% of dyes are lost during the dyeing process and released as effluent (Tripathi & Srivastava 2011). The non biodegradability of textile wastewater is due to high content of dyestuffs, surfactants and other additives. Several textile processing industries use conventional physico-chemical effluent treatment methods. These methods have some disadvantages such as high cost, formation of hazardous by-products and high energy requirements. Biological treatment is the only hope to overcome the above mentioned problems because of low cost and no harmful end products.

Most of the textile industries use azo dyes because of their attractive colour, ease and cost effectiveness in synthesis compared to natural dyes. Azo dyes are synthetic in origin, and have complex aromatic structure. They are widely used in textile, paper, food, cosmetics and pharmaceutical industries (Saranraj et al. 2010). They are classified as acid, direct and reactive azo dyes based on functional groups. Mostly azo dyes are xenobiotic compounds, very stable in acidic and alkaline conditions and recalcitrant to temperature and light (Baljeet & Poonam 2011). These dyes cause serious environmental problem because of its carcinogenic nature and reduced penetration of light in aquatic environment.

Textile wastewater is highly coloured which mainly block the penetration of sunlight thereby retarding the growth of aquatic animals and plants. It also contains the dissolved toxic substances and carcinogenic compounds. Therefore, it is mandatory to treat textile wastewater prior to its discharge to avoid public health hazards. There are many advanced non-biological methods commonly used to treat coloured textile wastewater, but biodegradation by using microorganisms is an attractive method. It does not produce any end products causing any biomagnification problem (Jadhav et al. 2008). In view of the above problems, the most potent bacterial cultures were selected in this study for maximum degradation of three different carcinogenic azo dyes and the cultures were acclimatized to the high concentration of azo dyes for effective degradation.

MATERIALS AND METHODS

Microorganisms and culture conditions: Pure cultures of *E. coli* and *P. putida* were obtained from KMCH, Erode and stored at 4°C for further studies.

Dyes and chemicals: Three different toxic azo dyes such as acid, direct and reactive azo dyes were used in this study. The dye samples (Acid orange 7, Congo red, Reactive black B) were purchased from Ponkamattchi Dye Chem Pvt. Ltd., Tirupur. All the chemicals used in the study were of analytical grade.

Acclimatization process: Initially, the dye solution was

prepared at a concentration of 3g/L. Then the selected organisms were acclimatized to the dye environment by gradually decreasing the nutrient broth concentration and increasing the dye concentration. So that it can effectively degrade the dye rich textile effluent compared with natural strains and considering the dye as a sole source of nutrient without additional energy supply.

Biodecolourisation analysis: The decolorization and degradation analysis was performed by supernatants obtained after centrifugation at 10,000rpm for 10min. Absorbance of the supernatant withdrawn at different time intervals was measured at maximum wavelength of the dyes (λ max for AO7 = 480nm, λ max for CR = 530nm, λ max for RB5 = 595nm) in the visible region on a UV-Vis spectrophotometer. The percentage of decolorization and degradation was calculated by the following formula.

$$DDP = (C_1 - C_2/C_1) \times 100$$

Where, DDP = Dye degradation percentage

C1 = Initial concentration of dye

C2 = Concentration of dye after reaction at time '*t*'

Concentration studies: Selected azo dyes were prepared in five different concentrations 200, 400, 600, 800 and 1000ppm and then sterilized. To that 24h adapted culture was inoculated in each different dye concentration in order to optimize the dye concentration and time required for effective degradation. The same procedure was repeated for three azo dyes and two cultures.

Optimization of parameters: In an attempt to study the effect of static and shaking condition on dye degradation, the selected, adapted 24h bacterial culture was inoculated in five different concentrations of acidic, direct and reactive azo dyes. To determine the effect of pH on degradation, the fully grown adapted culture was inoculated in conical flasks containing 100 mL optimized dye solution of varying pH 2, 4, 6, 8, 10 and 12. Similarly, the optimum temperature of dye degradation by selected culture was determined by evaluating the dye degradation at 30, 32, 34, 36, 38, & 40°C. After different time intervals 2mL of dye solution was withdrawn and supernatants obtained after centrifugation was used for degradation analysis by UV-Vis spectrophotometer.

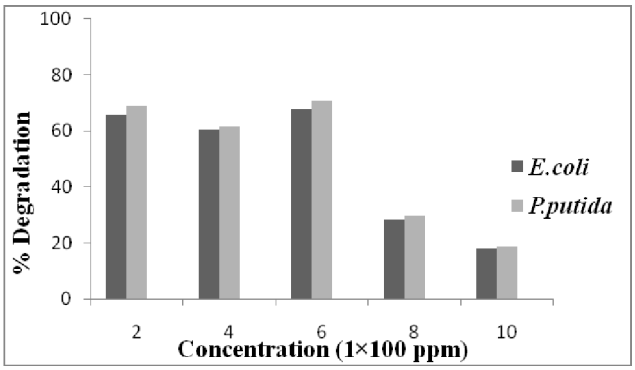

Fig. 1: Percentage degradation of AO7 in various concentration.

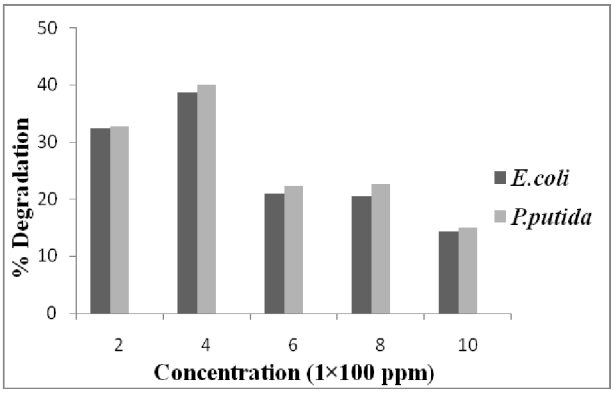

Fig. 2: Percentage degradation of CR in various concentration.

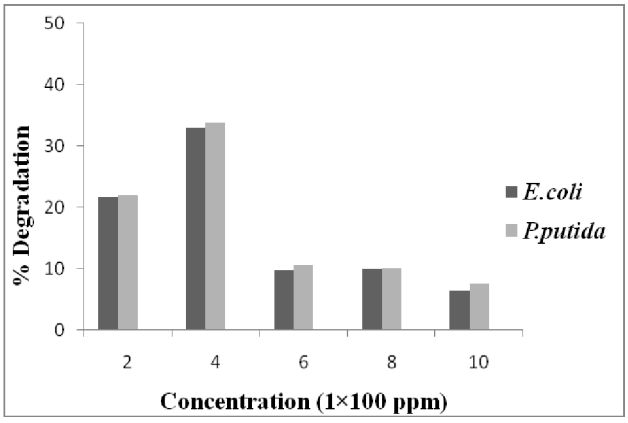

Fig. 3: Percentage degradation of RB5 in various concentration.

RESULTS AND DISCUSSION

The degradation of three different toxic azo dyes was studied in both static and shaking condition using two bacterial cultures *Escherichia coli* and *Pseudomonas putida*. In this study *E. coli* showed maximum degradation in shaking condition and *P. putida* showed effective degradation in static condition. Hence, shaking condition was preferred for *E. coli* and static condition was optimized for *P. putida* culture to investigate bacterial dye degradation in further experiments. Then the concentration of dye, pH and temperature were optimized for the three dye samples and two cultures. Fig. 1 shows the percentage degradation of acid azo dye (Acid Orange 7) in various concentrations. The optimum concentration of AO7 for effective degradation by bacterial cultures is 600ppm. By fixing the optimum concentration, the dye solution was adjusted to various pH 2, 4, 6, 8, 10 and 12. To that adapted bacterial cultures were

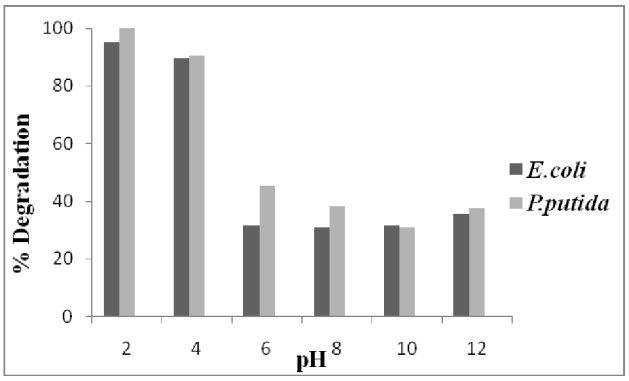

Fig. 4: Percentage degradation of AO7 in various pH.

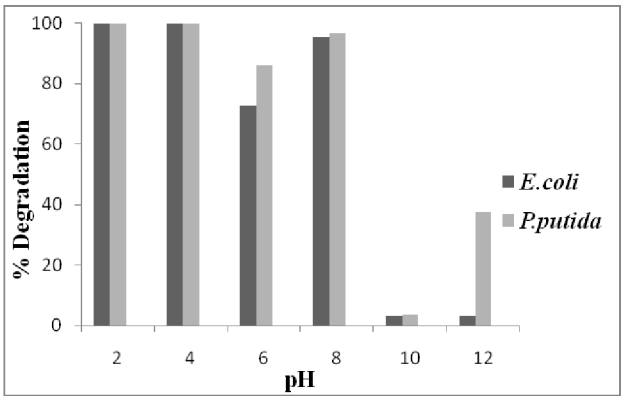

Fig. 5: Percentage degradation of CR in various pH.

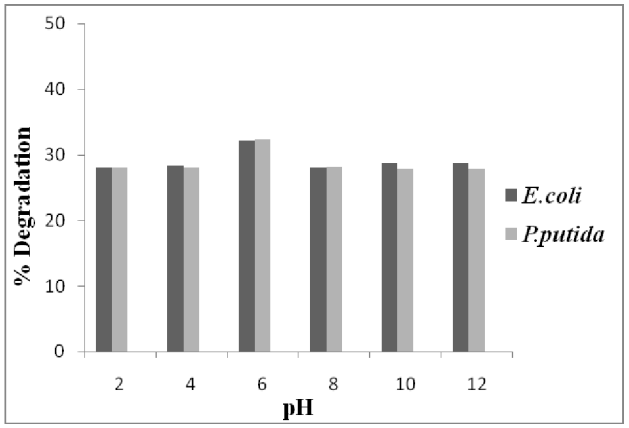

Fig. 6: Percentage degradation of RB5 in various pH.

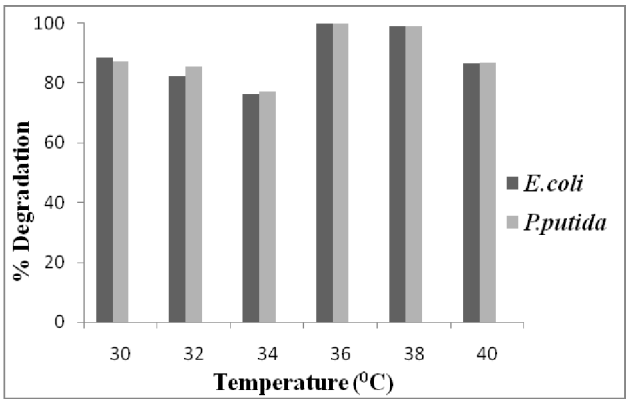

Fig. 7: Percentage degradation of AO7 at various temperatures.

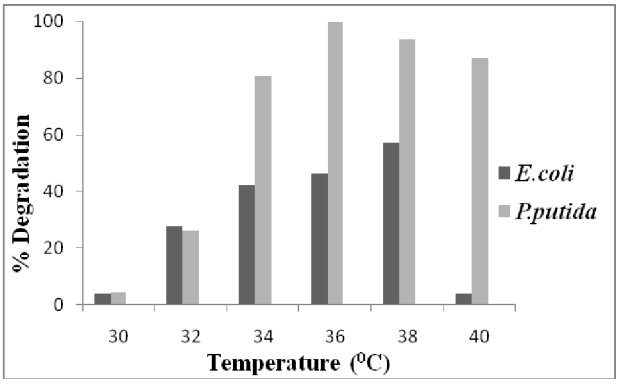

Fig. 8: Percentage degradation of CR in various temperatures.

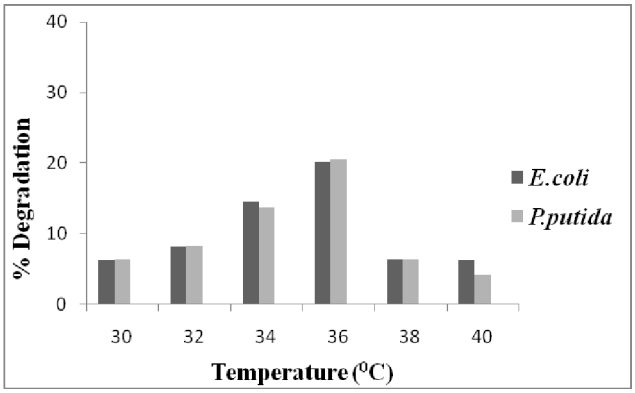

Fig. 9: Percentage degradation of RB5 in various temperatures.

inoculated and percentage degradation was calculated. Fig. 4 shows the optimum pH for maximum degradation of AO7, which was found to be pH 2. By fixing the dye concentration and pH, the dye solution was adjusted to various temperatures 30, 32, 34, 36, 38 and 40°C. Fig. 7 shows the percentage degradation of AO7 under different temperatures and maximum degradation was attained at 36°C. Fig. 2 shows the percentage degradation of direct azo dye (Congo red) in various concentrations and optimum concentration was found to be 400ppm. Then the pH was adjusted and optimized. Fig. 5 displays the degradation of CR in various pH and the maximum degradation was found to be in alkaline conditions, and the result was optimized at pH 8. Moreover, 100% degradation occurred at pH 2, 4 due to acidic environmental conditions. Fig. 8 shows the degradation of CR in various temperatures and the optimum was found to be at 36°C for *P. putida* and 38°C for *E. coli.* Fig. 3 shows the percentage degradation of reactive

diazo dye (Reactive black 5) in various concentrations and the maximum degradation was occurred at 400ppm. By fixing the optimum concentration, the RB5 dye solution was adjusted to various pH and adapted bacterial culture was inoculated for degradation study, and the optimum pH was found to be at pH 6. Fig. 6 shows the percentage degradation of RB5 in various pH solutions. By fixing the dye concentration, pH of the dye solution was adjusted to various temperatures and adapted bacterial culture was inoculated for degradation study and the optimum temperature was found to be at 36°C. Fig. 9 shows the degradation of RB5 in various temperatures. Further, FT-IR and UV spectral analysis was performed, which confirms the biodecolourisation.

CONCLUSION

The results, thus, obtained have characterized and identified dye degrading efficiency of *Escherichia coli* and *Pseudomonas putida*. The ability of the culture to tolerate, decolourise and degrade toxic azo dyes at high concentrations up to 3000ppm gives it an advantage for treatment of textile industry wastewater. In this study *E. coli* culture provides maximum degradation in acid azo dyes, whereas *P. putida* gives effective degradation in direct and reactive azo dyes. The degradation efficiency of the three azo dyes is in the order as follows.

AO7 > CR > RB5

Thus, by this study it is concluded that bacterial cultures of *E. coli* and *P. putida* can effectively degrade the dye rich textile effluent, and it can be used as a good microbial source for wastewater treatment.

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