BIODEGRADATION OF REACTIVE DYE USING MICROORGANISMS

[1]P. Parthiban, [2]V. Jayakumar, [3]Ashutosh Das

[1]Research assistant, [2]M.Tech student, [3]Professor

[1,2,3,4]Centre for Environmental Engineering, PRIST Deemed University, Thanjavur-613 403, Tamilnadu, India

Abstract : In the current era dyes play a vital role in industries. Extensive uses of dyes have led to enormous risks to all species including human beings. Biological treatment of these dyes is one of the most versatile options we have. This work is based on the utilization of microorganisms endowed with degrading abilities for the detoxification of harmful dye. The mixed cultures of Escherichia coli and Bacillus species are compared for their ability to degrade the dye. Experiments were conducted at 2 glucose concentrations (1 and 2 g/l) and three dye concentration (20, 30 and 50 ppm). Results show that dye degradation was most effective at 1 g/l glucose concentration. Although glucose is universally biodegradable compound, there is a complex interaction between different primary substrates and intermediate products resulting in contradicting effects. The results were also compared with immobilized cells of E. coli and Bacillus species. Moreover, there was variation in effect of glucose concentration on the degradation. In both cases, higher degradation was achieved at 2 g/l glucose concentration.

IndexTerms **– Mixed culture, Textile dye, Immobilization, Wastewater**

I. INTRODUCTION

The dyes are universal pollutants and persistent environmental problems due to the colour but also because of toxic chemicals release during the degradation process. Dyes have been treated using various methods such as ozonation, photochemical methods, coagulation, and adsorption and so on (dos Santos et al., 2007). However decolorization is a major problem are difficult to be treated by conventional methods. (Fiegelson et al., 2000; Wiegel et al., 1999; Armenante et al., 1999). Biological degradation is more versatile process having wider reach. Moreover, it is also environmentally amenable process compared to other process. This work is based on the utilization of microorganisms endowed with degrading abilities for the detoxification of harmful dye. The mixed cultures namely E. coli and Bacillus species are compared for their ability to degrade the dye.

II. METHODOLOGY

Congo red dye was obtained from Merck, India. The molecular structure is shown in Figure 1. Stock solution of dye was prepared b dissolving 1000 mg of dye in 1 L of distilled water. The monitoring and analysis work had been classified into two categories namely collection of samples and analysis of data for modelling studies.

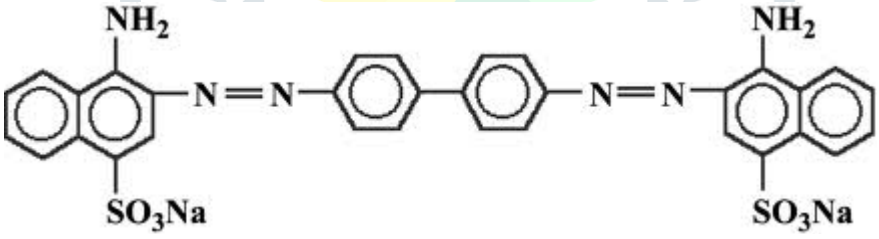

Figure 1 Molecular structure of Congo Red Dye

2.1 Glucose medium composition

The mixed culture was grown in a medium containing glucose and dye as the carbon source. The composition of this medium (1%) is given in Table.1. The concentration of glucose and the dyes were varied according to the experimental requirements; however, the nutrients concentrations were kept constant.

2.2 Analytical Methods

The pH of the synthetic wastewater is fixed using Systronics water analyser 371. The pH was adjusted using 0.1 N HCl and NaOH. The degradation analysis and biomass determination have been measured using Systronics UV – Vis spectrophotometer.

Table 1 Glucose medium composition

Component	(g/L)
Yeast extract	0.340
NH_4Cl	0.840
KH_2PO_4	0.134
K_2HPO_4	0.234
$MgCl_2.6H_2O$	0.084

2.3 Cultures

Cultures are kept in room temperatures with the required nutrient medium at regular intervals of time and are kept in shakers at 150-160 rpm for the good aerobic supply and good mixing of the nutrient medium in all sites of the reactor. Decolorization was studied using several concentrations of glucose at varying pH (2.5, 7 and 10) and at low dye concentration (50 ppm).

2.4 Immobilization

The cell suspension containing sodium alginate is added drop wise into the 0.2 M $CaCl_2$ solution by using filler. This result in formation of fine beads and there by enzyme is immobilized with calcium alginate as a support. Twenty five beads were taken for each experiments.

III. RESULTS AND DISCUSSION

Experiments were conducted for both immobilized as well as suspended cultures. Two different glucose concentrations (1 g/l and 2 g/l) were used to study the impact of glucose. At constant pH, dye initial concentrations were varied and the degradation was observed. Table 2 gives the details of different reactors. Similar set up was also used for *Bacillus sp*. For immobilization experiments, only 2 dye concentrations (10 and 20 ppm) were used.

Table 2 Reactor details for dye removal

Reactor No	Dye initial concentration (PPM)	Inoculum (ml)	1000 ppm dye stock solution (ml)	50 g/l Glucose stock solution	Media (without glucose)	Total volume (ml)
E1	30	5	0.6	0.4	5	20
E2	50	5	1	0.4	5	20
E3	20	5	0.4	0.4	5	20
E6	30	5	0.6	0.8	5	20
E7	50	5	1	0.8	5	20
E8	20	5	0.4	0.8	5	20

3.1 Suspended culture experiments

Microbes interaction as well as dye degradation is influenced by initial concentration. Figure 2 present the effects of initial concentration and glucose on the degradation of dye for *E. coli*. *B. subtilis* data are presented in Figure 3. It can be seen from Figure 2 that removal of dye was almost independent of initial concentration and higher removal was obtained at 1 g/l glucose. The dyes are poor in carbon content and are therefore glucose is a necessary requirement for biological degradation of dye effluents.

However, our results show that lower glucose concentration was favored for dye removal. Similar results were observed for dye removal using *B. subtilis*. Sahinkaya and Dilek (2006) also observed that the presence of biogenic substrates was not beneficial. They noted that increase in glucose concentration, though increasing the biomass, may not efficiently increase the degradation rate. In contrast, Kulkarni and Chaudhari (2006) reported a different phenomena indicating increased degradation of organics in the presence of biogenic substrates.

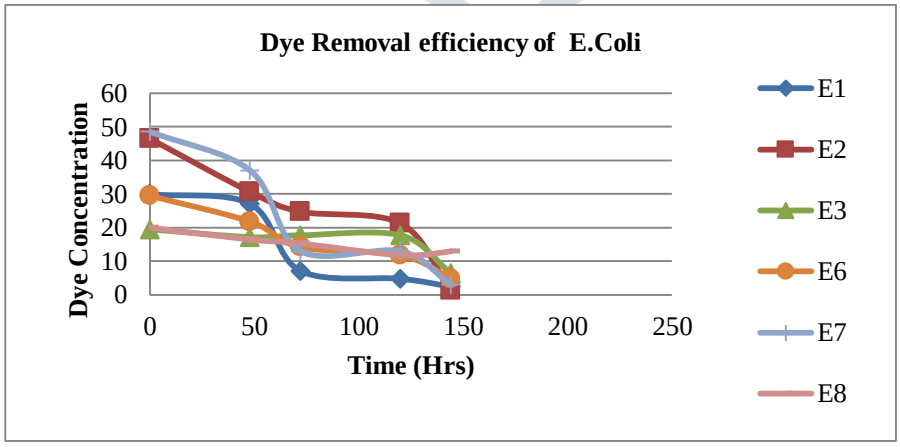

Figure 2. Dye removal using *E coli*

Figure 2 Dye Removal efficiency of E.coli

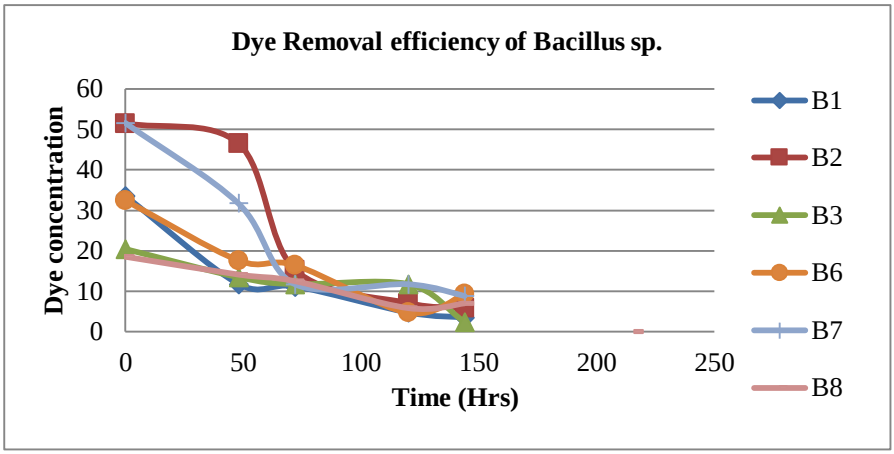

Figure 3 4 Dye Removal efficiency of Bacillus sp.

3.2 Immobilized culture experiments

As mentioned, immobilization experiments were conducted using sodium alginate beads. Results of dye removal data are presented in Figures 4 and 5. The maximum removal was obtained at 2 g/l glucose concentration for 20 ppm initial concentration. It could be that higher dye concentration demands a better microbes growth. Generally immobilization enhances the resistance of bacteria towards the inhibitory effect of dyes. During the fermentation the adsorbed cells built up a layer which covered the gel layer and cells accumulated especially in deeper areas, where they are protected from fluid shear forces and where they could degrade the dyes easily.

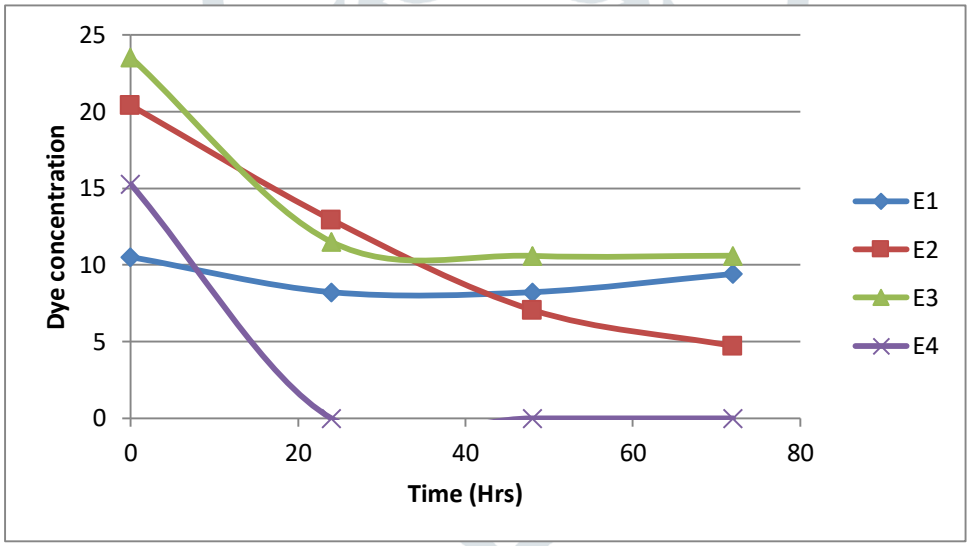

Figure 5 Dye removal using E.coli (immobilized)

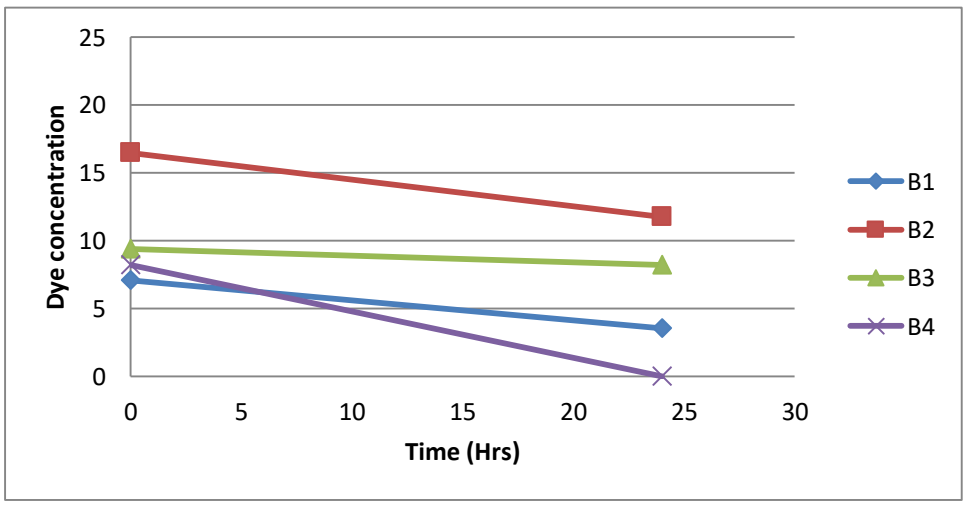

Figure 6 Dye removal using Bacillus sp. (immobilized)

References

[1] Armenante PM, Kafkewitz D, Lewandowski GA, Jou CJL. 1999. Anaerobic-aerobic treatment of halogenated phenolic compounds. Water Res. 33: 681-692.

[2] Dos Santos AB, Cervantes FJ, van Lier JB 2007. Review paper on current technologies for decolourisation of textile wastewaters: Perspectives for anaerobic biotechnology. BioresourceTechnol 98: 2369–2385.

[3] Feigelson L, Muszkat L, Muszkat KA. 2000. Dye photo-enhancement of TiO -photocatalyzed degradation of 2 organic pollutants: the organobromine herbicide bromacil. Water Sci Technol. 42(1-2): 275.

[4] Kulkarni M, Chaudhari A. 2006. Biodegradation of *p*-nitrophenol by *P. putida.* Bioresource Technol. 98: 982-988.

[5] Sahinkaya E, Dilek FB. 2006. Effect of biogenic substrate concentration on the performance of sequencing batch reactor treating 4-CP and 2,4-DCP mixtures. J Hazard Mater. 128: 258–264.

[6] Wiegel J, Zhang X, Wu Q. 1999. Anaerobic dehalogenation of hydroxylated polychlorinated biphenyls by *Desulfito bacterium dehalogens.* Applied and Environmental Microbiology. 65: 2217-2221.