Nature Environment and Pollution Technology © Technoscience Publications	Vol. 5	No. 2	pp. 239-242	2006

DECOLORIZATION OF SYNTHETIC AZO DYES AND DYE INDUSTRY EFFLUENT BY A WHITE ROT FUNGUS *PHANEROCHAETE CHRYSOSPORIUM* RP 78

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ABSTRACT

The white rot fungus, *Phanerochaete chrysosporium* RP 78 was obtained from Forest Products Laboratory, US Department of Agriculture, USA. The fungus was maintained on 2% malt extract medium and tested for decolorization of azo dyes such as evans blue (50μM), amido black 10B (25μM) and orange G (50μM). Decolorization using the fungus was 90.34%, 89.19% and 11.3% for evans blue, amido black 10B and orange G respectively. The effluents in dye industries were treated with the fungus in batch mode and continuous modes. The fungus in batch and continuous modes treated the dye industry effluent. A maximum decolorization of 70% was achieved on the 4th day in the batch mode, and a maximum decolorization of 48% was obtained on the 6th day in the continuous mode. These results suggest that the batch mode of treatment using *Phanerochaete chrysosporium* RP 78 may be more effective than the other mode for color removal.

INTRODUCTION

Dyes are colored substances used in food, cosmetics, paper, plastic and textile industries among others. They are retained on the substrates by physical adsorption, by making compounds with metals and salts, by mechanical retaining and solution or by making covalent bonds. About 10,000 different dyes have an assured future. Approximately a half of all known dyes are azo dyes, making them the largest group of synthetic colorants (Zollinger 1987). Azo dyes and their pigments are versatile and the most common synthetic colorants released in the environment. It has been reported that 90% of the reactive textile dyes, entering activated sludge sewage treatment plants, pass through unchanged and are discharged into rivers (Pierce 1994). Effluents are either treated by a biological process at the industries or treated together with the domestic wastewater in the municipal water treatment plant. The treatment of wastewater containing dyes and its discolorizaton involves serious problems. Among low cost viable alternatives available for effluent treatment and decolorization, the biological systems seem to be the best ones. Nowadays, biological systems are recognized by their capacity to reduce biochemical oxygen demand and chemical oxygen demand. By conventional aerobic biodegradation, microbial decolorization has been proposed as a less expensive and less environmentally intrusive alternative. Fungal decolorization of azo dyes has been reported by Bumpus et al. (1988), Cripps et al. (1990) Raffi et al. (1990) and Paszezynski et al. (1991).

The traditional textile finishing industry consumes about 100L of water to process about 1kg of textile materials. Young & Yu (1997) suggested binding of dyes to the fungal hyphae, physical adsorption and enzymatic degradation by extracellular and intracellular enzymes as reasons for the color removal. The saturated mycelium can be regenerated and used for repeated dye adsorption. The sequential adsorption and degradation dye rnolecules on living fungal hyphae may provide a

mechanism for feasible application of white rot fungi in a continuous treatment of industrial effluent (Wang & Yu 1998). In the present study, the white rot fungus, *Phanerochaete chrysosporium* RP78 was accessed for its potential to remove azo dyes from aqueous solutions and for decolorization of textile dye industry effluent.

MATERIALS AND METHODS

Maintenance of culture: The fungus *Phanerochaete chrysosporium* RP78 was obtained from Forest Product Laboratory, US Department of Agriculture and maintained on 2% malt extract medium (Malt extract 2g, agar 2g, in 100 mL of distilled water) as described by Watling (1971). The fungus was subcultured after 6 days at 37°C and maintained on malt agar slants. The spores were harvested and filter sterilized. The concentration of the spores was adjusted to 10^5 spores/mL and used as inoculam for further studies.

Decolorization of azo dyes: To study the ability of the fungus on azo dyes and removal from aqueous solution, C-limited medium containing evans blue (50μM), amido black 10B (25μM) and orange G (50μM) were inoculated with spore suspension of P. *chrysosporium* RP78 and incubated on a rotary shaker (120 rev/min) at 39°C for 6 days. Alter 6 days the samples were withdrawn and filtered through a G3 sintered glass filter. The optical densities of the filtrate were measured at 611, 618 and 479nm respectively for evans blue, amido black 10B and orange G in a spectrophotometer (Shimadzu TCC 240).

Decolorization of dye industry effluent: The ability of the fungus to decolorize dye industry effluent was assessed in batch and continuous mode in modified C-limited medium (Janshekar & Fiechter 1988). The medium was modified by taking dye industry effluent instead of distilled water for medium preparation. The pH of the solution was adjusted to 4.5. To this effluent medium (950mL), 50mL of spore suspension (10^5 spores/mL) was added and incubated 39°C. In batch mode study, the treatment was given for a specific period, and at the end of the treatment period samples were analyzed for color reduction. While in continuous mode of study, the process was continued for a week but the samples were withdrawn at regular time intervals. The color intensity of the samples was measured at 490nm (Adsorption maximum of the dye industry effluent) in a spectrophotometer.

RESULTS AND DISCUSSION

Synthetic azo dyes decolorization efficiency of the *Phanerochate chrysosporium* RP78 fungus is shown in Table 1. The dyes are aromatic compounds and the ligninolytic fungi degrade these dyes during secondary metabolism. The dye industry effluents were decolorized by white rot fungus in batch and continuous flow mode. The white rot fungi are the only microorganisms known to be capable of complete mineralization of lignocellulosic polymer. Cripps et al. (1990) reported that *P. chrysosporium* could remove 87-93% of orange II, tropedin O and congo red within 5 days. The result showed that *P. chrysosporium* has the potential to decolorize some azo dyes such as congo red or amido black 10B. Heinfling et al. (1997) reported that *Bierkendra adusta* and *T. versicolor* removed 95% of HRB 38 dye within 4 days. Selvam et al. (2003) reported that *Thelephora* sp. removed congo red 97% and amido black 10B 71% within 8 h. *Fomes lividus* was able to decolorize 30.8% of orange G within 9 days whereas congo red was removed up to 74% within 8 h and amido black 10B up to 98.9% in 6 h. In the present study it was found that *P. chrysosprium* was able to decolorize 90.34% Evan's blue within 6 days, whereas amido black 10B was removed up to 89.19%, but the removal of orange G was up to 11.3% within 3 days. Spadaro et al. (1992) established that *P. chrysosprium* was capable of

Table 1: Decolorization of azo dyes by *P. chrysosporium* RP78 liquid medium.

Incubation Period (Days)	Mycelial growth (mg/L)	% color removal
Evans blue (50 μM)		
1	74 ± 2.1	34.63 ± 0.8
2	78 ± 3.1	47.6 ± 0.7
3	78 ± 4.2	61.86 ± 0.5
4	79 ± 2.3	73.08 ± 0.6
5	80 ± 3.2	80.5 ± 0.7
6	82 ± 2.4	90.34 ± 0.8
Amido black 10B (25μM)		
1	73.5 ± 3.3	55.38 ± 0.5
2	76.0 ± 4.1	74.35 ± 0.7
3	77.6 ± 4.3	79.11 ± 0.8
4	80.1 ± 3.5	82.24 ± 0.7
5	80.5 ± 3.6	87.43 ± 0.9
6	81.1 ± 2.1	89.19 ± 0.7
Orange G (50μM)		
1	68.3 ± 3.1	6.8 ± 0.7
2	67.0 ± 4.1	8.3 ± 0.9
3	69.5 ± 2.6	11.3 ± 0.8
4	70.0 ± 3.6	10.2 ± 0.7
5	68 ± 4.1	9.2 ± 0.6
6	67 ± 3.5	8.4 ± 0.7

Values are mean of three replicates and ± standard deviation.

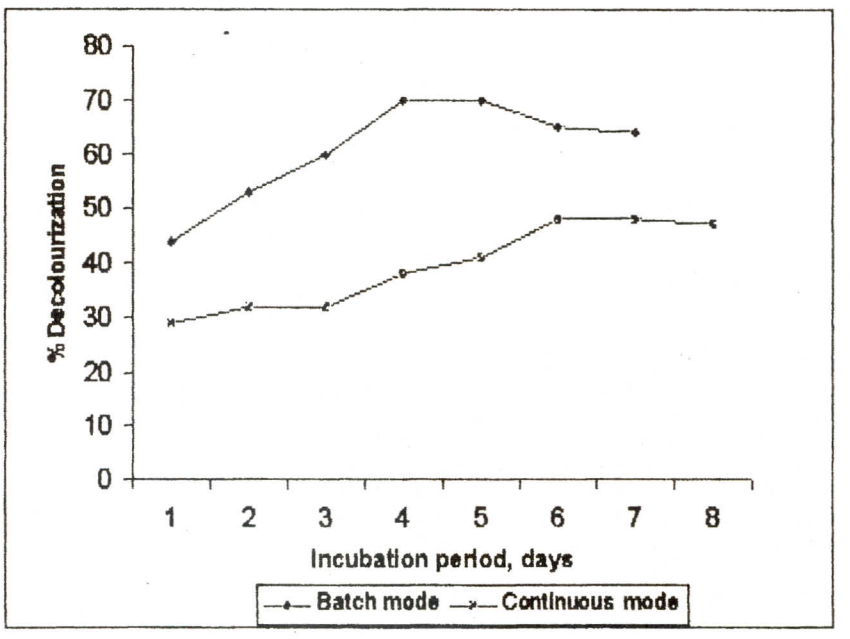

Fig. 1: Decolorization of the dye industry effluent by culture of Phanerochaete chrysosrium.

mineralizing a variety of toxic azo dyes and the mineralization of aromatic rings of azo dyes was dependent on the nature of the ring substituents.

The present study involved the treatment of a dye industry effluent by *P. chrysosporium* RP78 in two modes. In batch mode, the color removal was maximum of about 70% within 5 days of incubation, whereas in a continuous mode it was about 48% in 7 days (Fig 1). Wang & Yu (1998) reported that adsorption and degradation of dye molecules on living fungal hyphae might provide a mechanism for feasible application of white rot fungi in a continuous mode treatment of dye industrial effluent. Olikka et al. (1993) showed 54% decolorization of Congo red in presence of crude preparation of lignin peroxidase and hydrogen peroxidase as these enzymes utilize Congo red as a substrate. Rodrignez et al. (1999) reported that several industrial dyes were decolorized biocatatlytically by extracellular enzymes. Mycelium of this fungus was able to decolorize the azo dye and dye industry effluents. Selvam et al. (2003) reported that white rot fungi *Fomes lividus* and *Thelephora* sp. remove color in dye industry effluent in batch mode up to 84.4% and 61% and in continuous mode up to 37.5% and 50%. The purpose of the study was to investigate the possibilities of facilitating microbial decolorization of azo dyes and textile dye industry effluents. The results indicate that this is possible and potential application of the fungus in bioremediation of industrial effluents contamining azo dyes and other toxic compounds can be made.

ACKNOWLEDGMENT

The authors thank the Management and Principal, Dr. NGP Arts and Science College, Coimbatore for their encouragement and constant support for the research work.

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