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Microbial decolorization of azo dyes and dye industry effluent by *Fomes lividus*

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Summary

The white rot fungus, *Fomes lividus*, was isolated from the logs of *Shorea robusta* in the Western Ghats region of Tamil Nadu, India. The fungus was tested for decolorization of azo dyes such as orange G (50 μM) congo red (50 μM) amido black 10B (25 μM) and also for colour removal from dye industry effluents. The results revealed that the fungus could remove only 30.8% of orange G in the synthetic solution, whereas congo red and amido black 10B were removed by 74.9 and 98.9% respectively. A dye industry effluent was treated by the fungus in batch and continuous mode. In batch mode treatment, a maximum decolorization of 84.4% was achieved on day 4, and in continuous mode a maximum decolorization of 37.5% was obtained on day 5. The colour removal by the basidiomycete fungus might be due to adsorption of the dyes to the mycelial surface and metabolic breakdown. These results suggested that the batch mode treatment of *Fomes lividus* is one of the most efficient ways for colour removal in dye industry effluents.

Introduction

Approximately a half of all known dyes are azo dyes, making them the largest group of synthetic colorants. Azo dyes and their pigments are versatile and the most common synthetic colorants released into the environment. Approximately 10,000 different dyes and pigments are used in industries and over 7×10^5 tons of these dyes are annually produced worldwide (Zollinger 1987). Synthetic dyes are extensively used for textile dyeing, paper printing, colour photography and as additives in petroleum products. It has been reported that 90% of reactive textile dyes entering activated sludge sewage treatment plants pass through unchanged and are discharged into rivers (Pierce 1994). Not all dyes currently used could be degraded and/or removed by physical and chemical processes, and sometimes the degradation products are more toxic (Spadaro *et al.* 1994).

Microbial decolorization has been proposed as a less expensive and less environmentally intrusive alternative. Glenn & Gold (1983) first demonstrated that ligninolytic cultures of *Phanerochaete chrysosporium* decolorized several polymeric dyes. Various fungi have decolorizing abilities and an extensive review of microbiological decolorization has been made. Decolorization of the azo dyes orange II, tropeolin O, congo red, acid red 114, acid red 88, biebrich scarlet, direct blue 15, chrysophenine, tetrazine, and yellow 9 and the triphenylmethane dyes, basic green 4, crystal violet, brilliant green, cresol red, bromophenol blue and para rosanilines by various fungi has been reported (Cripps *et al.* 1990; Rafii 1990; Paszczynski *et al.* 1991). The degradation of azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes by *P. chrysosporium* has been extensively studied (Cripps *et al.* 1990; Kling & Neto 1991; Ollikka *et al.* 1993). *Trametes versicolor*, *Bjerkandera adusta* and *Phanerochaete chrysosporium* were able to decolorize commercially used reactive textile dyes, reactive orange 96, reactive violet 5 and reactive black 5 and two phthalocyanine dyes, reactive blue 15, and reactive blue 38 (Heinfling *et al.* 1997). Matthew & Bumpus (1998) observed the degradation of congo red by *Phanerochaete chrysosporium* in agitated liquid cultures.

The traditional textile finishing industry consumes about 100 l of water to process about 1 kg of textile materials. New closed-loop technologies such as the reuse of microbially or enzymatically treated dyeing effluents could help to reduce this enormous water consumption (Abadulla *et al.* 2000). Knapp *et al.* (1995) observed that in many cases adsorption of dye to the microbial cell surface is the primary mechanism of decolorization. Enzymes such as lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase all of which are involved in lignin degradation, participate in the decolorization of the dyes (Vyas & Molitoris

1995). Young & Yu (1997) suggested the binding of dyes to the fungal hyphae, physical adsorption and enzymatic degradation by extracellular and intracellular enzymes as reasons for the colour removal. The dye-saturated mycelium can be regenerated and used for repeated dye adsorption. The sequential adsorption and degradation of dye molecules 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, a white rot fungus, *Fomes lividus*, was isolated from the Western Ghats of South India and assessed for its potential to remove azo dyes from aqueous solutions and for decolorization of textile dye industry effluent.

Materials and methods

Culture and media

The fungus, *Fomes lividus* was isolated from *Shorea robusta* logs and maintained on 2% malt agar medium. The fungus was identified based on the keys provided previously (Bakshi 1971; Gilbertson & Ryvarden 1986). Fungal growth was cut out, sterilized with 1% mercuric chloride solution, repeatedly washed with sterile distilled water and incubated on 2% malt agar plates (malt extract 2 g and agar 2 g in 100 ml distilled water) as described by Watling (1971). The fungal growth on the plate was sub-cultured after 6 days at 37 °C and maintained on malt agar slants. Then, the spores were harvested without disturbing the mycelial growth using a camel hair brush and filter-sterilized. The spore concentration was adjusted to 10^5 spores/ml and used as inoculum for further studies. Growth kinetics and dye decolorization studies were carried out in C-limited medium (M14) of Janshekar & Fiechter (1988), to which spores in the one-tenth volume of the medium were inoculated.

Decolorization of azo dyes

To study the ability of the fungus to remove the azo dyes from the aqueous solution, C-limited medium containing orange G (50 μM), congo red (50 μM) and amido black 10B (25 μM) were inoculated with spore suspension of *F. lividus* and incubated in a rotary shaker (120 rev/min) at 39 °C for 6 days. After 6 days, the samples were withdrawn at regular time intervals and filtered through a G3 sintered glass filter. The optical density of the clear filtrate was measured at 479, 497 and 618 nm, respectively, for orange G, congo red and amido black 10B 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 (950 ml), 50 ml of spore suspension (10^5 spores/ml) was added and incubated at 39 °C. In batch mode study, the treatment was given for a specific period and at the end of the treatment period samples were analysed for colour reduction. In continuous mode, the treatment process was continued for a week but the samples were withdrawn at regular time intervals. The colour intensity of the samples was measured at 490 nm (the adsorption maximum of the dye industry effluent) in a spectrophotometer (Shimadzu, TCC 240). The untreated raw effluent served as control.

Results and discussion

The dyes are aromatic compounds and the ligninolytic fungi degrade these dyes during secondary metabolism. Cripps *et al.* (1990) reported that *P. chrysosporium* could remove 87–93% of orange 11, tropeolin O and congo red within 5 days. The growth and degradation efficiency of the test fungus is shown in Table 1. It was observed that *Fomes lividus* was able to decolorize only 30.8% of orange G within 9 days, whereas congo red was removed upto 74.0% within 8 h and amido black 10B upto 98.9% in 6 h. White rot fungi are the only microorganisms known to be capable of complete mineralization of lignocellulosic polymers. Spadaro *et al.* (1992) established that *P. chrysosporium* was capable of 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. Heinfling *et al.* (1997) reported that *Bjerkandera adusta* and *T. versicolor* removed 95% of HRB 8 dye within 4 days. When compared with previous results, as men-

Table 1. Decolorization of azo dyes by *Fomes lividus* in liquid medium.

Incubation period	Mycelial growth (mg/l)	Colour removal (%)	Dye removal /mg mycelium
[a]Orange G (50 μM)			
3	50 ± 1.1	12.1 ± 1.7	2.7
6	54 ± 2.4	20.50 ± 1.8	4.0
9	56 ± 1.1	30.8 ± 1.6	5.8
[b]Congo red (50 μM)			
2	50 ± 3.0	71.7 ± 1.7	25.1
4	50 ± 4.1	73.4 ± 1.5	25.7
6	49 ± 5.4	74.4 ± 2.3	25.9
8	45 ± 3.3	74.0 ± 1.2	25.9
[b]Amido black 10B (25 μM)			
2	53 ± 3.2	85.5 ± 1.3	32.7
4	54 ± 3.4	92.7 ± 1.2	34.0
6	51 ± 3.1	98.9 ± 1.6	36.3

Values are mean of three replicates and ± standard deviation.
[a] Incubation period in days.
[b] Incubation period in hours.

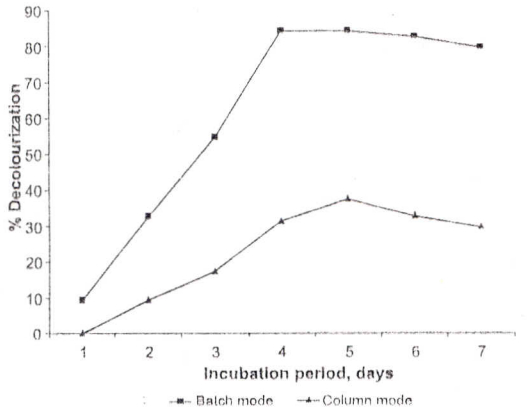

Figure 1. Decolorization of dye industry effluent by culture of *Fomes lividus*.

tioned above, the newly isolated *Fomes lividus* has a superior potential to decolorize some azo dyes such as congo red or amido black 10B.

The study on the treatment of dye industry effluent by mycelia of *F. lividus* showed that in batch mode 84.4% of colour was removed from the effluent on day 4 of incubation whereas in a continuous mode only 37.5% at maximum on day 5 (Figure 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 treatment of industrial effluent. Ollikka *et al.* (1993) showed 54% decolorization of congo red in the presence of crude preparation of lignin peroxidases and hydrogen peroxide as these enzymes utilize congo red as a substrate. Vyas & Molitoris (1995) reported that lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase, all of which are involved in lignin degradation, participate in the decolorization of the dyes. Rodriguez *et al.* (1999) reported that several industrial dyes were decolorized biocatalytically by extracellular enzymes. This is the first report on azo dye degradation and effluent colour removal by a *Fomes lividus*. Mycelium of this fungus was able to decolorize the azo dyes and dye industry effluents. The purpose of this study was to investigate the possibilities of facilitating microbial decolorization of azo dyes and textile dye industry effluents. The results indicate that this is a possible and potential application in the bioremediation of industrial effluents contaminated with azo dyes and other toxic compounds.

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