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Biological treatment of a pulp and paper industry effluent by *Fomes lividus* and *Trametes versicolor*

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Summary

White rot fungi *Fomes lividus* and *Trametes versicolor*, isolated from the Western Ghats region of Tamil Nadu, India, were used to treat pulp and paper industry effluents on a laboratory scale and in a pilot scale. On the laboratory scale a maximum decolourization of 63.9% was achieved by *T. versicolor* on the fourth day. Inorganic chloride at a concentration of 765 mg/l, which corresponded to 227% of that in the untreated effluent, was liberated by *F. lividus* on the 10th day. The chemical oxygen demand (COD) was also reduced to 1984 mg/l (59.3%) by each of the two fungi. On the pilot scale, a maximum decolourization of 68% was obtained with the 6-day incubation by *T. versicolor*, inorganic chloride 475 mg/l (103%) was liberated on the seventh day by *T. versicolor*, and the COD was reduced to 1984 mg/l corresponding to 59.32% by *F. lividus*. These results suggested that *F. lividus* seems to be another candidate efficient for dechlorination of wastewater.

Introduction

The pulp and paper industry is one of the major industries in India causing water pollution. The manufacture of paper yields a significant quantity of wastewater. It is estimated that 273–450 m^3 of water is required to produce 1 ton of paper and about 60–300 m^3 of wastewater is discharged (Subramanium 1976; Thompson *et al.* 2001). The discharge of an untreated effluent from the industry into water bodies causes poor water quality and the colour from an untreated effluent is detectable over long distances. The pulp and paper mill effluent is highly coloured, imparting a black/brown colour to receiving water bodies. The effluent colour may increase water temperature and decrease photosynthesis, both of which probably lead to a decreased concentration of dissolved oxygen (Kingstad & Lindstrom 1984). The wastewater colour is primarily due to lignin and its derivatives, which are discharged in the effluents mainly from the pulping, bleaching and chemical recovery stages of the plant.

White rot fungi can degrade lignin and its derivatives and therefore have potentials in the lignin/phenolic wastewater treatment (Eaton *et al.* 1980; Eriksson *et al.* 1980). They have proved role ideal organisms for decolourization as well as for the reduction of adsorbable organic halides (AOX) and the chemical oxygen demand (COD). *Trametes versicolor* is one of the white rot fungi known to decolourize kraft mill effluents from sulphate pulping (Livernoche *et al.* 1981, 1983). Colours in such effluents can be removed with mycelial pellets or calcium alginate-immobilized mycelium in batch cultures or in a continuous process (Livernoche *et al.* 1981; Royer *et al.* 1983; Archibald *et al.* 1990). The maximum colour removal of baggasse-based paper mill effluent has been achieved by *T. versicolor* (Modi *et al.* 1998). Another white rot fungus, *Phanerochaete chrysosporium*, produces isoenzymes, including lignin peroxidases and Mn-dependent peroxidases (MnPs) which are capable of degrading not only lignin, but also chlorinated lignins found in pulp-bleaching effluents (Kirk *et al.* 1986; Lankinen *et al.* 1990). *Phanerochaete chrysosporium* mycelium immobilized on the surface of polyurethane foam can be used for the treatment of bleach plant effluents by the trickling filter reactor called the MYCOPOR process (Messner *et al.* 1990). Upon screening of 12 basidiomycetous fungi, the most efficient strains for decolourization of paper mill wastes have been identified as *P. chrysosporium* strains and *P. flavidoalba* strains that produce extracellular ligninases, lignin peroxidase and

MnP in the culture filtrates (Pereze *et al.* 1997). In this work, two newly isolated white rot fungi, *F. lividus* and *T. versicolor*, were examined on a laboratory scale and on a pilot scale to evaluate them for application to the treatment of bleach plant effluent from a large paper mill. In particular, their potentials in decolourization, the reduction of the COD and the increase in the inorganic chloride content were analysed.

Material and methods

Strains and their maintenance

Two white rot fungi *F. lividus* and *T. versicolor* used in this study were isolated from a *Shorea robusta* log and an *Acacia nilotica* log, respectively, collected from the Western Ghats region of Tamil Nadu, India. The fungi were identified based on the keys provided previously (Bakshi 1971; Gilbertson & Ryvarden 1986). Fungi on the logs were cut out, sterilized with 1% mercuric chloride solution, repeatedly washed with sterile distilled water as described previously and cultured on 2% malt agar medium for 6 days at 37 °C (Watling 1971). Spores were harvested using a camel hair brush and filter-sterilized. The spore concentration was adjusted to 10^5 spores/ml and used as an inoculum for further studies.

Effluent source

The effluent from the first extraction of the bleaching sequence was sewage from a paper mill, in Tamil Nadu, India, utilizing eucalyptus as a main raw material, stored at 4 °C and filtered through a 0.5 mm sieve to remove large suspended particles. Production paper involves chemical digestion of wood and allied materials to convert them to pulp and chemical refining of the pulp. For these processes high amounts of alkali and chlorine compounds are used. Hence the wastewater obtained in this process was dark brown in colour with charring wood and chemical odour. Moreover, since chlorine compounds are used, the wastewater contained high amounts of COD and organic chlorides that are carcinogenic.

Treatment of the effluent using a rotating biological contactor

To analyse the efficiency of the wastewater treatment, the above two fungi were grown in media described elsewhere (Pellinen *et al.* 1988). In a rotating biological contactor, (890 ml) was mixed with 10 g of glucose and 60 ml of aqueous nutrient solution containing KH_2PO_4 2 g, $MgSO_4 \cdot 7H_2O$ 5 g, $CaCl_2$ 0.1 g, NH_4Cl 0.116 g, thiamine-HCl 0.001 g. The solution was sterilized and the pH was adjusted to 4.5 with concentrated H_2SO_4. The reactor was inoculated with 50 ml of spore suspension (10^5 spores/ml) and maintained at 39 °C for 4 days; on day 5, the medium was replaced by effluent 820 ml, nutrient solution without NH_4Cl 60 ml, NH_4Cl 35.3 mg, benzyl alcohol 0.84 ml, Tween80 1.0 and 90 ml of mineral solution containing nitrilotriacetic acid 1.5 g, $MnSO_4 \cdot H_2O$ 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.1 g, $CoSO_4$ 0.1 g, $ZnSO_4$ 0.1 g, $CuSO_4 \cdot 5H_2O$ 0.01 g, $AlK(SO_4)_2$ 0.01 g, H_3BO_3 0.01 g, $NaMoO_4$ 0.01 g. The solution pH was adjusted to pH 4.5 with concentration H_2SO_4 and the reactor was maintained at 39 °C and continuously flushed with oxygen. After treatment the mycelia were harvested and their efficiency for reducing the colour, increasing the inorganic chloride content, and reducing the COD were analysed according to the methods reported previously (NCASI 1971; APHA 1976). On the laboratory scale, the activities were measured every other day and on the pilot scale everyday for 7 days.

Results and discussion

To assess the potentials of the two ligninolytic fungi, a pulp and paper mill effluent was treated on two scales, since different scales can show different efficiencies in the treatment. Furthermore, colour, the chloride content and the COD in effluents are regarded as important factors to evaluate the water quality. Therefore, those factors in a pulp and paper effluent were measured during treatment of each of the two ligninolytic fungi, *F. lividus* and *T. versicolor*, on a laboratory scale and on a pilot scale. In the laboratory scale experiments with *F. lividus*, colour was reduced at maximum by 66.7% of that in the untreated effluent by the 4-day incubation. The liberation of inorganic chloride was increased upto 227% (765 mg/l) of that in the untreated effluent during 10 days and the COD was reduced to 1984 mg/l (59.7%) (Figure 1A). In the *T. versicolor* treatment, colour removal was 63.9% by 4-day incubation, the liberation of inorganic chloride was increased upto 197% (695 mg/l) during 10 days and the COD was reduced to 2240 mg/l (67%) (Figure 1B). In a pilot scale, *F. lividus* removed the colour at maximum by 72.8% during 6 days, 57.7% (369 mg/l) of inorganic chloride was liberated on the seventh day and the COD reduction was 1984 mg/l (59.3%) (Figure 1C). *Trametes versicolor* removed the colour by 68.0, 103% of inorganic chloride was liberated and the COD was reduced to 2313 mg/l (69.2%) during 7 days (Figure 1D). These results revealed that the pilot scale experiment is not as efficient as the laboratory scale treatment and that the pilot scale experiment needs to be improved further. In the previous studies, *P. chrysosporium* and *T. versicolor* decolourized a pulp and paper mill effluent by 40–80% (Pellinen *et al.* 1988; Bergbauer *et al.* 1991; Fukui *et al.* 1992; Manzanares *et al.* 1995; Lee *et al.* 1995 a, b; Modi *et al.* 1998). *Phanerochaete chrysosporium* increases inorganic chloride content by 34% (Pellinen *et al.* 1998). The COD of 32–70% was reduced with *P. chrysosporium* and *T. versicolor* (Pellinen *et al.* 1988; Martin & Manzanares 1994).

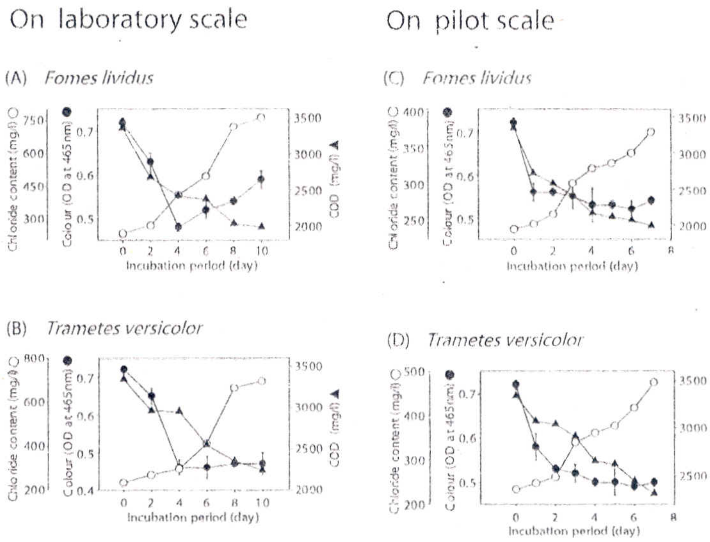

Figure 1. Biological treatment of pulp and paper mill effluent using ligninolytic fungi. Colour (OD at 465 nm) – values decrease over control, chloride content (mg/l) – values increase over control, COD (mg/l) – values decrease over control. Values are mean of three replicates and standard deviation.

Table 1. Comparison of the efficiencies of the treatments.

Treatment	Remaining content after treatment		
	Colour[a] (%)	Chloride mg/l (%)	COD mg/l (%)
None	0.72 (100)	234 (100)	3344 (100)
F. lividus			
Lab scale	0.48 (66.7)	765 (227)	1984 (59.3)
Pilot scale	0.52 (72.2)	369 (57.7)	1984 (59.3)
T. versicolor			
Lab scale	0.46 (63.9)	695 (197.0)	2240 (67.0)
Pilot scale	0.49 (68.0)	475 (103)	2313 (69.2)

Colour – % decrease over control, Chloride content – % increase over control, COD – % decrease over control.
[a] Absorbance at 465 nm.

When compared with previous results, the newly isolated *F. lividus* and *T. versicolor* have a superior potential to dechlorinate lignin and/or lignin derivatives (Table 1). This study is the first to report that *F. lividus* seems to be the best organism for dechlorination of lignin in pulp and paper mill effluent and therefore, using this organism may prove to be a very simple and inexpensive methodology to remove organic chloride in wastewater efficiently.

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