

Treatment of a Pulp and Paper industry effluent by *Daldenia concentrica*, *Lepiota sp.* and *Trametes serialis* – A biological approach

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Abstract-

Wood rot fungi *Daldenia concentrica*, *Lepiota sp.* and *Trametes serialis* were isolated from the Western Ghats region of Tamil Nadu, India. They 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 58.98% was achieved by *Daldenia concentrica* on the Tenth day. Inorganic chloride at a concentration of 772 mg/l, which corresponded to 217% of that in the untreated effluent, was liberated by *Daldenia concentrica* on the Tenth day. The chemical oxygen demand (COD) was also reduced to 1994 mg/l (59.5%) by each of the *Daldenia concentrica*. On the pilot scale, a maximum decolourization of 61.54% was obtained with the Tenth day incubation by *Daldenia concentrica*, inorganic chloride 620 mg/l (155.14%) was liberated on the Tenth day and the COD was reduced to 1915 mg/l corresponding to 57.1% by *Daldenia concentrica*. These results revealed that *Daldenia concentrica* proved to be more efficient for the treatment of pulp and paper industry effluent in lab scale when compared to pilot scale.

Index Terms - COD, Wood rot fungi, pulp and paper industry effluent.

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1 INTRODUCTION

Pulp and paper production is a major industry in India, with a total capacity of over 3 million tonnes per annum (CPCB 2001). In India there are 380 paper industries which produces a variety of different paper, paperboard as well as newsprint products. The pulp and paper industry is one of the major industries in India causing water pollution. It is estimated that 273-450 m³ of water is required to produce 1 ton of paper and about 60-300m³ of wastewater is discharged (Addison et al. 2005). Biological treatment is known to be effective in reducing the organic load and toxic effects of pulp and paper mill effluent (Blair and Davis 1980; Chupal et al. 2005). Significant work has been reported on the problem of colour removal from pulp and paper mill wastes at a global level (Abhay Raj et al. 2009; Manzanares et al. 1995).

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). 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 waste-water.

The effluent colour may increase water temperature and decrease photosynthesis, both of which probably lead to a decreased concentration of dissolved oxygen (Kings-tad & Lindstrom 1984). The waste water 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. *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 mycelia 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). The newly isolated *F.lividus* have a superior potential to dechlorinate lignin and lignin derivatives and *F.lividus* seems to be the best organism for dechlorination of lignin in pulp and paper mill effluent.(Selvam et al. 2002).

In this work, three newly isolated white rot fungi, *Daldenia concentrica*, *Lepiota sp.* and *Trametes serialis* 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.

2 MATERIALS AND METHODS

2.1 Fungal strains and preparation of culture media

Three wood rot fungi *Daldenia concentrica*, *Lepiota sp.* and *Trametes serialis* used in this study were isolated from the decayed wood of 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 10⁵ spores/ml and used as an inoculum for further studies.

2.2 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 waste water contained high amounts of COD and organic chlorides that are carcinogenic.

2.3 Waste water treatment using rotating biological contractor

To analyse the efficiency of the wastewater treatment, the above three fungi were grown in media described elsewhere (Pellinen et al. 1988). In a rotating biological contractor, (890 ml) was mixed with 10 g of glucose and 60 ml of aqueous nutrient solution containing KH_2PO_4 - 2 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 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, Tween 80 1.0 and 90 ml of mineral solution containing nitrilotriacetic acid- 1.5 g, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ - 0.5 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.1 g, CoSO_4 - 0.1 g, ZnSO_4 - 0.1 g, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ - 0.01 g, $\text{AlK}(\text{SO}_4)_2$ - 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 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 10 days.

3 RESULTS

To assess the potentials of the three 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 three ligninolytic fungi, *Daldenia concentrica*, *Lepiota sp.* and *Trametes serialis* on a laboratory scale and on a pilot scale.

In the laboratory scale experiments with *Daldenia concentrica*, colour was reduced at maximum by 58.98% of that in the untreated effluent by the 10-day incubation. The liberation of inorganic chloride was increased upto 117% (772 mg/l) of that in the untreated effluent during 10 days and the COD was reduced to 1994 mg/l (59.05%) (Figure 1 A). In the *Lepiota sp.* treatment, colour removal was 53.85% by 8-day incubation, the liberation of inorganic chloride was increased upto 113% (761 mg/l) during 10 days and the COD was reduced to 1860 mg/l (55.46%) (Figure 1 B). In the laboratory scale experiments with *T.serialis*, colour was

reduced at maximum by 51.29% of that in the untreated effluent by the 10-day incubation. The liberation of inorganic chloride was increased upto 698 mg/l (87.24%) during the 10-day incubation and the COD was reduced to 1880 mg/l (56.06%) at 10-th day of incubation (Figure 1 C).

In a pilot scale, *Daldenia concentrica* removed the colour at maximum by 61.54% during 10 days, 155.14% (620 mg/l) of inorganic chloride was liberated on the Tenth day and the COD reduction was 1915 mg/l (57.1%) (Figure 1 D). *Lepiota sp.* removed the colour by 58.98, 122.22% of inorganic chloride was liberated and the COD was reduced to 2050 mg/l (38.87%) during 10 days (Figure 1 E). *Trametes serialis* removed the colour by 62.83%, 113.99% of inorganic chloride was liberated and the COD was reduced to 2060 mg/l (61.42%) during 10 days (Figure 1 F).

4 DISCUSSION

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. *Pleurotus ostreatus* removed the colour of kraft mill effluent by 69.0% and COD was reduced to 66.9% after fed batch treatment of kraft mill effluent (Choudhury et al. 1998). In the previous studies, *Phanerochaete chrysosporium* and *Trametes 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 *Phanerochaete chrysosporium* and *Trametes versicolor* (Pellinen et al. 1988; Martin & Manzanares 1994).

Pleurotus sajor caju decolourized the paper mill effluent by 66.7% on day 6 of incubation. Inorganic chloride liberated by *Pleurotus sajor caju* was 230.9% and chemical oxygen demand (COD) was reduced by 61.3% on 10 day treatment. In pilot scale treatment maximum decolourization was obtained by *Pleurotus sajor caju* 60.1% on 6 day of incubation. Inorganic chloride was increased by 524.0 mg/l and the COD was reduced by 1442.0mg/l (57.2%) by *Pleurotus sajor caju* on day 7 of incubation (Ragunathan et al. 2004). *Trametes versicolor* on the fourth day of treatment showed a maximum decolourization of 63.9% in laboratory scale, Inorganic chloride at a concentration of 765mg/l, was liberated by *Fomes lividus* on the 10th day. The Chemical oxygen demand was also reduced to 1984mg/l by *Fomes lividus*. On the pilot scale, a maximum decolourization of 68% was obtained with

the 6 day incubation by *Trametes versicolor*, inorganic chloride 475mg/l (103%) was liberated on the 7th day by *Trametes versicolor* and the COD was reduced by 1984 mg/l by *Fomes lividus*.(Savant et al. 2006) *Daedaleopsis sp.* and *Phanerochaete chrysosporium* exhibited the highest ability to decolourize waste water by 52% and 86% respectively, COD was reduced by 59-71% and 66-83%.(Prasongsuk et al. 2009).

The treatment of paper mill effluent in laboratory scale with *Thelephora sp. a*, maximum decolourization of 43.1% was observed on 4th day treatment. Inorganic chloride at the concentration of 751mg/l was liberated 10th day. The chemical oxygen demand was also reduced to 1840 mg/l in laboratory scale. In pilot scale, a maximum decolourization by 23.6% was obtained on 10th day incubation, inorganic chloride 361mg/l was liberated on the 6th day and the chemical oxygen demand was reduced to 2,000mg/l. (Selvam et al. 2006). *Trametes versicolor* reduced biological oxygen demand and chemical oxygen demand of paper mill effluent by 52% and 42% respectively.(Saetang & Babel 2009) A maximum colour removal of 57% and 67% reduction of chemical oxygen demand after 14th day of incubation when treated with the white rot fungus *Pleurotus sajor caju* was reported (Belem et al. 2008). *Ceriopsis subvermispora* could decolourize kraft-bleaching effluent at 90% and also resulted in reduction of COD of up to 45%.(Ghoreishi & Haghghi 2007). In laboratory scale experiments with, *Schizophyllum commune* the colour was reduced at a maximum by 73.9 % when compared with untreated effluent by 6 day incubation. The liberation of inorganic chloride was increased upto 282.0% (539 mg/l) of that in the untreated effluent on the 6th day of treatment and the COD was reduced to 3996mg/l (67.0%). In *Lenzites eximia* the percent of colour removed was at a maximum of 65.0% when compared to untreated effluent by 6 day incubation. The liberation of inorganic chloride was increased upto 534 mg/l (278.1%) when compared with untreated effluent by the 6th day incubation and the COD was reduced to 4317 mg/l (65.0%) (Selvam et al. 2012).

5 CONCLUSION

The newly isolated *Daldenia concertrica*, *Lepiota sp.* and *Trametes serialis* have a superior potential to dechlorinate lignin and/or lignin derivatives (Table 1). This study is to report that *Daldenia concertrica*, *Trametes serialis* and *Lepiota sp.* 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 waste water efficiently. *Daldenia concertrica* was identified

as the ideal organism for the biological treatment of pulp and paper industry effluent.

TABLE 1.
COMPARISON OF THE EFFICIENCIES OF THE TREATMENTS

Treatment	Remaining content after treatment		
	Colour ^a (%)	Chloride mg/l (%)	COD mg/l (%)
None	0.78 (100)	243 (100)	3354 (100)
<i>Daldenia concentrica</i>			
Lab scale	0.46 (58.98)	772 (217)	1994 (59.5)
Pilot scale	0.48 (61.54)	620 (155.1)	1915 (57.1)
<i>Lepiota sp.</i>			
Lab scale	0.42 (53.85)	761 (213.1)	1860 (55.46)
Pilot scale	0.46 (58.98)	540 (122.22)	2050 (38.87)
<i>Trametes serialis</i>			
Lab scale	0.40 (51.29)	698 (187.2)	1880 (56.06)
Pilot scale	0.49 (62.83)	520 (113.99)	2060 (61.42)

Colour - % decrease over control, Chloride content - % increase over control, COD - % decrease over control.

^a Absorbance at 465 nm.

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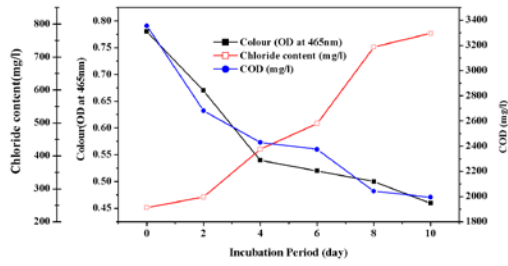
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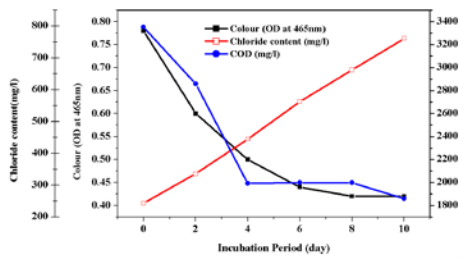
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ON LABORATORY SCALE

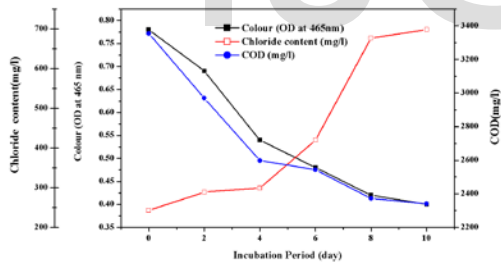
(A) *Daldenia concentrica*



(B) *Lepiota sp.*

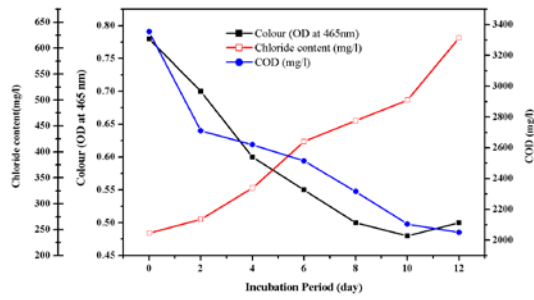


(C) *Trametes serialis*

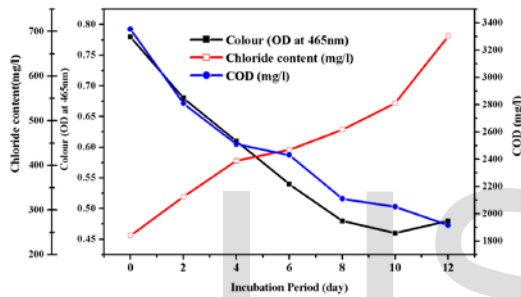


ON PILOT SCALE

(D) *Daldenia concentrica*



(E) *Lepiota sp.*



(F) *Trametes serialis*

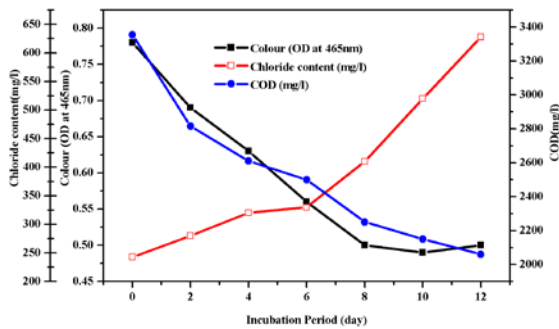


Figure 1: Biological treatment of pulp and paper mill effluent using ligninolytic fungi.

Colour (OD at 465 nm) - values decrease over control, Chloride content (mg/l) – values increase over control, COD (mg/l) – values decrease over control.