

ORIGINAL RESEARCH ARTICLE

Bioremediation of Pulp and Paper Mill Effluent by Newly Isolated Wood Rot Fungi from Western Ghats Area of South India

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ABSTRACT

Fifty six samples were collected from Western Ghats area, of Tamilnadu and Karnataka, South India. The collected fungi were isolated and identified based on the key provided previously. *Phanerochaete chrysosporium*-787 was obtained from Microbial Type Culture Collection, (MTCC) Chandigarh, India and was used as the reference fungus. The fungi were screened for their ligninolytic activity based on their ability to oxidize dyes, poly R-478 and remazol brilliant blue, to degrade native lignin and further confirmation was done by the liberation of ethylene from KTBA (2-keto-4-thiomethyl butyric acid). The color removals in 57 samples were in the range of 11.5 to 38.4% in poly- R dye and 11.1 to 72.0 % in remazol brilliant blue. The mycelial growth rates were in the range of 1.24 to 3.67 mg/day and percent degradation of lignin was found to be in the range 20.4 to 68.8. The ligninolytic activity of the fungi were further confirmed by their ability to release ethylene from KTBA and the results were found to be in the range of 1.210 to 3.121 ppm. From the above screening results three best white rot fungi *Polyporus hirsutus*, *Daedalea flavida*, *Phellinus sp* were selected for bioremediation of pulp and paper mill effluents on laboratory scale and pilot scale. On the laboratory scale a maximum decolourization of 62.2 % was achieved by *Phellinus sp* on 10th day of treatment. Inorganic chloride 820mg/l (189.7 %) was liberated by *Phellinus sp* on the 10th day of treatment.

The chemical oxygen demand (COD) was also reduced to 3010 mg/l (42.1%) by *Phellinus sp*. In pilot scale, a maximum decolourization of 66.2% was achieved by *Polyporus hirsutus* on 10th day, inorganic chloride 582mg/l (105%) was liberated on the 10th day by *Phellinus sp* and the chemical oxygen demand (COD) was reduced to 3260mg/l corresponding to 37.3 % by *polyporus hirsutus*. These results suggest that *Polyporus hirsutus* and *Phellinus sp* are efficient for the treatment of pulp and paper industry effluent.

Key words: Chemical oxygen demand, decolourization, effluent treatment, pulp and paper industry.

INTRODUCTION

The pulp and paper mill generates wastewater with very high biological oxygen demand (BOD), chemical oxygen demand (COD), toxic substances, recalcitrant organics, turbidity, high temperature and intense colour. The colouring body present in the wastewater from pulp and paper mill is organic in nature and is comprised of wood extractives, tannin resins, synthetic dyes, lignin and its degradation products formed by the action of chlorine on lignin^[1]. It is estimated that 273-450m³ of water is required to produce 1 ton of paper and about 60-300 m³ of waste water is discharged^[2]. One of the significant problem is the persistent dark brown color in the released effluent from waste water treatment facilities of

which the major contributors are lignin and its derivatives, such as chlorolignin, discharged from the pulp bleaching process.³ Black liquor which originates from the chemical pulping stage contains lignin, carbohydrates, organic acids, sulfur compounds, phenolic compounds, terpenes and resin.¹ The bleaching agents used in pulp and paper industries are chlorine, alkali, hypochlorite and hydrogen peroxide. The use of chlorine based chemicals in the bleaching process generates chlorophenol compounds which are completely resistant to microbial attack and remain as recalcitrants^[3]. The pollution load in terms of biological oxygen demand (BOD) from small paper mills is 2.5 times higher than that of large paper mills, which employ the soda recovery

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process The release of bleach plant waste into the receiving waters which contains high adsorbable organic halogen (AOX) has become one of the major environmental problems for the pulp and paper industry. Efforts are made to reduce the chloroorganics and chloride discharges by the substitution of chlorine based bleaching chemicals with other environment friendly agents^[4]. In response to environmental concerns and stringent emission standards, modification of the production process at the pulping and bleaching stages include the cooking time for additional lignin removal, introduction of oxygen delignification as a pretreatment step, elemental chlorine free (ECF) and totally chlorine free (TCF) bleaching^[5]. Several physical and chemical processes for colour removal extensively studied include combination of ultrafiltration, reverse osmosis techniques, ion exchange chromatography, lime precipitation, but these processes are expensive and are not considered to be economical^[6]. Conventional treatment processes like chemical pre-treatment and lagooning are not adequate to meet the regulatory effluent standards for being discharged into sewers^[7]. Biological methods of the effluent treatment have the advantage of being cost effective and in addition to colour removal, they can also reduce both the biological oxygen demand (BOD) and chemical oxygen demand (COD) of waste water^[8]. White rot fungi are used for bioremediation processes since these organisms have the ability to degrade a wide range of environmental pollutants^[9,10] reported that white rot fungi have been used for the biological treatment of pulp and paper industry effluent^[11] reported that white rot fungi are the ideal organisms for decolorization, reduction of absorbable organic halides and chemical oxygen demand. The decolorization can be achieved either by adsorption or oxidative degradation by the enzymes. Several strains of white rot fungi have been found to decolorize wood processing wastewater^[12, 13]. developed a MYCOR (Mycelial Colour removal) process for the treatment of spent chlorine bleaching liquor using *Phanerochaete chrysosporium* in rotating biological contractor. The MYCOR process efficiently reduces the amount of toxic low molecular weight in the effluent. *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^[14,15]. reported that

Phanerochaete chrysosporium produces isoenzymes including Lignin peroxidase (LiP), Manganese dependant peroxidase (MnP) which are capable of degrading chlorinated compounds in pulp bleaching effluents^[16] reported the decolourization and dechlorination of pulp and paper industry effluent by *Thelephora sp.* The treatment of effluent from Kraft bleach plant with white rot fungi *Pleurotus sajor caju* and *Pleurotus ostreatus* was also studied^[17,18] and reported the decolourization of pulp mill wastewater using thermotolerant white rot fungus *Daedaleopsis sp.*^[19] reported that purified lignin peroxidase and manganese peroxidase were effective in the decolourization of kraft bleach plant effluent.^[5] reported waste water bioremediation by *Phanerochaete chrysosporium* in pulp and paper industry. *Pleurotus ostreatus* and *pleurotus sajor-caju* are known to decolourize kraft mill effluent and bioconversion of paper mill sludge to useful raw materials.²⁰In the present study three newly isolated fungi *Polyporus hirsutus*, *Daedalea flavida*, *Phellinus sp.* were examined on laboratory scale and pilot scale to evaluate them for the treatment of paper industry effluent. In particular their potentials in decolourization, the reduction of the COD and the increase in the inorganic chloride content were analyzed.

MATERIALS AND METHODS

Collection and Isolation

Fifty six samples of wood rot fungi were collected from decayed wood and living trees from Western Ghats Area of Tamilnadu and Karnataka, South India. The fruit bodies were collected along with supporting wood. The samples were marked with information such as collection number with names, procurement location and date of collection. Fruit bodies were wrapped in Ziploc polythene bags and brought to the laboratory by maintaining aseptic conditions. The collection sight is situated in the latitude of 11.58°S and longitude of 76.93° East at 420 ± 50 M MSL. It receives rainfall of about 300 mm per year with high humidity and even temperature. The fungal growth was cut sterilized with 1% mercuric chloride solution, repeatedly washed with sterile distilled water²¹ and inoculated on 2 % malt agar medium in petriplates. The fungal growth which occurred on the plates were subcultured and maintained in malt agar slants.

Identification and preparation of spore suspension of the isolated fungi

The isolated samples were identified based on the key provided previously^[22,23]. The malt agar

medium was prepared by dissolving 20g of malt extract and 20g of agar in 1000ml of distilled water. The pH was maintained as 4.5. The fungi were grown on malt agar plates for 6 days at 37°C. Then the plates were flooded with sterile distilled water and brushed with camel brush smoothly without disturbing the mycelia growth and filtered through sterile filter. The concentration of the filtrate was adjusted to 10⁵ spores/ml and used as inoculums for further studies.

Screening for ligninolytic activity

The fungi were screened for their ligninolytic activity based on their ability to oxidize dyes, poly R^[24] and remazol brilliant blue^[25], to degrade native lignin^[26] and further confirmation was done by the liberation of ethylene from KTBA (2-keto-4-thiomethyl butyric acid).

Strains selected for treatment of pulp and paper mill effluent

From the above screening results three best white rot fungi *Polyporus hirsutus*, *Daedalea flavida*, *Phellinus sp* were selected. These newly isolated high potential white rot fungi were used for the treatment of pulp and paper industry effluent.

Effluent source

The effluent from the first extraction of the bleaching sequence was sewage from a Tamilnadu News print and papers Limited, (TNPL) karur, Tamilnadu, 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 waste water obtained in this process was dark brown in color with charring wood and chemical odour. Since chlorine compounds are used, the waste water contained high amounts of COD and organic chlorides that are carcinogenic.

Treatment of effluent using a rotating biological contractor

To analyze the efficiency of the waste water treatment, the selected fungi were grown in media by the method²⁷. In a rotating biological contractor, (890ml) was mixed with 10g of glucose and 60 ml of aqueous nutrient solution containing KH₂PO₄ 2g, MgSO₄.7H₂O-5g, CaCl₂-0.1g, NH₄Cl-0.116g, thiamine HCl-0.001g. The solution was sterilized and the pH was adjusted to 4.5 with concentrated H₂SO₄. The reactor was inoculated with 50ml of spore

suspension (105spores/ml) and maintained at 39°C for 4 days. On day 5, the medium was replaced by effluent of 820ml, nutrient solution without NH₄Cl 60 ml, NH₄Cl 35.3mg, benzyl alcohol 0.84ml, tween80 1.0 and 90ml of mineral solution containing nitriloacetic acid 1.5g, MnSO₄.H₂O-0.5g, FeSO₄.7H₂O- 0.1 g, CoSO₄- 0.1 g, ZnSO₄-0.1 g, CuSO₄.5H₂O- 0.01 g, AlK(SO₄)₂- 0.01 g, H₃BO₃- 0.01 g, NaMoO₄- 0.01 g. The pH of the solution was adjusted to 4.5 with concentrated H₂SO₄ 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^[28]. Samples were withdrawn at regular intervals on laboratory scale and pilot scale for 10 days.

RESULTS AND DISCUSSION

The color removals in 57 samples were in the range of 11.5 to 38.4% in poly- R dye and 11.1 to 72.0 % in remazol brilliant blue. The mycelial growth rates were in the range of 1.24 to 3.67 mg/day and percent degradation of lignin was found to be in the range 20.4 to 68.8. The ligninolytic activity of the fungi were further confirmed by their ability to release ethylene from KTBA and the results were found to be in the range of 1.210 to 3.121 ppm. From the above screening results based on the ligninolytic activity of the fungi three best white rot fungi *Polyporus hirsutus*, *Daedalea flavida*, *Phellinus sp* were selected. These newly isolated high potential white rot fungi were used for the treatment of pulp and paper industry effluent. The potential of three white rot fungi namely *Polyporus hirsutus*, *Daedalea flavida*, *Phellinus sp*, were assessed when the paper mill effluent was treated with these selected fungi on two scales namely laboratory scale and pilot scale. Since different scales can show different efficiencies in the treatment. The color, the chloride content and the COD in effluents are regarded as important factors to evaluate its quality. To determine these factors, the pulp and paper industry effluent was treated with three white rot fungi *Polyporus hirsutus*, *Daedalea flavida*, *Phellinus sp*, on laboratory scale and pilot scale. In laboratory scale experiments with, *Polyporus hirsutus* the colour was reduced at a maximum by 63.6% of untreated effluent by 10 days incubation. The liberation of inorganic chloride was increased upto 165.0% (750mg/l) of that in the untreated effluent by the

10 day incubation and the COD was reduced to 2960 mg/l (43.0%). In *Daedalea flavida* the percentage of color removed was at a maximum by 63.6% of untreated effluent by 10 days incubation. The liberation of inorganic chloride was increased upto 784mg/l (177.0) of that in the untreated effluent by the 10 day incubation and the COD was reduced to 2980mg/l (42.6). In *Phellinus sp* the color was reduced at a maximum by 62.2% of untreated effluent by 10 days incubation. The liberation of inorganic chloride was increased upto 820mg/l (189.7) of that in the untreated effluent by the 10 day incubation and the COD was reduced to 3010mg/l(42.1) (Fig 1:a,b,c). In pilot scale treatment with *Polyporus hirsutus* the colour was reduced at a maximum by 66.2% of untreated effluent by 10 days incubation. The liberation of inorganic chloride was increased upto 535mg/l (89.0) of that in the untreated effluent by the 10 day incubation and the COD was reduced to 3110mg/l (40.1). In *Daedalea flavida* the color was removed at a maximum by 67.57% of untreated effluent by 10 days incubation. The liberation of inorganic chloride was increased upto 570mg/l (101.4) of that in the untreated effluent by the 10 day incubation and the COD was reduced to 3210 mg/l(38.2). In *Phellinus sp* maximum colour removal was by 68.92% when compared to that of untreated effluent by 10 days incubation. The liberation of inorganic chloride was increased up to 582 mg/l (105.7) of that in the untreated effluent by the 10 day incubation and the COD was reduced to 3260mg/l(37.3) (Fig 2:a,b,c). These results revealed that laboratory scale experiments are more efficient when compared to pilot scale experiments. ²⁹ reported that *Pleurotus ostreatus* removed the color of Kraft mill effluent by 69.0% and COD was reduced to 66.9% after fed batch treatment of Kraft mill effluent. *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. ^{10 30} reported that in laboratory scale treatment of paper industry effluent, a maximum decolourization of 63.9% was achieved by *Trametes versicolor* on the 4th day. Inorganic

chloride at a concentration of 765mg/l, which correspond to 22.7%, was liberated by *Fomes lividus* on the 10th day. The Chemical oxygen demand was also reduced to 1984mg/l by these two fungi. 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 1984mg/l corresponding to 59.32% by *Fomes lividus*^[18]. reported that *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%. ^[16] reported that 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 corresponds to 220.9% on 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 with 1 day incubation, inorganic chloride 361mg/l was liberated on the 6th day and the chemical oxygen demand was reduced to 2,000mg/l corresponding to 40.2%. ^[31] reported that *Trametes versicolor* reduced biological oxygen demand and chemical oxygen demand of paper mill effluent by 52% and 42% respectively^[17] reported 57% colour removal and 65-67% reduction of chemical oxygen demand after 14 days of incubation when treated with the white rot fungus *pleurotus sajor caju*^[32] reported that some white rot strains such as *Ceriopsis subvermispora* could decolourize kraft-bleaching effluent at 90% and also resulted in reduction of COD of up to 45%. When compared with previous results, the newly isolated *Polyporus hirsutus*, *Daedalea flavida*, *Phellinus sp* have superior potential to dechlorinate lignin and /or lignin derivatives (Table 1).

Table 1: Comparison of the efficiencies of the treatment in lab scale and Pilot scale

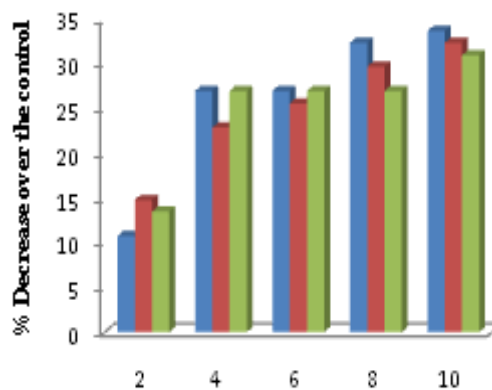
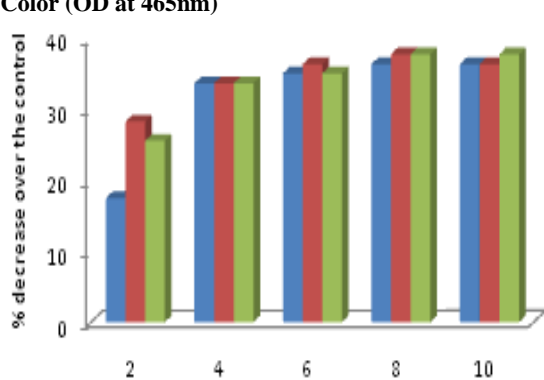
Treatment	Remaining content after treatment		
	Color ^a (%)	Chloride (mg/l)	COD (mg/l)
Control	0.74 (100)	283(100)	5200(100)
<i>P.hirsutus</i>			
Lab scale	0.47 (63.6)	750 (165.0)	2960(43.0)
Pilot Scale	0.49(66.2)	535(89.0)	3110(40.1)
<i>D.flavida</i>			
Lab scale	0.47(63.6)	784(177.0)	2980(42.6)
Pilot Scale	0.50(67.57)	570(101.4)	3210(38.2)
<i>Phellinus sp</i>			
Lab scale	0.46 (62.2)	820(189.7)	3010(42.1)
Pilot Scale	0.51(68.92)	582(105.7)	3260(37.3)

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

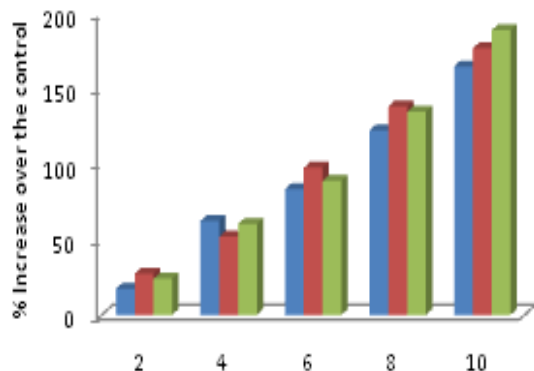
^a Absorbance at 465 nm

Fig 1: Treatment of pulp and paper mill effluent by *Polyporus* b) Chloride content (mg/l) *hirsutus*, *Dadella flavida* and *Phellinus* sp on laboratory scale

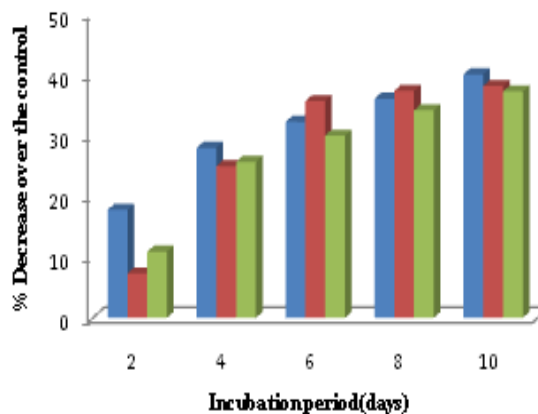
(a) Color (OD at 465nm)



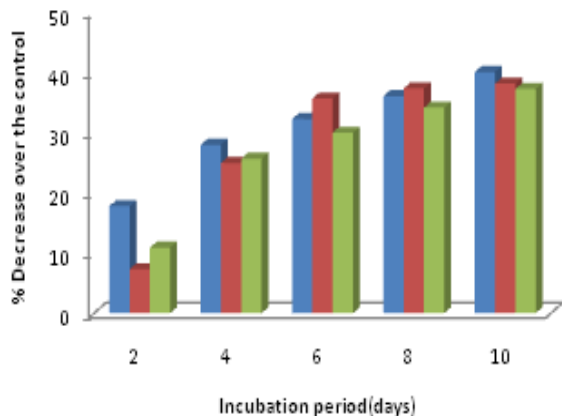
(b) Chloride content (mg/l)



(c) COD (mg/l)



(c) COD (mg/l)



■ *Ph-Polyporus hirsutus*; ■ *Df-Daedalea flavida*; ■ *P-Phellinus.Sp*

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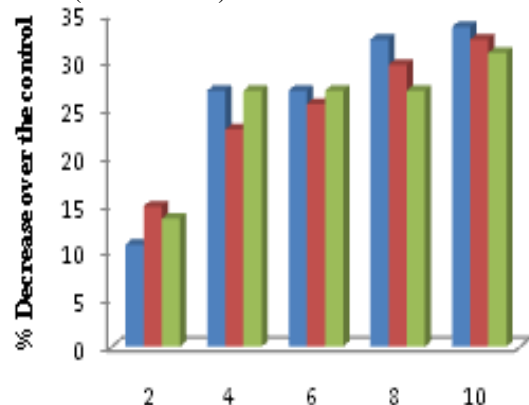
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Fig 2: Treatment of pulp and paper mill effluent by *Polyporus hirsutus*, *Dadella flavida* and *Phellinus* sp on pilot scale

(a) Colour (OD at 465nm)



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