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ORIGINAL RESEARCH ARTICLE

Biological Treatment of Pulp and Paper Industry Effluent by White Rot Fungi *Schizophyllum commune* and *Lenzites eximia*

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ABSTRACT

White rot fungi, *Schizophyllum commune and Lenzites eximia* were collected from the Western Ghats region of Tamil Nadu, India from the living tree of *Tamarindus indica* and burnt tree respectively. The fungi were isolated using 2% malt extract agar medium and the fungal growth were sub cultured and incubated for 6 day at 37^0C and maintained on Malt agar slants. Then, the spores were harvested without disturbing the mycelial growth using a camel hairbrush and filter sterilized. The spore concentration was adjusted to 10^5spores/ml and used as inoculums to treat pulp and paper industry effluents on a laboratory and pilot scales. In laboratory scale a maximum decolourization of 73.9% and 69.5% was achieved by *Schizophyllum commune* and *Lenzites eximia* on 6^{th} day respectively. Inorganic chloride at a concentration of 539 mg/l and 534mg/l was liberated by *Schizophyllum commune* and *Lenzites eximia* respectively on 6^{th} day of treatment. The chemical oxygen demand (COD) was reduced to 3996mg/l (67.0%) by *Schizophyllum commune* and 4317mg/l (65.0%) by *Lenzites eximia*. In Pilot scale, a maximum decolorization of 62.3% was obtained on 6^{th} day of incubation by *Schizophyllum commune* and 56.20% by *Lenzites eximia* on 6^{th} day. Inorganic chloride at a concentration of 477 mg/l and 469 mg/l was liberated by *Schizophyllum commune* and *Lenzites eximia* respectively on 6^{th} day. The chemical oxygen demand (COD) was reduced to 4798mg/l (61.5%) by *Schizophyllum commune* and 5196mg/l (56.6%) by *Lenzites eximia* on 6^{th} day treatment. These results revealed that *Schizophyllum commune* proved to be more efficient for the treatment of pulp and paper industry effluent in lab scale when compared to pilot scale.

Key words: White Rot Fungi, COD, pulp and paper industry effluent.

1. INTRODUCTION

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 273-450m^3 of water is required to produce 1 ton of paper and about 60-300m^3 of wastewater is discharged. [1] Each pulp and paper industry utilizes large amounts of water, which reappear in the form of an effluent containing large amounts of organic compounds. These higher molecular weight compounds are biologically inactive since they cannot penetrate inside the cellular membrane of living organisms and the degradation of such compounds results in lower molecular weight compounds which could be active and toxic to living organisms.[10] The toxic solvents and chlorine compounds lead to the formation of certain xenobiotic compounds like dioxin, biphenyls and polybrominated diphenyl ethers.[1] When these compounds are introduced in aquatic life as well as humans the Adsorbable organic halides (AOXs) exhibit toxicity and it may bioaccumulate in fish tissue causing clastogenic, carcinogenic, endocrine and mutagenic effects.[25] This is a major risk to human health if large amounts of fish exposed to these substances are consumed. So, the effluent generated by pulping industries is a major threat to the environment as well as human health.[29] The effluent colour may increase in temperature and decrease photosynthesis, both of which may probably lead to decreased concentration of dissolved oxygen.[17] The wastewaters from pulp and paper industry are toxic and cause extensive pollution. A survey within the United Kingdom

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paper industry has found that the chemical oxygen demand (COD) of the pulp and paper effluent can be very high. [32] Biological oxygen demand (BOD) and Total suspended solids (TSS) are the other two key parameters for the measurement of pollution.[19] The physico-chemical methods are efficient only for removal of high molecular weight compounds and also the treatment cost is quiet high. So there arises a need to develop biological methods which can solve both the problem of time and cost thereby treating the effluent in effective manner.[29] Biological methods for treating pulp and paper industry effluent are of great concern over physico-chemical methods due to their economical and ecofriendly impact.[23] Biological treatment is categorized into aerobic treatment, anerobic treatment and fungal treatment. The aerobic treatment includes activated sludge processes, aerated lagoons and biological reactors. The microorganisms reported for aerobic treatment are *Pseudomonas putida, Citrobacter sp.* and *Enterobacter sp.*[6] In aerated lagoons, the chlorinated effluents stream are mixed with general mill wastewaters and 70% of AOX removal was reported.[30] In bioreactors moving bed biofilm reactor and suspended carrier biofilm reactor were the recent development and fungal bioremediation by *Phanerochaete chrysosporium. Lentinus edodes, Trametes versicolor*, has also been reported.[31,15,11] White rot fungi are used for bioremediation processes because they have the ability to degrade a wide range of environmental pollutants.[28] Bioremediation of pulp and paper industry effluent by *pleurotus sp*. has been widely reported.[22] White rot fungi are the ideal organisms for decolourization, reduction of AOX and chemical oxygen demand (COD) and this can be achieved either by adsorption or oxidative degradation by the enzymes.[12] Several strains of white rot fungi have been found to decolourize wood processing wastewater.[18] The MYCOR (Mycelial Colour Removal) process for the treatment of spent chlorine bleaching liquor using *Phanerochaete chrysosporium* in rotating biological contractor was developed and this process efficiently reduces the amount of toxic low molecular weight compounds in the effluent.[7] Extensive studies for waste water bioremediation by *Phanerochaete chrysosporium* in pulp and paper industry were reported.[9] The decolourization of pulp mill wastewater using thermotolerant white rot fungus *Daedaleopsis* sp was also studied.[21]

The biological treatment of pulp and paper industry effluent by the white rot fungi *Fomes livdus* and *Thelephora sp* in both lab scale and pilot scale was reported.[26]. In the present study two newly isolated fungi *Schizophyllum commune* and *Lenzites eximia* were examined for their potential in decolourization, the reduction of COD and increase in organic chloride in both laboratory scale and pilot scale.

2. Materials and methods

2.1 Microorganism and media

The fungi, *Schizophyllum commune* and *Lenzites eximia* were collected from Western Ghats region in Tamil Nadu, India. The fungi were isolated from living tree of *Tamarindus indica,* burnt tree and used for the treatment of pulp and paper industry effluent. The fungi were identified based on the key provided previously.[3,14] The fungal growth was cut and then sterilized with 1% mercuric chloride solution, repeatedly washed with sterile distilled water as described previously.[33] and inoculated on 2% Malt agar medium. The fungal growth was sub cultured and incubated for 6 day at 37^0C and maintained on Malt agar slants. Then, the spores were harvested without disturbing the mycelial growth using a camel hairbrush and filter sterilized. The spore concentration was adjusted to 10^5 spores/ml and used as inoculum for further studies.

2.2 Effluent source and Treatment using a rotating biological contractor

The pulp and paper industry effluent was collected from Seshasayee paper mills, Pallipalayam, Erode, Tamilnadu, India and utilizing *Eucalyptus grandis* wood chips as a main raw material. The effluent was stored at 4ºC and filtered through a 0.5mm sieve to remove large suspended particles.

To analyze the efficiency of the waste water treatment, the selected fungi were grown in media by the method [20]. In a rotating biological contractor, (890ml) was mixed with 10g of glucose and 60 ml of aqueous nutrient solution containing KH_2PO_4 2g, $MgSO_4.7H_2O$-5g, $CaCl_2$-0.1g, NH_4Cl-0.116g, thiamine HCl-0.001g. The solution was sterilized and the pH was adjusted to 4.5 with concentrated H_2SO_4. The reactor was inoculated with 50ml of spore suspension (10^5spores/ml) and maintained at 39ºC for 4 day. On day 5, the medium was replaced by effluent of 820ml, nutrient solution without NH_4Cl 60 ml, NH_4Cl 35.3mg, benzyl alcohol 0.84ml, tween80

1.0 and 90ml of mineral solution containing nitrilioacetic acid 1.5g, $MnSO_4H_2O$- 0.5g,

$FeSO_4.7H_2O$- 0.1 g, $CoSO_4$- 0.1 g, $ZnSO_4$- 0.1 g, $CuSO_4.5H_2O$- 0.01 g, $AIK(SO_4)_2$- 0.01 g, H_3BO_3- 0.01 g, $NaMoO_4$- 0.01 g. The pH of the solution was adjusted to 4.5 with concentrated H_2SO_4 and the reactor was maintained at 39ºC 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 Chemical Oxygen Demand were analyzed according to the methods reported previously.[2] Samples were withdrawn at regular intervals every day.

3. RESULTS AND DISCUSSION

The potential of two white rot fungi namely *Schizophyllum commune* and *Lenzites eximia* were assessed when the paper mill effluent was treated with these selected fungi on two scales namely laboratory scale and pilot scale because different scales can show different efficiencies in the treatment. The colour, the chloride content and the COD in effluents are regarded as important factors to evaluate its quality. 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/ll) 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%) (**Fig 1**). In pilot scale treatment with *Schizophyllum commune* the colour was reduced at a maximum by 62.3% of untreated effluent by 6 day incubation. The liberation of inorganic chloride was increased upto 477 mg/l (238.0%) of that in the untreated effluent by the 6 day incubation and the COD was reduced to 4798mg/l (61.3%). In *Lenzites eximia* the colour was removed at a maximum by 56.2% when compared to untreated effluent on 6 day incubation. The liberation of inorganic chloride was increased upto 477mg/l (231.2%) of that in the untreated effluent by the 6 day incubation and the COD was reduced to 4798 mg/l (61.3%). (**Fig 2**). These results revealed that laboratory scale experiments was more efficient when compared to pilot scale experiments. *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. [8] *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 [22]. *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.*[26] *Daedaleopsis sp* and *Phaneochaete chrysosporium* exhibited the highest ability to decolourize waste water by 52% and 86% respectively, COD was reduced by 59-71% and 66-83%.[21]. 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.[27] *Trametes versicolor* reduced biological oxygen demand and chemical oxygen demand of paper mill effluent by 52% and 42% respectively.[24] 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[5]. *Ceriopsis subvermispora* could decolourize kraft-bleaching effluent at 90% and also resulted in reduction of COD of up to 45%.[13] In the present study newly isolated white rot fungi *Schizophyllum commune* and *Lenzites eximia* have superior potential to dechlorinate lignin and or lignin derivatives when compared to previous reports (**Table 1**).

Table 1: Comparision of the efficiencies of the treatment in labscale and Pilot scale

Treatment	Content removed during treatment		
	Colour [a] (%)	Chloride (mg/l)	COD (mg/l)
Control	0.69	141	12000
Schizophyllum commune			
Lab scale	0.18(73.9)	539(282.0)	3996(67.0)
Pilot Scale	0.26 (62.3)	477(238.0)	4798(61.5)
Lenzites eximia			
Lab scale	0.21(69.50)	534(278.1)	4317(65.0)
Pilot Scale	0.30(56.20)	469(232.0)	5196(56.6)

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

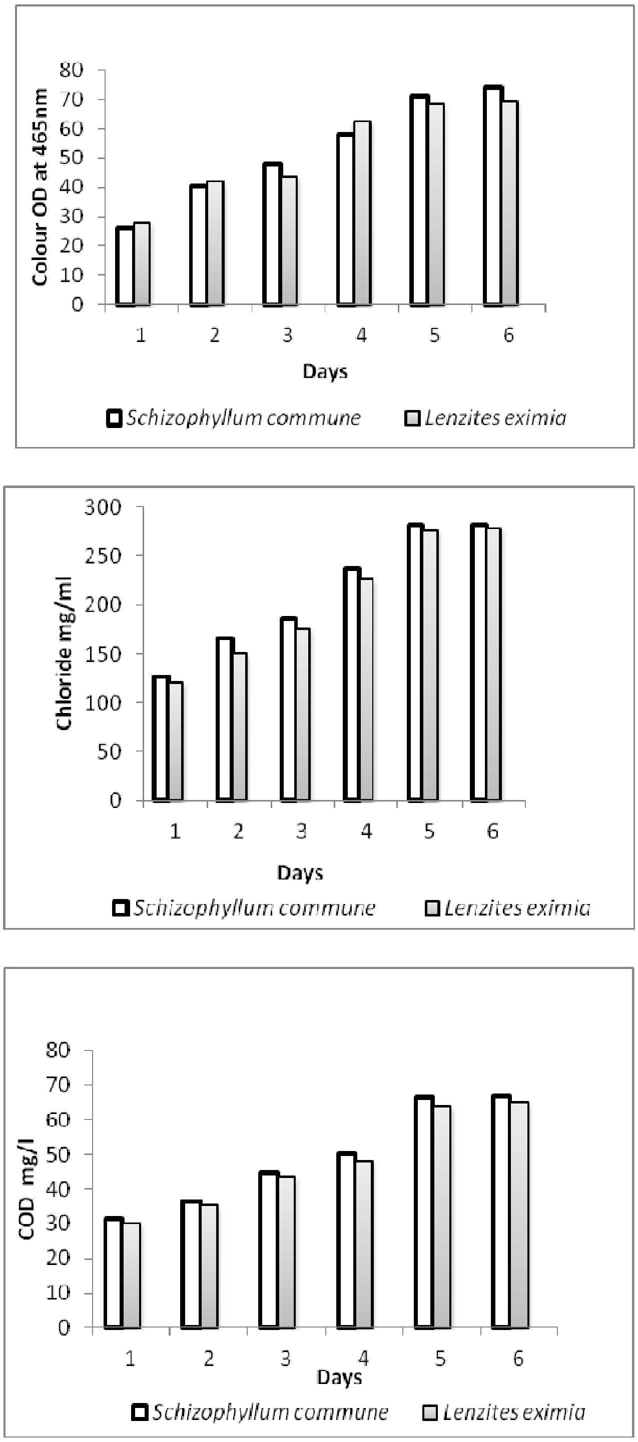

Fig 1: Colour removal of pulp and paper mill effluent by *Schizphyllum commune* and *Lennzites eximia* on laboratory scale. Colour (OD at 465nm)-values decrease over control, chloride content (mg/l)-values increase over control. COD (mg/l)- values decrease over control.

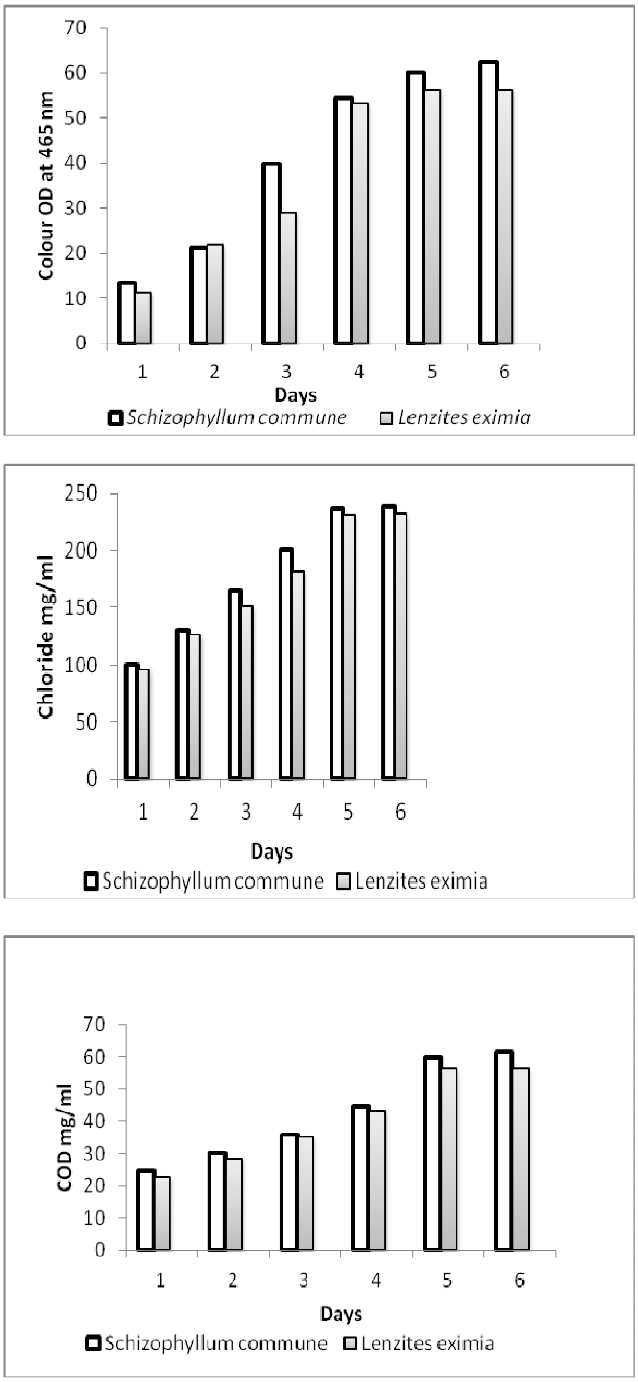

Fig: 2 Treatment of pulp and paper mill effluent by *Schizophyllum commune and Lenzites eximia* on pilot scale. Colour (OD at 465nm)-values decrease over control, chloride content (mg/l)-values increase over control. COD (mg/l)- values decrease over control.

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