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Short Communication

Biological Decolourization of Higher Concentrations of Synthetic Lignin by Native Fungi

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Lignin is an organic compound abundantly found in wood. Degradation of lignin poses major problems for paper mill industries all over the world with regard to the dark- brown color of lignin in wastewater, which is also a major problem facing the environment today. The decolourization capability was tested with species like *Phanerocheate chrysosporium*, *Trametes hirsuta* and an isolated native Marine fungi – M1 using synthetic lignin. The result showed that, isolated strain decreased the lignin color by up to 20% after cultivation for 7 days. Where the decolourisation ability of the fungi *Phanerocheate chrysosporium* and *Trametes hirsuta* was observed to 80 and 70% respectively.

Key Words: Lignin, decolourisation, *Phanerocheate chrysosporium, Trametes hirsuta.*

Introduction

Bioremediation is defined as the application of biological processes to the treatment of pollution. Most research within the field of bioremediation has focused on bacteria, with fungal bioremediation (Mycoremediation) attracting interest just within the past two decades. White rot fungi can withstand toxic levels of most organopollutants (Aust et al., 2003). White rot fungi is a physiological grouping of fungi that can degrade lignin (and lignin – like substances). Four main genera of white rot fungi have shown potential for bioremediation: *Phanerocheate sp, Trametes sp, Bjerkandera sp* and *Pleurotussp* (Hestbjerg et al., 2003). The main mechanism of biodegradation employed by this group of fungi, however, is the lignin degradation system of enzymes. These extracellular lignin – modifying enzymes have very low substrate specificity so they are able to mineralize a wide range of high recalcitrant organo pollutants that are structurally similar to lignin (Cajthaml et al., 2002; Mansur et al., 2003; Pointing, 2001; Veignie, 2004).

The fact that these fungal enzymes work extracellularly allows them to access many of the non-polar, non-soluble toxic compounds that intracellular processes cannot (Reddy and Mathew, 2001; Levin et al., 2003). The three main lignin – modifying enzymes are lignin peroxidases, Mn-dependent peroxidase and laccase. All three of these enzyme groups are stimulated by nutrient limitation (Mansur et al., 2003; Aust et al., 2003). They are most effective at degrading lignin and lignin-like substances when certain nutrient levels, primarily nitrogen are low. Conversely, activities of these enzymes are completely suppressed in media containing high levels of nitrogen. This characteristic is advantageous for the fungi inhabiting highly contaminated sites with very low productivity due to toxic levels of organo pollutants (Reddy and Mathew, 2001).

In recent years, many researches indicated that white rot fungus is a promising microbe in wastewater treatment. The basidiomycete *Phanerochaete chrysosporium* in white rot fungi is the most extensively studied and discussed (Tien, M. and Kirk, T. K, 1983). The extracellular ligninolytic enzymes of white rot fungi have the ability to degrade a wide spectrum of recalcitrant organo-pollutants such as chlorinated phenols and various types of synthetic dyes because of their nonspecific characteristics (Heinfling, A et al., 1997; Novotny, C., et al., 2001). And some significant mineralization (20%&48%) was observed during degradation of azo dyes (Spadaro, J. T et al., 1992).

The study of biodegradation of lignin by wood-rotting fungi is limited not only by lack of taxonomy in referring to genetic variety, but also in their potential for industrial use. The studies have mainly focused on

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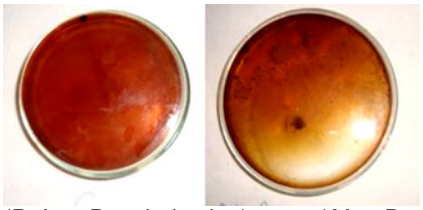
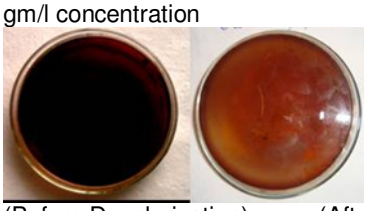


(Before Decolorization) (After Decolorization)
(a)
Decolorization by *Phanerochaete chrysosporium* in 5 gm/l concentration



(Before Decolorization) (After Decolorization)
(b)
Decolorization by *Phanerochaete chrysosporium* in 10gm/l concentration

(Before Decolorization) (After Decolorization)
(c)
Decolorization by *Phanerochaete chrysosporium* in 15gm/l concentration

Figure 1. Decolorization by *Phanerochaete chrysosporium*

Phanerochaetechrysosporium (Boominathan and Reddy, 1992). Until now, selectively few species of basidiomycetes have been used in lignin degradation studies, such as *Bjerkandera, Pleurotus, Polyporus, Pycnoporus* and *Trametes* (Addleman and Archibald, 1993; Paice et al., 1993; Eggert et al., 1996). Species of marine fungi that may have potential capacity for use in the pulp and paper industry can possibly be used effectively, since these are available in great number along the coastal zones. Studies are therefore required in order to determine not only variations in fungal species and lignin degradation capabilities, but also to establish species or strains that may be suitable for biotechnological applications. The purpose of this work was to study the ability of white rot fungi *Phanerochaete chrysosporium, Trametes hirsute* and a marine fungus to decolorize synthetic lignin.

Methodology

Chemicals

All the chemicals used were procured from Himedia Laboratories Pvt.Ltd, Mumbai, India.

Microorganisms

Commercial strains of *Phanerocheate chrysosporium* (MTCC 787), *Trametes hirsuta* (MTCC 136) were procured from IMtech, Chandigarh, India. A native marine fungal species (Here by named as M1) was isolated from the coastal waters of Visakhapatnam.

Culture Conditions

All the cultures were cultured on PDA. The cultures were maintained at 27^0C and were sub-cultured periodically.

Decolorization Studies

Comparative decolorization ability of the three fungi was studied by adding commercial lignin. The experimental procedure was as follows. The 7 days, 10 days and 7 days old cultures of *Phanerochaete chrysosporium* and *Trametes hirsuta* and M1 respectively, were inoculated in the medium containing synthetic lignin under sterile conditions. The concentration of synthetic lignin in the culture medium was 2 $mg{\cdot}L^{-1}$, 4 $mg{\cdot}L^{-1}$, 6 $mg{\cdot}L^{-1}$. The decolorization potential of the fungi was observed after 7 days.

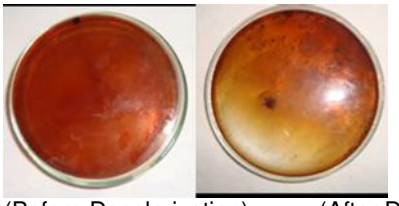
Results

The main colored substances of general kraft pulp bleaching effluent were lignin and its related compounds. Decolorizing activity by the test fungal species toward a commercial lignin on plate assay is obvious, as shown in Figures. In this solid state decolorization, the mycelial growth of the microorganisms and the decolorization patterns were observed to be as follows:

***Phanerochaete chrysosporium*:** Ligninocellulosic materials were able to induce ligninolytic enzyme production in many fungi. The increase in lignin concentration is correlated with the decolorization capacity of extracellular medium. The growth of the fungus started on the third day in the concentrations 5 $g{\cdot}L^{-1}$, 10$g{\cdot}L^{-1}$, where as no growth was observed in 15$g{\cdot}L^{-1}$ till seventh day, which may be attributed to higher concentration of the lignin. The growth was observed to be less in comparison with the other two fungi. The decolorization of synthetic lignin by *P. chrysosporium* was observed to be faster than the decolorization by *T. hirsuta* and the Marine fungi. The decolorization by *P. chrysosporium* was observed to be more than the other two fungi on the 21^{st} day of incubation in the concentrations 5 $g{\cdot}L^{-1}$, 10$g{\cdot}L^{-1}$, however the ability decreased at higher concentration. (Figure 1).

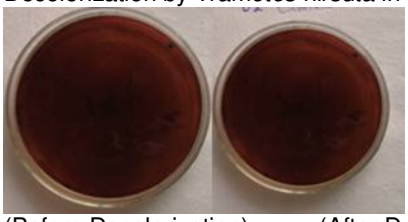
Many workers have reported that Lignin peroxidase has been shown to be involved in dye decolorization, mainly in *P. chrysosporium* cultures (Bumpus and Brock, 1988; Cripps et.al., 1990; Ollikka et.al.,1993; Paszczynski and Crawford 1991 and Young and Yu, 1997).

***Trametes hirsuta*:** The growth of the fungus started on the Eighth day in the concentrations 5$g{\cdot}L^{-1}$, 10$g{\cdot}L^{-1}$,



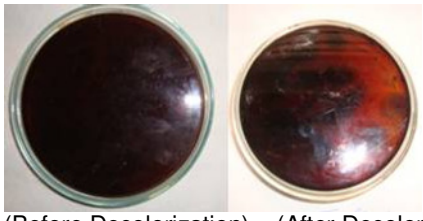
(Before Decolorization) (After Decolorization)
(a)
Decolorization by *Trametes hirsuta* in 5gm/l concentration

(Before Decolorization) (After Decolorization)
(b)
Decolorization by *Trametes hirsuta* in 10gm/l concentration

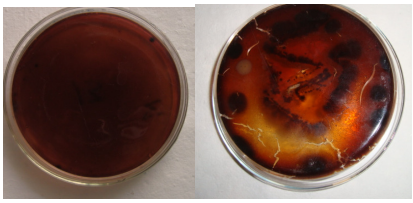


(Before Decolorization) (After Decolorization)
(c)
Decolorization by *Trametes hirsuta* in 15gm/l concentration

Figure 2. Decolorization by *Trametes hirsute*



(Before Decolorization) (After Decolorization) (a)
Decolorization by Marine fungi in 5gm/l concentration



(Before Decolorization) (After Decolorization) (b)
Decolorization by Marine fungi in 10gm/l concentration

(Before Decolorization) (After Decolorization) (c)
Decolorization by Marine fungi in 15gm/l concentration

Figure 3. Decolorization by Marine fungi

where as no growth was observed in 15g·L^{-1} till Eleventh day, which may be attributed to higher concentration of the lignin. The growth was observed to be luxurious when compared with other two fungi. The decolorization of synthetic lignin by *T. irsute* was observed to be slower than the decolorization by *P chrysosporium* and the Marine fungi. The decolorization by *T. irsute* was observed to be lesser than *P.chrysosporium* on the 21st day of incubation in all the three concentrations. Even though the growth was observed to be luxurious the ability to decolorize has reduced with the increase in concentrations of 10g·L^{-1} and 15g·L^{-1} (Figure 2).

Sathiya Moorthi et.al., 2007 has studied the decolorization capability of T.hirsuta and proved the laccase activity from this fungus through a Dark brown color that indicated laccase activity of culture filtrate (well-1 Glass distilled water; well-2 20 µl of crude enzyme and well-3 30 µl crude enzyme).

***Marine Fungi*:** The growth of the fungus started on the Second day in the concentrations 5g·L^{-1}, 10g·L^{-1}, and in 15g·L^{-1}. The growth was observed to be luxurious when compared with *P. chrysosporium* and less when compared to *T.hirsuta*. The decolorization of synthetic lignin by marine fungi was observed to be slower than the decolorization by *P. chrysosporium*. The decolorization by marine fungi was observed to be lesser than the other two fungi on the 21st day of incubation in all the three concentrations. (Figure 3).

Discussion

Pointing (1999) showed that qualitative assays are powerful tools used in screening fungi for lignocellulose degrading enzyme production. Such tests give a positive or negative indication of enzyme production. They are particularly useful in screening large numbers of fungal isolates for several classes of enzyme, where definitive quantitative data are not required. All fungal isolates were screened for the presence of laccase, lignin-peroxidase and manganese dependent peroxidase activities by using an agar plate assay as a qualitative method for the determination of lignocellulolytic enzyme production (Atalla et.al., 2010).

With the aim of finding decolorization activity at higher concentrations and because extracellular ligninolytic enzymes have been shown to be induced by growth on natural lignin substrates, the three fungal species were grown in media containing different concentrations of synthetic lignin substrates. In the present study the synthetic lignin decolorization ability of *P. chrysosporium,*

T. hirsuta and marine fungi were studied. Compared with *T. hirsuta* and marine fungi, *P. chrysosporium* have shown the highest ability to decolorize. However, the extent of color removal decreased with increase in concentration. Decolorization depends upon on the laccase production, media and dyes. Similar observation regarding dye degradation by the white rot fungus *P. chrysosporium* has been observed by Spardaro et al. (1992). These results also show that in conclusion, several industrial dyes were decolorized by extra cellular enzymes from different strains of fungi. This appears to be a good application for immobilization and use as a bioreactor for effluent treatment from the dye and printing industries.

Conclusion

Lignin, which is widespread in nature, especially in all-higher plants, is a hydrocarbon aromatic compound. Its complex structure makes lignin very difficult to degrade and therefore it can be persistent in the environment. Lignin degradation is important in the pulp and paper industry worldwide, which uses chemical substances to breakdown lignins in pulp processing. The process releases hazardous lignin-compound effluents into the environment that are toxic and carcinogenic (Harazono et al., 1996; Elizabeth Rodrý´guez, 1999*). P. chrysosporium* showed the highest ability to decolorize synthetic lignin, the study indicated that the lignin decolorization by marine fungus was competent with the standard species and can provide a cost-effectivess in using native organisms. But further studies are required to prove the ability of the fungi to degrade such very high concentrations of lignin, so that it can be adopted and applied for lignin degradation in paper and pulp mill effluents.

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