



## BIODEGRADATION OF PHENOL USING SPENT SUBSTRATE OF *PLEUROTUS SP.*

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### ABSTRACT

Phenol is an aromatic hydrocarbon which exists as a colorless or white solid in its pure state. Over the past several decades, there is growing concern about wide spread contamination of surface and ground water by phenol, due to rapid development of chemical and petrochemical industries. Phenol affects aquatic life even at relatively low concentration (5-25mg/L). Treatment for removal of phenol includes chemical as well as biological processes. Studies show that ligninases such as Lignin Peroxidase and Laccase, produced by *Pleurotus sp.*, can degrade phenol. Spent substrate of *Pleurotus* mushrooms consists of ligninases. Present work was to investigate the potential of spent substrate of edible mushroom *P. ostreatus* for biodegradation of phenol. *P. ostreatus* was cultivated on paddy straw. After harvest, spent substrate was utilized for phenol degradation. According to the enzyme profile of two ligninases present in the spent substrate of *P. ostreatus*, maximum specific activity for Laccase was observed in 35 day old spent substrate and LiP activity was maximum in 56 day old spent substrate, which together contributed significantly for removal of phenol. Spent substrate of 35<sup>th</sup> and 56<sup>th</sup> day were each incubated with phenol sample (1:1w/v) for one day, which resulted in degradation of phenol by 48% and 45% respectively. From these results it appears that, spent substrate of *P. ostreatus* can be used effectively to remove phenol from industrial effluents.

**KEY WORDS:** Biodegradation, Phenol, Spent substrate, *Pleurotus sp.*

## INTRODUCTION

Phenol, a toxic organic compound, is a common and potential contaminant in water effluent of industries like coke oven units, oil refineries, plastics, leather and paint industries and paper and pulp industries. <sup>[1]</sup> Naturally it occurs in human and animal wastes or in decomposing organic material. This aromatic compound, which is constantly ingested into our environment, is highly toxic and found to affect aquatic life, causing ecological imbalance. It has high bioaccumulation rate along the food chain. Phenol can be absorbed by our body through the respiratory organ, skin and alimentary canal. It can restrain the central nervous system and interact with the liver and nephridium. <sup>[2]</sup> Hence, in 1985, WHO imposed a stringent effluent discharge limit of 0.2 mg/l. <sup>[3]</sup> Thus its removal is essential for environmentally sustainable existence. Conventional treatments to remove phenol from water include chemical oxidation, solvent extraction as well as biological processes. Studies show that extracellular ligninases such as Lignin Peroxidase (LiP) and Laccase produced by certain *Pleurotus sp.* can degrade phenol. <sup>[4]</sup>

*Pleurotus* is a genus of gilled mushroom, commonly called as oyster mushroom. They belong to kingdom Fungi, family Tricholomataceae and class Basidiomycetes. They are found in tropical/temperate climates at a temperature range of 22-30°C and humidity 80-95%. Oyster mushrooms are the second most common mushroom produced in India, as well as worldwide. These mushrooms are edible as well as medicinal. Commercially, *Pleurotus* mushrooms are cultivated in bags, on lignocellulosic agriwaste such as paddy straw. After cultivation, a considerable amount of mushroom substrate (spent substrate) remains as residual material.

Presently, spent substrate (SS) is used for cultivation of other mushroom species or vegetables, for preparation of vermiculture, or as animal feed. But, it needs heat treatment before being removed from the mushroom house. Being expensive, some mushroom growers discard the spent substrate far from the farm. Without proper treatment, spent substrate can pose health problems. Conversely, recycling of spent substrate can increase sustainability, and also help farm economy. Spent substrate is observed to be a good source of fungal extracellular ligninases. Several studies have shown the potential use of the spent substrate in purification of water and soil. In the present study, an attempt was made to investigate the potential of spent substrate of *Pleurotus ostreatus* for biodegradation of phenol in waste water.

## MATERIALS AND METHODS

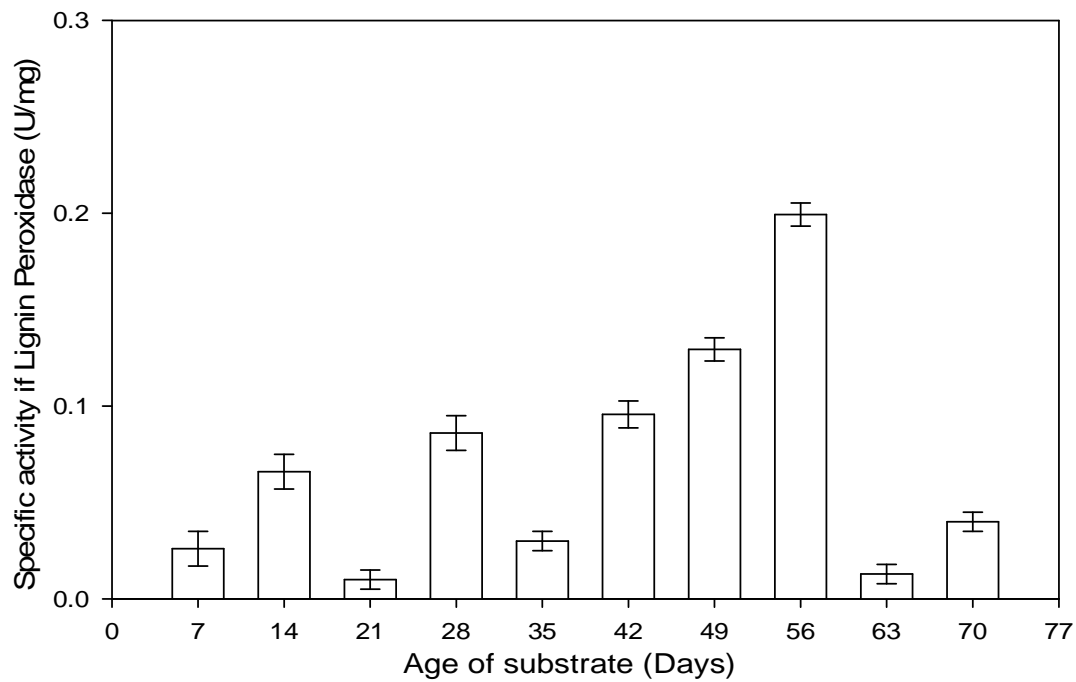
*Pleurotus ostreatus* culture (NCIM-1200) was collected from NCIM, Pune. Spawn was prepared and mushroom was cultivated on paddy straw.<sup>[5]</sup> Spawn to substrate ratio was 1:10 (w/w). Mushroom substrate was utilized for quantitative assays of extracellular ligninases (Lignin Peroxidase (LiP) and Laccase) from 7<sup>th</sup> day onwards till 70<sup>th</sup> day (at 7 days interval).<sup>[6,7]</sup> Substrate was mixed with citrate buffer(1:1w/v) and 0.05% Tween 80, given shaking for 30min, centrifuged and used as crude enzyme extract for assays (at room temperature and pH of mushroom bed). Protein was estimated simultaneously.<sup>[8]</sup> Mushrooms were harvested on 18<sup>th</sup> and 21<sup>st</sup> day. After harvest, the SS was incubated with phenol sample (1:1w/v) for one day, at room temperature. Initial and final phenol concentrations of the sample were estimated.<sup>[9]</sup> All the experiments were done in triplicate.

## RESULTS AND DISCUSSION

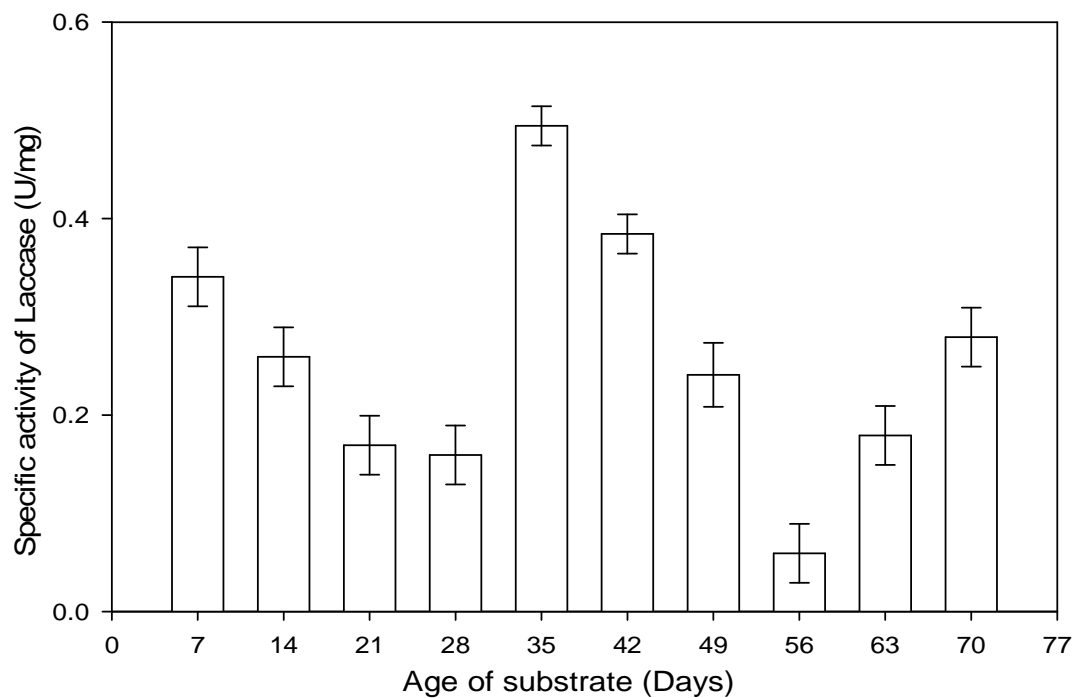
It has been hypothesized that the addition of LiP or Laccase enzyme can reduce the concentration of phenolic compounds in wastewater. The process of removing these phenolic compounds by such enzymes has been researched by several researchers. The main purpose of biological (enzymatic) treatment is to accelerate the oxidation process of the organic matter. Present research has shown that the extracellular ligninase enzymes present in the spent substrate of *P. ostreatus* can react with aqueous phenolic compounds to simply remove them from the aqueous phase.

According to the enzyme profile of two ligninases in SS of *P. ostreatus*, LiP activity increased till 56<sup>th</sup> day and then showed a steep decrease, while Laccase production followed a wave pattern with three distinct peaks on 7<sup>th</sup>, 35<sup>th</sup> and 70<sup>th</sup> day (Fig.1&2). Even after the harvest, *P. ostreatus* continued to produce both ligninases and the enzymes gave their activity peaks only after the harvest. LiP showed maximum activity on 56<sup>th</sup> day of cultivation (0.2U/mg), and Laccase activity was maximum on 35<sup>th</sup> day (0.5 U/mg).

Incubation of the phenol sample (1:1w/v) with 35 day old SS and 56 day old SS for one day, resulted in degradation of phenol by 48% and 45% respectively (Tab 1). *P. ostreatus* was observed to produce more Laccase on comparison with LiP; and on 35<sup>th</sup> day, Laccase production was very high, when compared to LiP. Hence, even though the two ligninases present in the SS contributed together for the biodegradation of phenol; among the two enzymes, Laccase is found to be more efficient in degrading phenol.



**Fig. 1** Specific activity of lignin peroxidase in the substrate of *Pleurotus ostreatus*



**Fig. 2** Specific activity of laccase in the substrate of *Pleurotus ostreatus*

**Table 1 Percentage of reduction in phenol concentration by the use of spent substrate of *Pleurotus ostreatus***

Age of spent substrate (days)	Phenol conc. (mg/L)	
	Initial	After 1 day incubation (Percentage of reduction)
35		6364.62 ± 23.40 (48%)
56	12281.7 ± 23	6770.70 ± 82.03 (45%)

## CONCLUSION

Conventional cultivation practice is to harvest 2-3 flushes of mushrooms, and after which the spent substrate is discarded. From the above results it appears that the spent substrate of *Pleurotus ostreatus* can be used effectively to remove phenol from waste water. In addition to being entirely ecofriendly, the process described here assures double benefit from oyster mushroom cultivation – production of edible/medicinal nutritious oyster mushrooms, and the utilisation of spent substrate for bioremediation. Also, the process offers a viable solution for solid waste management of spent mushroom substrate. This work could make the recycling of spent mushroom substrate commercially attractive. In further research the selection of lignocellulosic substrate and the optimization of conditions need to be addressed.

## ACKNOWLEDGEMENT

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