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# Decolorization and Degradation of Phenolic Paper Mill Effluent by Native White Rot Fungus *Phanerochaete chrysosporium*

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**Abstract:** A white rot fungus isolated from soil samples enriched by continuous pulp and paper mill effluent irrigation and identified as *Phanerochaete chrysosporium* was capable of decolorization and degradation of chlorinated phenol from paper mill effluent. There was 84% effluent decolourisation along with 79% COD reduction by *Phanerochaete chrysosporium*. The effluent chlorinated phenol degradation was 91% by the fungus when added with 1% glucose as co-substrate.

**Key words:** Paper mill effluent, colour, COD, chlorinated phenol

## INTRODUCTION

Phenolic effluents, which colour receiving waters and toxic to mammals and fish[1], are produced by pulp and paper, coal conversion, petrochemical, dyeing and textile industries etc.[2] Among these, effluent from pulp and paper plant is one of the major environmental bugbears in India, contributing to soil and water pollution. In India, pulp and paper industry ranks third in terms of fresh water withdrawal and is brought under the list of 17 most polluting sectors as identified by the Central Pollution Control Board[3]. Today there are about 406 pulp and paper industries with an annual installed capacity of 6.2 million tones. The wastewater generated per ton of paper produced is 220 $m^3$ in large mills manufacturing 2000 t $d^{-1}$[4]. A major source of the phenolic wastes is the alkaline-extraction stage effluent from wood-pulp bleaching, which contains over 50% of colour load. The untreated and partially treated paper mill effluent, by its brown colour, causes aesthetic problem and contaminates the surroundings by their toxic components[5]. It makes the downstream water unfit for domestic and irrigation purposes. The phenol rich wastewaters from paper mill effluent are harmful to both flora and fauna, even at relatively low concentrations of 10-12 mg $L^{-1}$. Conventional treatment methods, such as aerated lagoons and activated sludge plants are ineffective in removing the colour and phenol. Microorganisms are known to utilize phenolic substances and hence in the present study, the fungus isolated from soil samples enriched with continuous paper mill effluent irrigation over 20 years for its colour removal, COD reduction and phenol degradation of paper mill effluent were investigated.

## MATERIALS AND METHODS

**Microorganisms, inoculum development, culture medium and conditions:** The white rot fungus was isolated from enriched soil samples with continuous pulp and paper mill effluent irrigation over 20 years by employing standard serial dilution plating technique[6]. The isolated fungus was tested for its ligninolytic activity by observing the growth on media containing phenol red. Plates were observed for growth and colour change from yellow to red around the culture growth, which indicate the ligninolytic nature of the culture.

**Identification of isolated fungi:** The screened fungal culture was grown at 30°C for 5 days on CPDA medium [20.0 g glucose, 1.0 g $KH_2PO_3$, 1.5 g $MgSO_4.7.H_2O$, 50 μg vitamin B1, 15.0 g agar powder and 1,000 mL potato extract liquid (20%)]. A glass slide was taken and a drop of lactophenol plus cotton blue fluid was mounted on the centre of a glass slide. A portion of mycelial mat from the colony was transferred into the drop of lactophenol plus cotton blue with the help of flamed and cooled needle. With the help of two needles the propagules were gently spread, so that the mycelia mixed with the stain. The slides were observed under low and high power objectives of a microscope (Nikon, Japan) and the types of conidia, hyphae and their arrangement were noted and based on colony and cell morphology, physiological and

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biochemical characteristics the fungus was identified. The pure strain were stored at 4°C in CPDA slants and inoculated once in every 3 months.

**Decolourisation of effluent using isolated fungi:** Pulp and paper mill effluent was scanned in a spectrophotometer to ascertain the wavelength and the maximum absorbance was observed at 465 nm and the rate of decolourisation was monitored at this wavelength. For colour determination, the effluent sample was centrifuged at 10000 rpm for 30 min to remove all the suspended matter. The pH of the supernatant was adjusted to 7.6 with 2 N NaOH and then used for the measurement of absorbance at 465 nm against distilled water as blank. Absorbance values were transformed into colour units (CU) using the following equation:

$$CU = 500A_2/A_1$$

Where, $A_1$ is the absorbance of 500 CU platinum-cobalt standard solution ($A_{465}$ = 0.132) and $A_2$ is the absorbance of effluent sample.

The isolated *Phanerochaete chrysosporium* was used for the decolourization. The fungal isolate was grown in 250 mL Erlenmeyer flask containing 100 mL of Czapek-Dox mineral solution (pH 6.5). After five days, the mycelial pellets was washed with sterile phosphate buffer (pH 7.0) and then the mycelial mat was homogenized by grinding with acid washed sand via pestle and mortar. Two milliliter of the homogenized fungal mycelial mat of isolated culture was added to 250 mL Erlenmeyer flask containing 100 mL effluent alone and 5 mL samples were drawn from the inoculated treatment and centrifuged at 7000 rpm for 10 min. The supernatant was collected and the CU values were measured for seven days at 1, 2, 3, 4, 5, 6 and 7 days after inoculation at 465 nm in the spectrophotometer. The same experiment was carried out by using isolated fungi with different carbon sources viz., glucose, fructose and starch and nitrogen sources viz., ammonium sulphate, sodium nitrate and diammonium phosphate alone and in combination. The treatment schedule is indicated below. $T_1$-Control (Effluent alone); $T_2$-Effluent + Glucose (2.5 g $L^{-1}$); $T_3$-Effluent + Starch (2.5 g $L^{-1}$); $T_4$-Effluent + Fructose (2.5 g $L^{-1}$); $T_5$-Effluent + Ammonium sulphate (1.25 g $L^{-1}$); $T_6$-Effluent + Sodium nitrate (1.25 g $L^{-1}$); $T_7$-Effluent + Diammonium phosphate (1.25 g $L^{-1}$); $T_8$ -Effluent + Glucose (2.5 g $L^{-1}$)+ Ammonium sulphate (1.25 g $L^{-1}$); $T_9$-Effluent + Glucose (2.5 g $L^{-1}$) + Sodium nitrate (1.25 g $L^{-1}$); $T_{10}$-Effluent+ Glucose (2.5 g $L^{-1}$) + Diammonium phosphate (1.25 g $L^{-1}$); $T_{11}$-Effluent + Starch (2.5 g $L^{-1}$) + Ammonium sulphate (1.25 g $L^{-1}$); $T_{12}$-Effluent + Starch (2.5 g $L^{-1}$) + Sodium nitrate (1.25 g $L^{-1}$); $T_{13}$-Effluent + Starch (2.5 g $L^{-1}$)+ Diammonium phosphate (1.25 g $L^{-1}$); $T_{14}$-Effluent+ Fructose (2.5 g $L^{-1}$) + Ammonium sulphate (1.25 g $L^{-1}$); $T_{15}$-Effluent + Fructose (2.5 g $L^{-1}$) + Sodium nitrate (1.25 g $L^{-1}$); $T_{16}$-Effluent + Fructose (2.5 g $L^{-1}$)+ Diammonium phosphate (1.25 g $L^{-1}$). The treatments were replicated twice with control. Five mL samples were drawn from the inoculated treatments and centrifuged at 7000 rpm for 10 min. The supernatant was collected and the CU values were measured for seven days at 0, 2, 4 and 7 days after inoculation at 465 nm in the spectrophotometer.

**Colour removal percentage:** The percent colour reduction was measured as per the method described by Thanga *et al.*[7]:

$$\text{Colour removal (percent)} = \frac{\text{(A)-Absorbance of residual broth}}{\text{Absorbance of uninoculated broth (A)}} \times 100$$

**Degradation of chlorinated phenol in effluent:** The assay for degradation of chlorinated phenol in the effluent was carried out by taking 100 mL of effluent in 250 mL Erlenmeyer flasks. Glucose was added at 1% level and the whole content was sterilized by autoclaving and then inoculated individually with 2 mL of fungi spore suspension along with heat killed culture. The inoculated flasks were incubated at room temperature and samples were collected at 3, 5, 7 and 9 Days After Inoculation (DAI). For the analysis of remaining phenol, the effluent was extracted by acidifying 10 mL effluent sample with 5 N HCl. The chlorinated phenol was extracted three times with 10 mL of dichloromethane medium. The organic phase was reextracted with 0.5 N NaOH. The aqueous phase was taken and analysed by 4-aminoantipyrine method.

## RESULTS

**Identification of fngi:** The isolated fungus was identified as *Phanerochaete chrysosporium* based on its morphology, physiological and biochemical characteristics and verified at Mycology Department of Indian Agricultural Research Institute, New Delhi.

**Colour removal of pulp and paper mill effluent using the isolated fungi with co-substrates:** In general addition of carbon and nitrogen sources either alone or in

Table 1: Effluent colour removal and COD reduction by *Phanerochaete chrysosporium*

| | *Phanerochaete chrysosporium* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Colour removal (colour units) | | | | COD reduction | | | |
| Treatments | 0 DAI | 2 DAI | 4 DAI | 7 DAI | 0 DAI | 2 DAI | 4 DAI | 7 DAI |
| $T_1$ | 1485 | 1256 (15.3) | 957 (35.5) | 854 (42.4) | 3981 | 3475 (12.7) | 3018 (24.2) | 2425 (39.1) |
| $T_2$ | 1589 | 1207 (24.0) | 941 (40.7) | 623 (60.7) | 4115 | 3250 (21.0) | 2159 (47.5) | 1521 (63.0) |
| $T_3$ | 1497 | 1187 (20.6) | 989 (33.8) | 754 (49.3) | 3815 | 2950 (22.7) | 2200 (42.3) | 1821 (52.3) |
| $T_4$ | 1459 | 1184 (18.8) | 935 (35.8) | 801 (45.1) | 3758 | 3054 (18.7) | 2450 (34.8) | 1842 (51.0) |
| $T_5$ | 1495 | 1087 (27.2) | 827 (44.6) | 487 (67.4) | 3621 | 2766 (23.6) | 2054 (43.3) | 1525 (57.9) |
| $T_6$ | 1509 | 1058 (29.8) | 769 (49.0) | 528 (65.0) | 4015 | 3057 (23.9) | 2308 (42.5) | 1547 (61.5) |
| $T_7$ | 1587 | 1209 (23.8) | 897 (43.5) | 523 (67.0) | 4127 | 2987 (27.6) | 2412 (41.6) | 1820 (55.9) |
| $T_8$ | 1614 | 1131 (29.9) | 742 (54.0) | 250 (84.4) | 3817 | 2354 (38.3) | 1314 (65.6) | 848 (77.8) |
| $T_9$ | 1587 | 1158 (27.0) | 795 (49.9) | 301 (81.0) | 4274 | 2890 (32.4) | 1658 (61.2) | 880 (79.4) |
| $T_{10}$ | 1549 | 1138 (26.5) | 810 (47.7) | 320 (79.2) | 4250 | 2578 (39.3) | 1540 (63.8) | 1120 (73.6) |
| $T_{11}$ | 1597 | 1241 (22.3) | 822 (48.5) | 329 (79.3) | 3745 | 2589 (30.9) | 1810 (51.7) | 880 (76.5) |
| $T_{12}$ | 1610 | 1202 (25.3) | 780 (51.5) | 375 (76.7) | 3893 | 2889 (25.8) | 2087 (46.4) | 945 (75.7) |
| $T_{13}$ | 1528 | 1095 (28.3) | 675 (55.8) | 339 (77.8) | 4155 | 3048 (26.6) | 2150 (48.3) | 1040 (74.9) |
| $T_{14}$ | 1457 | 1088 (25.3) | 794 (45.5) | 367 (74.8) | 3624 | 2650 (26.9) | 1958 (46.0) | 940 (74.0) |
| $T_{15}$ | 1477 | 1062 (28.1) | 799 (45.9) | 395 (73.2) | 3757 | 2620 (30.3) | 1758 (53.2) | 1095 (70.8) |
| $T_{16}$ | 1509 | 1066 (29.3) | 792 (47.5) | 325 (78.4) | 3819 | 2879 (24.6) | 1917 (49.8) | 1130 (70.4) |

Figures in parenthesis indicate per cent colour reduction; DAI-Days After Inoculation

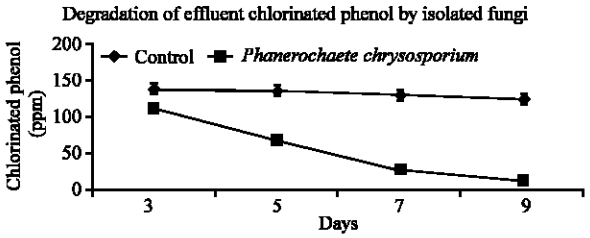


Fig. 1: Degradation of effluent chlorinated phenol by *Phanerochaete chrysosporium*

combination had considerable effect on colour removal of effluent. The results revealed that, *Phanerochaete chrysosporium* responded more to glucose and the colour removal was 60.7%, whereas addition of fructose and starch removed 49.3 and 45.1% of colour, respectively (Table 1). Among the N sources tested, *Phanerochaete chrysosporium* responded more to ammonium sulphate and the colour removal was 67.4%, whereas it was 67 and 65% for diammonium phosphate (DAP) and sodium nitrate, respectively. Combination of carbon and nitrogen sources caused maximum decolourisation than their individual additions. The maximum decolourisation (84.4%) was achieved in glucose + ammonium sulphate combination followed by glucose + sodium nitrate (81%). The lowest (42.4%) was recorded in control (without co-substrate).

**Effluent COD reduction:** The COD of the effluent declined continuously up to 7 days and 79.4% COD reduction by adding co substrates glucose and sodium nitrate in the effluent (Table 1). The COD reduction was 39.1% only in effluent without co substrate.

**Chlorinated phenol removal:** The chlorinated phenol reduced from 138 to 13 ppm by *Phanerochaete chrysosporium* which accounted 90% chlorinated phenol degradation (Fig. 1).

## DISCUSSION

Lignin is the single most important activity in the biological cycle of carbon. The multitude of interunit bonds and functional groups and the heterogeneity of the polymer is the main reason for the resistance of lignin to microbial attack and it is in fact one of the most recalcitrant naturally occurring biological material. In the present study, we describe the isolation of a lignin degrading fungus from the soil enriched by irrigation over 20 years by paper mill effluent.

**Colour removal of pulp and paper mill effluent using isolated fungi with co-substrates:** The fungus *Phanerochaete chrysosporium* showed better colour reduction (61%) when glucose was used as a carbon source. It was reported that addition of glucose @ 1% (W/V) triggered the decolourisation potential of *Phanerochaete chrysosporium*[8]. Similarly in the straw soda pulping effluent using *Trichoderma viride* removed 70% of the colour with addition of 1% glucose has been reported by Martin and Manzanares[9]. Leung Pui Chi and Ponting[10] reported that fast decolourisation rates was observed in the Poly R 478 dye in the polysaccharide monomers (glucose and xylose) as carbon source. Among the N sources, the fungus *Phanerochaete chrysosporium* responded more to ammonium sulphate which was in line

with the findings of Prasad and Gupta[11], who found a significant increase in the efficiency of colour removal by *Phanerochaete chrysosporium* upon supplementation of a small quantity of nitrogen. The nitrogen addition in small quantities increased ligninolytic activity through increased Lignin Peroxidase (LiP) and Manganese Peroxidase (MnP) activity and therefore enhanced decolourisation rates. Among the combination, glucose 2.5 g $L^{-1}$ + ammonium sulphate 1.25 g $L^{-1}$ recorded the highest colour removal (84%) for *Phanerochaete chrysosporium*. Similar results were also reported by Jothimani and Prabakaran[12], who found that glucose at 0.15 and urea at 0.05% increased decolourisation of the dye effluent by 65 and 70.3%, respectively when *Bacillus* and *Phanerochaete chrysosporium* cultures were used.

The microbial system along with co-substrates were found to be efficient in reducing COD of the pulp and paper mill effluent. In the present study the fungal system reduced the COD of the effluent considerably when added with co-substrates. The addition of glucose induced the LiP and MnP enzyme activity of fungal system and enhanced the COD reduction. Bajpai *et al*.[13] also reported 35% COD reduction in paper mill effluent by *Trametes versicolor*. Similarly Nagarathnamma and Bajpai[14] reported 50% reduction in COD of decolourised chlorine bleaching of Kraft pulp effluent.

**Chlorinated phenol removal of pulp and paper mill effluent using isolated fungi:** There was 91% chlorinated phenol degradation of pulp and paper mill effluent by *Phanerochaete chrysosporium* when it was added with 1% glucose. The glucose added in the effluent might be used as the energy source for the rapid growth of fungi and degraded the chlorinated phenol present in the effluent. Also the extracellular enzyme produced by the fungi is responsible for the chlorinated phenol degradation. Similar results were reported by Peralta-Zamora *et al*.[15] who observed the decolourization and pollutant degradation of pulp mill effluents with immobilized lignin and MnP from *Phanerochaete chrysosporium*.

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