Influence of culture parameters on paper mill effluent decolourization by a white rot fungus *Ganoderma lucidum*

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Efficacy of a white rot fungus *G. lucidum* for reduction of colour of paper mill effluent under various growth conditions was evaluated. *G. lucidum* cultured in IBME medium supported maximum colour reduction on 18th day of fungal growth. The optimization of growth parameters further improved colour reduction. The 18 day old culture at 4 g/l inoculum concentration resulted in maximum decolourization (89%) of the effluent with pH adjusted to 6.5 at 35°C along with maximum reduction in biological oxygen demand and chemical oxygen demand. Relative contribution of lignin peroxidase and laccase to the decolourization of paper mill effluent by *G. lucidum* was also observed.

Pulp and paper industries generate significant amount of untreated effluent from the digestors. The discharge of untreated effluent into water bodies damages the water quality and colour is detectable over long distances. It is estimated that 273-450 m³ of water is required to produce a tonne of paper resulting in the generation of 300 m³ as waste water. These effluents are dark brown in colour and have a high biological and chemical oxygen demand (BOD and COD), insoluble total solids along with lignin compounds and their derivatives. The characteristic dark brown colour of these effluent is due to the formation of lignin degradation products during the processing of lignocellulosics for paper and pulp manufacture. Lignin derivatives are highly resistant to microbial attack and consequently escape through the current waste water treatment. Biological treatment of such effluents has been tried by using various strains of bacteria and fungi. Recently white rot fungi have been used for decolourization of paper mill effluent. In view of this, the present study describes the efficacy of a white rot fungus *Ganoderma lucidum* to decolourize paper mill effluents.

**Materials and Methods**

**Fungal strains and culture condition**—*G. lucidum* (PTK) maintained on potato dextrose agar slants (pH 6.5) was transferred to Czapek's dox agar medium (pH 6.5) for 5 days. Mycelial disc (5 mm diam) of 5 days old fungus was taken as initial inoculum for the experiments.

The effluent was collected from Karur Paper Mill Ltd, Tamil Nadu, at three different places and designated E₁, E₂ and E₃. The paper mill utilizes sugarcane bagasse (*Saccharum officinarum*) and eucalyptus (*Eucalyptus spp.*) as the main raw material. The effluents collected were brought to the laboratory and filtered through a sieve (0.5 mm) to remove suspended particles and stored at 4°C. The physicochemical characteristics of the effluent before and after treatment in the laboratory are presented in Table 1.

The culture media—(a) BM-basal medium, contained glucose (10 g), potassium hydrogen phosphate (1 g), ammonium tartrate 180 mg, magnesium sulphate (0.5 g), potassium chloride (0.5 g), ferrous sulphate (0.1 g), veratryl alcohol 1 mM and asparagine (1 g); (b) IBME-medium contained, the same ingredients as that of basal medium but dissolved only in effluents (80% v/v) and (c) E-medium, effluents (E₁, E₂ and E₃ 80% v/v) without the addition of ingredients of the basal medium, were chosen for the present study.

The effect of different carbon sources on decolourisation of E₁ effluent, the IBME medium devoid of glucose was added with one percentage of xylose, maltose, sucrose, carboxymethyl cellulose and starch, individually and the test fungus *G. lucidum* was inoculated into the respective medium.

The influence of nitrogen source on E₁ effluent by *G. lucidum* was tested in IBME medium which was devoid of nitrogen source and the other media were prepared with different concentrations of ammonium nitrate added with 1% glucose as carbon source.
To study the influence of different pH on decolourization of E₁ effluent, IBME medium was initially adjusted with different pH such as 3.5, 4.5, 6.5, 7.5, 8.5 and 9.5. To study the effect of different temperature on decolourization of E₁ effluent, the IBME medium was prepared, inoculated with test fungus and kept at different temperature such as 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C.

To study the effect of various inoculum concentrations on decolourization of E₁ effluent, different concentrations (g/l) of inoculum such as 0.5 to 4.5 with gradual increase of 0.5 g/l, was added in IBME medium.

Five days old 5 mm diameter of mycelium disc of C. lucidum inoculated into 250 ml Erlenmeyer flask containing 50 ml of respective medium described above were incubated at 37°C for of 18 days. The mycelium was harvested at every 3 days interval by filtering through a preweighed Whatman No.1 filter paper, washed with distilled water and oven dried at 80°C for 48hr and its mycelial dry weight was recorded.

Analytical parameters

During the above studies, lignin content, BOD, COD, pH and colour present in both raw and treated effluents were estimated. The lignin content in the effluent at different time intervals was estimated with the help of a Beckmann DU 40 - Spectrophotometer by measuring the optical density at 700 nm. Culture filtrate was used to estimate lignin peroxidase and laccase activity, in a Philips Recording Spectrophotometer.

Results and Discussion

Pulp and paper mill effluents are recognised as environmental hazards. Because of their ability of degrade lignin under laboratory conditions, several microorganisms have been used to decolourize effluent from different sources. Eaton et al., and Kirk et al., have reported degradation of lignin and its derivatives from effluents by using Phanerochaete chrysosporium and Tremetes versicolor. Another white rot fungus Schizophyllum commune has also been reported to decolourize about 9% of the Kraft waste liquor.

In the present study G. lucidum grown in E₁, E₂ and E₃ stages of effluent (80% v/v) resulted in maximum mycelial growth (1.78, 1.84, 1.86 g/l) was recorded on day 18, 18 and 15 for E₁, E₂ and E₃ effluents respectively (Fig. 1a). During the fungal growth, maximum percentage of colour reduction (79, 72, 94%) was determined on day 18 for 3 effluent stages re-
respectively (Fig. 1b). The peak reduction of COD (96, 96, 98%) was recorded on day 18 for E₁, E₂ and E₃ stages effluent samples (Fig. 1c). As the fungus grew the residual lignin was also utilized and the maximum amount of lignin from the effluent was removed on day 18 (Fig. 1d).

Further to optimize the medium for higher rate of decolourization, removal of COD and removal of lignin in E₁ effluent, G. lucidum was treated with BM, IBME and E media. Effluent from E₁ stage was used in this experiment since it contained maximum COD, BOD, lignin and colour (Table 1). The highest degree of decolourization was observed in IBME medium (Fig. 2b). The maximum reduction in COD (98%) was in IBME medium was observed on day 18 (Fig. 2c). There were a considerable activity in lignin peroxidase (88, 90 U/L) and laccase (310, 94 U/L) in IBME and E medium respectively (Fig. 2a, 2d). The fungus G. lucidum also produced extracellular lignin peroxidase and laccase in basal medium amended with lignin isolated from sugarcane bagasse. P. chrysosporium grown in olive mill waste effluent supplemented with basal salts medium produced lignin peroxidase and manganese peroxidase; the olive mill waste decolourization and COD removal were 76% and 68% respectively.

IBME medium added with different carbon sources stimulated the test organism for maximum decolourization, reduction of COD and lignin content in the effluent. With addition of glucose in IBME medium, a reduction of 82% and 98% of colour and COD respectively was recorded. Xylose was also found to be very efficient in reducing colour and COD (Fig. 3a). Starch was able to remove maximum amount of lignin (480 mg/l) compared to other co-substrates (Fig. 3b). Even without addition of co-substrates, 38% and 20% of decolourization and COD reduction were observed in medium treated with G. lucidum. The highest amount of mycelial growth was recorded with addition of glucose, maltose and xylose (Fig. 3b).

The E₁ effluent (in IBME) with various concentrations...
tion of ammonium tartrate amended resulted in maximum reduction of COD (98% at 1.25% of ammonium tartrate) and colour (82% decolourization) (Fig. 4). The effect of pH and temperature on E1 effluent shown in Fig.5 and G. lucidum tolerated pH 4.5 to 8.5 in E1 effluent. Maximum growth (1.89 g/l) was obtained on day 12 at pH 6.5 (Fig.5a). Colour removal (86%) and COD (89%) were recorded maximum at pH 6.5 and also recorded at pH 4.5 to 8.5 (Fig.5b). The growth, colour removal and COD were significantly suppressed at pH values below 4.5 and above 8.5. The maximum colour and COD reduction was recorded at temperature 35°C. Comparable colour and COD reduction at 30° and 40°C. Any temperature above 45°C suppressed the colour and COD reduction (Fig.6).

Effect of inoculum concentration on E1 effluent is shown in Table 2. The colour removal was found to increase from 32 to 89% when inoculum concentration was increased from 0.5 to 4 g/l. The optimal inoculum concentrations was 4 g/l for effective reduction of colour and COD.

Thus in the above experiments the test fungus G. lucidum indicated the dependency on decolourization and COD removal by the added co-substrate. Without an added co-substrate, only 38% of colour removal reduction and 20% of COD removal occurred. However, glucose and xylose supplemented to the medium remarkably reduced the colour and COD. Eaton et al. compared the suitability of 3 sludges and combined sludge with that of cellulose powder for use as a carbon source for P. chrysosporium cultures and his studies showed that decolourization obtained after 7 days was 82-86, 63 and 92% in the case of primary, combined sludge and cellulose powder. Ramaswamy observed that addition of 1% bagasse pith resulted in 80% colour reduction within 7 days by S. communes. Blesare and Prasad reported that decolourization by S. communes with addition of different carbon sources could be resulted the following order viz, sucrose (60%) glucose (48%) cellulose (35%) and pulp (20%). Tremetes versicolor and Trichoderma sp. did not improve decolourization by addition of nitrogen. However, P. chrysosporium,
under nitrogen limiting conditions found to stimulate
decolourization. So far, the potential of G. lucidum has
not been for the decolourization of paper mill effluent.

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