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SCREENING OF WHITE ROT FUNGI FOR DECOLORIZATION OF PULP AND PAPER INDUSTRIAL WASTEWATER

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ABSTRACT

Six fungal strains collected from decomposed woods in the Northern areas of Pakistan (Khanspur) were evaluated for their ability to decolorize pulp and paper production wastewater. All the strains, IPP01, IPP02, IPP03, IPP04, IPP05 and IPP06 were able to grow well in Kirk media with pulping wastewater containing lignin without glucose indicating that they could utilize lignin as nutrient. However, when they were tested for their effectiveness on decolourization of the wastewater from pulp and paper industry, it was indicated that strains IPP01, IPP04 and IPP06 were the most efficient strains able to decrease the color of the pulping wastewater by 47.8%, 47.2% and 47.0%, respectively. These strains were identified by basic morphology as *Pleurotus* sp (IPP01), *Coriolus* sp. (IPP04), and *Phenerochaete* sp (IPP06).

Key Words: decolourization, white-rot fungi, pulp and paper, wastewater

INTRODUCTION

In Pakistan there are more than sixty pulp and paper mills with nearly annual production of 400,000 tones. The production is mainly based on local grass, wheat straws, waste paper etc. The pulping process being employed in Pakistan is NSSC (Neutral Sulfide Semi-Chemical) pulping. Majority of the mills do not have wastewater treatment facility and discharge the effluent into the receiving streams without any treatment, thus posing a great threat to the eco-system (Pak-EPA, 1999). Recently, the impact of this industry on the environment has been closely examined. Even though the color of effluent from pulp and paper industry does not directly affect the environment, it causes psychological trouble for people living in those areas. A number of researches demonstrated that the effluent from pulping process contains lignin compounds as major degraded compounds. These compounds are largely removed from the woody raw material in an alkali pulping stage. Some of these degradation products have exhibited toxicity and mutagenicity and may accumulate in the tissues of animals, fish and human beings (Bajpai *et al.,* 2000).

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 *Pleurotus* sp. 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 *et al.*, 2004).

A number of studies have provided information on white-rot fungi that can secrete enzymes for degrading natural lignin as summarized by Kondo (1998). For example, Fukuzumi (1980) was the first group who studied on utilization of white-rot fungi for wastewater treatment. They fed varieties of the fungi with wastewater from pulping process containing essential nutrients and found that from total of 35 fungal strains, *Tinctoporia* sp. was the best for wastewater decolourization. Furthermore, Eaton *et al.* (1980) carried out similar experiments using *Phlebia brevispora*, *P.subserialis*, *Poria cinerascens* and *Trametes versicolor*. Results demonstrated that the tested strains efficiently decolorized the wastewater. Another experiment was done by Livernoche *et al.* (1983). They screened and isolated the white-rot fungi that were able to decolorize wastewater from pulp bleaching process. From 15 strains, only six strains: *T. versicolor*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Polyporus versicolor* and unidentified strain, showed ability of wastewater decolourization. In this experiment, microorganisms from natural sources were screened for their capability to produce ligninolytic enzyme to decolorize the effluent from pulp and paper production process.

MATERIALS AND METHODS

Analysis of wastewater quality

The wastewater of paper and pulping process from Century paper and board mill Jamber Khurd, Bhai Pheru, Lahore, Multan road, District Kasure was used in this study. Chemical Oxygen Demand (COD) was analyzed by

closed reflux method. The color and turbidity were determined using spectrophotometer model UV-UIS dual beam UVS-2800. The pH value and temperature were determined using pH-conductivity meter model 3510. Dissolved Oxygen (DO) was analyzed using DO 3510 Microprocessor Auto Cal DO meter. Total solid and suspension solid were analysed according to the standard method for the Examination of Water and Wastewater, APHA. AWWA. Edition 19th, 1992.

Isolation of White-rot fungal strains

The mushroom samples were collected from Northern areas of Pakistan. Two methods were used for fungal spore separation: the first technique was done by cutting fresh mushroom samples into small pieces, place them into sodium hypochlorite by forming different concentrations (0.5%, 1.0%) and washed with dist. water thoroughly and then placing on Kirk media ($CaCl_2$ 0.4g, $MgSO_4$ 0.3 g, Ammonium Sulphate 2 g, Potassium dihydrogen phosphate 2.1 g, and Yeast extract 0.4g per wastewater 1 L), pH of the medium was adjusted to 5 and containing usual antibacterial substances. The second technique (also called drop spore or shoot technique) concerned directly spore shooting from fresh mushroom samples onto Kirk media. The agar plates containing the mushroom samples were then incubated at room temperature for 72 h. For the first technique, the resulting spores were observed as groups which would be afterwards isolated using needle under microscope and placed on Kirk media. For the second technique, the growth was demonstrated as fungal mycelium on the agar which afterwards were isolated by cutting the agar containing growing mycelium and placed on Kirk media. The resulting fungal mycelium from both were subcultured until the purified fungal strains were obtained.

Screening of fungal strains capable of wastewater Decolourization

Preliminary screening of the fungi could be done by growing the stains on Kirk media with Pulping wastewater containing lignin without glucose at room temperature for a period of time instead of using synthetic lignin. The strains that were capable of decolorizing the wastewater would be theoretically able to survive and grow well on the media containing waste water lignin. The purified strains were cultured on Kirk media for 5-7 days at room temperature and then three pieces of 2-cm media containing fungal mycelium were subjected into 50 ml of the waste water from pulp and paper process containing essential nutrients ($CaCl_2$ 0.4g, $MgSO_4$ 0.3 g, Ammonium Sulphate 2 g, Potassium dihydrogen phosphate 2.1 g, and Yeast extract 0.4g per wastewater 1 L) and grow at room temperature with shaking at 100 rpm for 9 days. The samples were collected at intervals for quality analysis.

RESULTS AND DISCUSSION

Analysis of wastewater quality

From Table 1, it was shown that the wastewater from pulping process contained quality as follows: pH 5.2, temperature 20.5 °C, BOD 850 mg/L, COD 3,216 mg/L, total dissolved solids 1,065 mg/L and color 1,017 Pt Co.

Table 1. Quality of wastewater from pulping process.

PARAMETERS	WASTEWATER SAMPLE
pH	6.5
Temperature (°C)	20.5
BOD (mg/L)	850
COD (mg/L)	3,216
Total dissolved solids (mg/L)	1,065
Suspended solids (mg/L)	45
Color (Pt Co)	1,016

Isolation of white-rot fungal strains

Starting from six white rot fungi samples collected from Northern areas of Pakistan. These fungal strains were white-rot fungi and labeled as IPP01-06. These strains were kept as culture stocks on Kirk media in Petri plates at 20 °C.

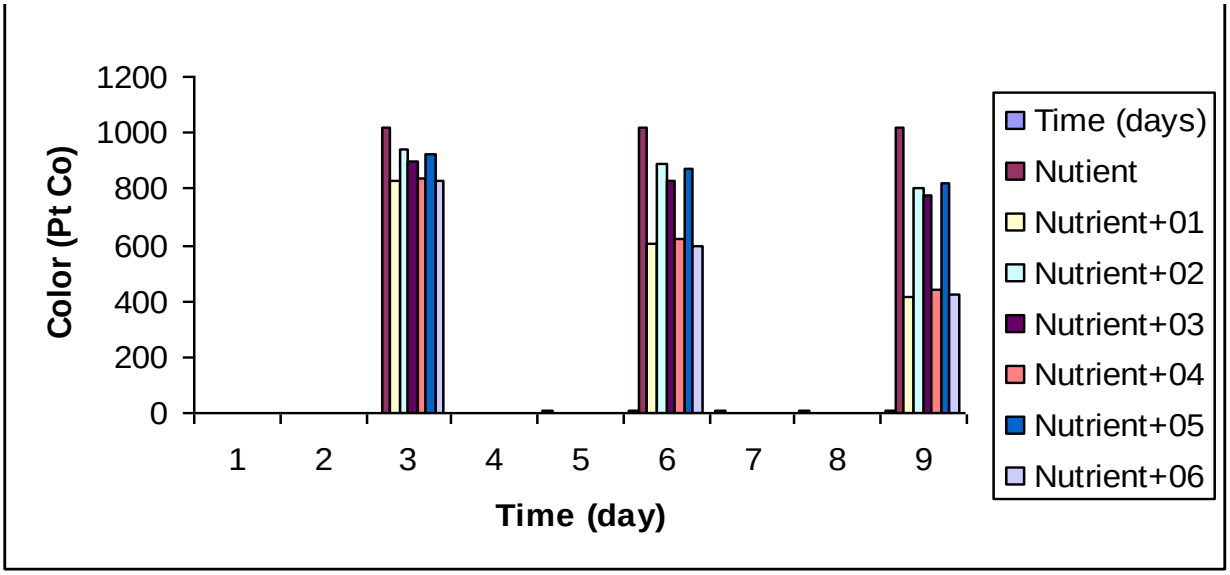

Fig 1. Effectiveness of fungal strains *Pleurotus ostreatus* (01), *Ganoderma lucidum* (02), *Schizophyllum commune* (03), *Coriolus versicolor* (04), *Fomes fomentarius* (05), *Phanerochaete chrysosporium* (06), on colour value (decolourization) of treated waste-water from pulp and paper industry.

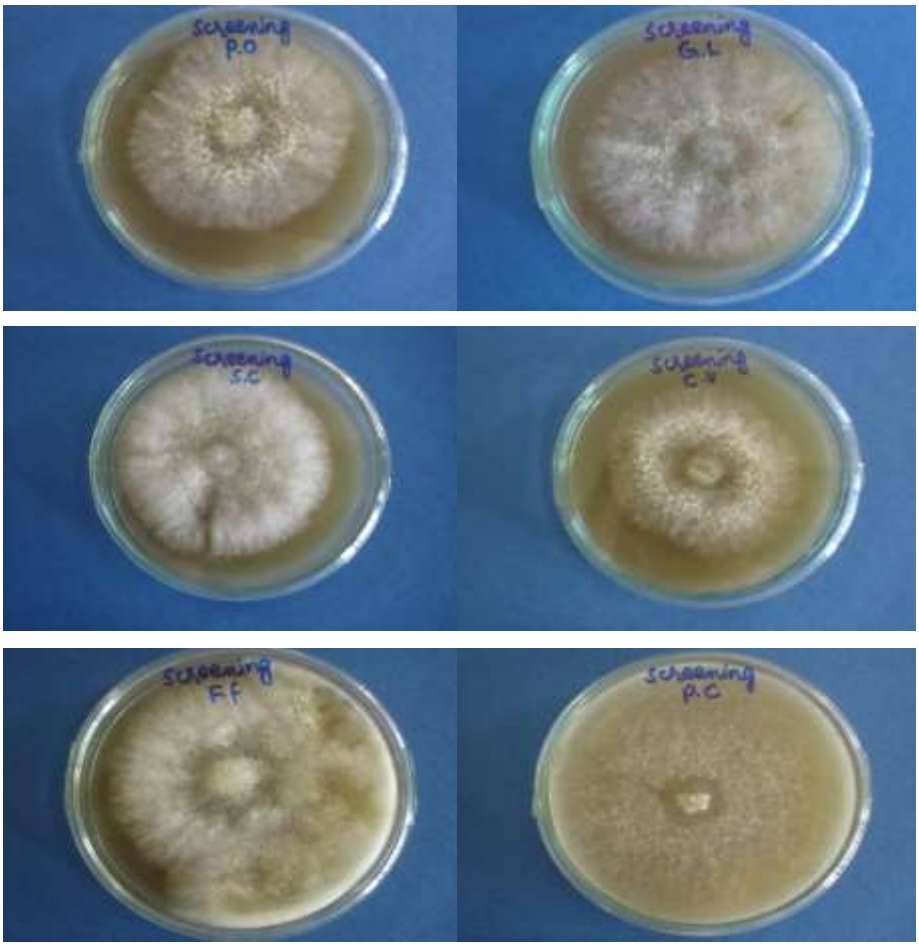

Fig. 2. Preliminary screening of white rot fungi *Pleurotus ostreatus* (PO), *Ganoderma lucidum* (GL), *Schizophyllum commune* (SC), *Coriolus versicolor* (CV), *Fomes fomentarius* (FF), *Phanerochaete chrysosporium* (PC), against waste-water of pulp and paper industrial effluent.

Screening of fungal strains capable of wastewater Decolourization

The six fungal strains were cultivated on Kirk media with Pulping wastewater containing lignin without glucose at room temperature for a period of time in order to confirm their capability of surviving in condition with lignin. Fig. 2 demonstrated that all six strains as indicated [*Pleurotus ostreatus* (IPP01), *Ganoderma lucidum* (IPP02), *Schizophyllum commune* (IPP03), *Coriolus versicolor* (IPP04), *Fomes fomentarius* (IPP05), *Phanerochaete chrysosporium* (IPP06)] were able to grow well in lignin as observed by their growing mycelium. The selected strains were tested for their effectiveness on decolourization of wastewater. It has shown that three (IPP01, IPP04, IPP06) out of six tested strains were the most efficient strains capable of lightening the wastewater color (Figure 1). The strains IPP01 and IPP06 decreased the color by about 47.8% and 47.0% from 1,016 Pt Co to 486 Pt Co and from 1,016 to 478 Pt Co respectively, while the color was reduced by strain IPP04 by 47.2% from 1,016 to 480 Pt Co. This revealed that the three fungal strains as mentioned above were able to decolorize the wastewater from pulping process or able to degrade lignin compounds in wastewater. However, the mechanism of this is still unknown and needs more research.

CONCLUSION

Starting with six mushroom samples collected from the Northern areas of Khanspur, Pakistan. These six fungal strains were obtained including white-rot fungi. Preliminary screening showed that three strains (IPP01, IPP04 and IPP06) were able to grow on Kirk media with Pulping wastewater containing lignin and decolorize the wastewater from pulp and paper process by 47.8%, 47.2% and 47.0% respectively. After being identified by basic morphology, the strains IPP01, IPP04 and IPP06 showed identity to Genus *Pleurotus* sp., *Coriolus* sp., and *Phenerochaete* sp., respectively. Accordingly, the next experiments would focus on determination of enzymatic activities and optimization of nutrients appropriate for fungal growth in bioreactor for the treatment of pulp and paper industrial effluent on large scale

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