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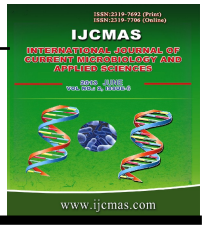

Original Research Article

Potential role of *Pleurotus ostreatus* in the decolorization and detoxification of the dye Synozol red

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A B S T R A C T

The present paper focuses on the use of fungus, *Pleurotus ostreatus*, to decolorize and degrade azo dye, Synazol Red. Decolorization study showed that *P. ostreatus* was able to decolorize 96% Synazol Red in 24 days. It was also found that 94% Synazol Red containing dye effluent was decolorized by *P. ostreatus* after 30 days of incubation at room temperature. The enzyme exhibited highest activity at 70°C and at pH 6.0. The absence of zone of inhibition on agar plates indicated that the fungal degraded dye metabolites are nontoxic to beneficial micro-flora. Therefore, *P. ostreatus* has promising potential in colour removal from textile wastewater containing azo dyes.

Keywords

Azo dyes; decolorization; *Pleurotus ostreatus;* bioremediation

Introduction

A great number of dyes and other chemicals are used in textile industry. There are more than 100,000 commercially available dyes with over 10,000 different dyes and pigments used in industries, representing an annual con-sumption of around 7×105 tonnes worldwide (Akhtar *et al*., 2005). Azo dyes represents the 70% of total dye produced per year and are considered most important.They are extensively used in textile dyeing due to their favorable characteristics such as superior fastness to the applied fabric, high stability resistance to microbial attack, water-fastness and simple application techniques. However, nearly 50% of reactive dyes may be lost in the effluent after the dyeing of cellulose fibers, and are highly recalcitrant to conventional waste water treatment processes (Aksu and Cagatay, 2006).

Effluents from the textile industries containing dye are highly colored and are therefore visually identifiable (Kilic *et al*., 2007). The discharge of these industrial effluents into aquatic ecosystems and their efficient removal from textile industry is still a major environmental challenge not only for aesthetic reasons, but also for the alteration of the solubility of gases in water, their bio-recalcitrant nature, and their effects on the ecosystem due to the toxic intermediates produced (mutagenic

and/or carcinogenic). In recent years, several microorganisms have been investigated for decolorization of reactive dyes, and the effectiveness depends on the adaptability and the activity of selected microorganisms (Kodam *et al*., 2005). The role of fungi in the treatment of wastewater has been extensively researched and due to an increased cell-to-surface ratio, fungi have a greater physical and enzymatic contact with the environment. Basidiomycetes are considered to be efficient laccase producers, (Martí-nez *et al.,* 2005) in particular, the white rot fungi produce large repertoire of extracellular lignin-modifying enzymes (Arora and Sharma, 2009) which are able to degrade and detoxify a wide range of xenobiotic compounds under aerobic conditions.

Laccase, EC 1.10.3.2, p-diphenol: oxygen oxido-reductase, is part of a larger group of enzymes termed the multicopper oxidases (MOC), (Komori *et al*., 2009) belonging to the group of blue-copper proteins. It is an important class of enzyme found in many organisms, including plants fungi, bacteria and humans (Augustine *et al*., 2008). This enzyme is generally extracellular and catalyzes the oxidation of several phenolic compounds, aromatic amines, thiols and some inorganic compounds using molecular oxygen as electron acceptor (Arora and Sharma, 2009) and have a great potential in various biotechnological processes. Fungal laccases have been confirmed for their ability to degrade several azo dyes (Husain, 2006).

The aim of the present work was to exploit the biodecolourization of Synazol Red by *P. ostreatus* with the following objectives: to assess the ability of the fungal cultures to decolorize and degrade the dye the actual dye industry waste, confirmation of degradation of the dye and to assess the toxicity of the degraded products.

Materials and Methods

Microorganisms and growth conditions

The fungus, *P. ostreatus*, was isolated from soil. The solid medium used for fungal growth contained per liter: 10 g of malt extract, 4 g of yeast extract, 4 g of glucose and 20 g of agar (pH 5.5). For laccase production and induction studies, 3 ml of homogenized mycelium were used for inoculation of 1000-ml Erlenmeyer flask containing 300 ml of culture medium having 20 mg /l of synazol red, glucose, 10 g; peptone, 5 g; yeast extract, 1 g; ammonium tartrate, 2 g; KH2 PO4, 1 g; MgSO4.7H2O, 0.5 g; trace elements solution, 1 ml. The pH was adjusted to 5.5.

Decolorization study

The fungus *P. ostreatus* was inoculated in the medium having a known concentration of the dye ,the medium was incubated at room temperature for a period of one month ,every week the dye concentration of the decolourized broth was quantified by comparing its absorbance with the absorbance of known concentrations of Synazol Red and this was used to calculate the dye removal rate (mg L-1) and expressed in percentage of decolorization (Chen *et al.,* 1999).

I - F Decolorization (%) × 100

Where, *I* is the initial absorbance and *F* is the absorbance of decolorized medium.

Decolorization of dye from industrial effluent

To check the efficacy of fungus to decolorize the dye from industrial effluent,

a laboratory-scale experiment was set up. Two plastic containers were taken. The first container, was labeled as treated and it contained 2 L of dye effluent along with 500ml of *P. ostreatus*. the second container was labeled as control and it contained , only 2 L of dye effluent (temperature, 33°C; pH, 7.6; dissolved oxygen, 0.154 ± 0.04 g /l was taken and 20 mg / L of Synazol Red stress was maintained in each container. The experiment was carried out at room temperature (28 ± 2°C). After 10, 20 and 30 days of incubation, samples were taken, centrifuged and supernatants were used to estimate the amount of Synazol in dye effluents by ultraviolet visible spectroscopic analysis by measuring the optical density at 463 (Khalaf, 2008). The percent decolourization was calculated by taking untreated dye solution as control (100%).

Enzyme assay

Laccase activity was determined by using azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as substrate at 420 nm. The measurements were made with 100 mM sodium acetate buffer (pH 5.0) at 30°C for 30 min. One unit of enzyme activity was defined as the amount of enzyme that oxidized 1 μmol of ABTS per min.

Effect of temperature and pH on the enzyme activity

The optimum temperature of the laccase was determined by incubating the reaction mixture for 30 min at different temperatures ranging from 30 to 90°C. The pH profile of the enzyme was evaluated by incubating the reaction mixture for 30 min at optimum temperature in the presence of appropriate buffers: 50 mM sodium acetate (pH 4.5 to 6.0), 50 mM sodium phosphate (pH 6.0 to 8.0), and 50 mM Tris-HCl (pH 8.0 to 10.0). The activity of each sample was quantified by the assay method as earlier described.

Microbial assay

The decolorized dye at the concentration of 100 mg L-1 was tested for its toxic effect on the agriculturally important soil bacterial flora according to Mali *et al*., (2000). *Bacillus cereus* and *Azotobacter* sp. were inoculated on minimal salt medium. Two wells of 2 mm diameter were made on the minimal salt medium containing plates. Both were filled with 1.0 mg/L of decolorized centrifuged broth. The plates were incubated at 30°C for 48 h. Zone of inhibition surrounding the well represented the index of toxicity.

Results and Discussion

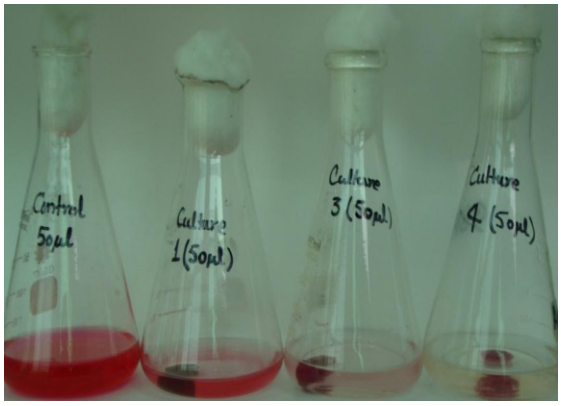
Decolorizing ability of the fungus

The synazol red decolorizing ability of the fungus was checked and it was found that as the period of incubation increased the rate of dye declorization also increased maximum decolorization (96%) was observed after 24 days of incubation. (Table 1, Figure 1).

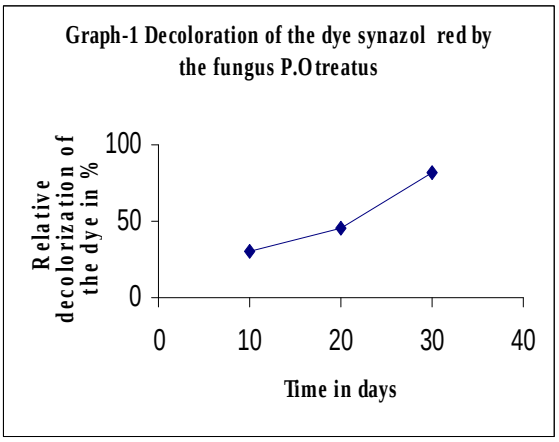
Table.1 % Decoloration of the dye Synazol red by the fungus *P.Otreatus*

Time period in days	% Decoloration
6	40
12	66
18	82
24	96

Figure.1 % Decoloration of the dye synazol red by the fungus *P.otreatus*



dye from the industrial effluent after 10, 20 and 30 days (Figure.1).



Bio-decolorization of dyeing wastewaters by microbial enzymes is a promising, eco-friendly and cost competitive alternative. Usman *et al*., (2011) described that *Corynebacterium* sp. could decolorize 60% (Reactive Black5) and 76% (Reactive Yellow15) from the medium containing dye at a concentration of 100 mg /l after two days. Degradation of azo dyes by filamentous fungi, such as white rot fungi have already been reported (Martins *et al*., 2001). Compared to other fungal oxidative enzymes, laccases can act oxidatively, non-specifically at the aromatic rings and has the potential to degrade a wide range of compounds. Laccases have gained much attention over the last number of years in many industrial and environmental fields due to their wide substrate specificity (Sadhasivam *et al*., 2009).

Decolorization of Synazol Red from industrial effluent

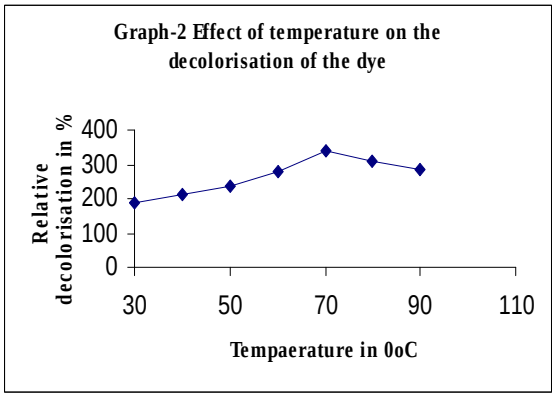
The ability of *P. ostreatus* to decolourize the dye Synazol Red from dye contaminated Industrial effluents, was done and it was observed that *P. ostreatus* was capable to decolorize 32, 48, and 94%

Ali e*t al*., (2008) reported that the decolorization of acid red 151, orange II, sulfur black and Drimarine blue K2RL was 68.64, 43.23, 21.74, and 39.45%, respectively by *Asper-gillus niger* in liquid medium under static condition. Jin et al. (2007) reported 89.9% optimum decolorization rate of reactive black RC, reactive yellow HF2-GL, reactive blue BGFN and reactive black B-150 at pH 3.0 after 48 h of incubation. Similarly, removal of Congo red from an aqueous solution by fungus *A. niger* was reported by (Fu and Viraraghavan, 2002).

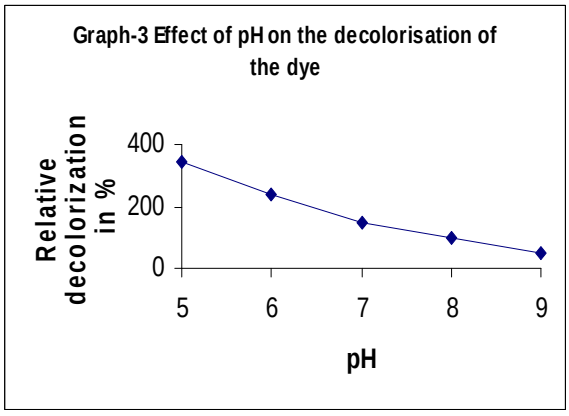
Effect of temperature on enzyme activity

Extracellular laccase produced by *P. ostreatus* was characterized with regard to pH optimum and thermostatbility. The activity of crude laccase isolated from culture filtrate of *P. ostreatus* was determined at various pH values and temperatures. *P. ostreatus* laccase activity was maximum at 70°C (348%) whereas at 30°C (218%), 40°C (254%), 50°C (290%) and 90°C (311%) (Figure 2). The temperature optimum of the laccase was 70°C with ABTS as a substrate in buffer

of pH 5. The optimum temperature of laccase I from *Pleurotus eryngii* was 65°C and that of laccase II from the same organism was 55°C (Munoz *et al.,* 1997). As in the present study, laccase from *P. ostreatus* showed maximum activity at 70°C. So it belongs to laccase 1 category of the enzyme. Zouari-Mechichi *et al.*, (2006) reported that *Trametes trogii* laccase in crude form showed optimum activity at pH 7 at room temperature for 24 h but retained more than 50% of its activity at pH 5. The laccase in the crude extract was also stable for 24 h at 50°C.



Effect of pH on enzyme activity

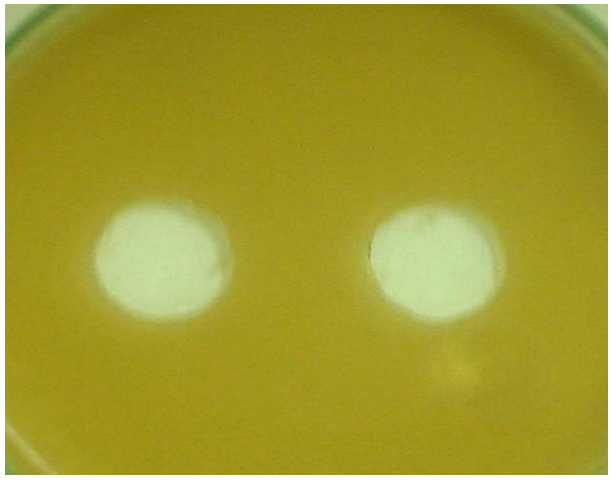


Experiments were performed to elucidate whether pH interfere laccase activity or not, and it was assessed that *P. ostreatus* laccase activity was maximum at pH 5 (350%). In contrast, pH 6 (256%), pH 7 (186%), pH 8 (100%), and pH 9 (58%) showed decrease in enzymatic activity (Figure 3). Jung *et al*., (2002) reported that laccase of *Trichophyton rubrum* was more stable at pH 6, although pH optima depend on the substrate used (Fukushima and Kirk, 1995). The activity of many laccases decrease beyond optimum pH (Jung *et al*., 2002), but this laccase showed a high relative activity over a broad pH range from 5 to 9. This could be a very useful characteristic for various industrial applications.

Toxicity assay

No zone of inhibition was observed in the treated dye, indicating that the biodegraded or decolourized products were non-toxic to the tested beneficial bacterial flora of the soil (Figure 4).

Figure.4 No zone of inhibition was observed around the well.



Brilliant green, fast green, methylene blue, and Congo red removal and their toxicity after biological treatment have been reported by Mali et al. (2000). Despite this fact, untreated dyeing effluents may cause serious environmental and health hazards.

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