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Biodegradation of Orange II dye by *Phanerochaete chrysosporium* in simulated wastewater

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This study presents decolorization of textile azo dye, Orange II, by white rot fungus, *Phanerochaete chrysosporium*. Orange II (85%) was removed in 7 days (optimum decolorization on 5th day at 28-30°C and pH 5.0) in liquid cultures under shaking aerobic conditions using *P. chrysosporium*. Higher dye concentration in simulated dye showed inhibitory effects on decolorization. Decolorization ability of fungus was correlated to lignolytic enzyme activity.

Keywords: Azo dye, Decolorization, Mn- peroxidase, *Phanerochaete chrysosporium*, White rot fungi

Introduction

Azo dyes account for most textile dyestuff produced and are most commonly used synthetic dyes in textile industry[1]. About 10-15% of dyes goes unused in textile effluents[2,3]. In mammals, azo dyes are reduced to aryl amines by cytochrome p450 and a flavin dependent cytosolic reductase[4]. Anaerobic breakdown of azo dyes can lead to reduction of azo bond producing mutagenic and carcinogenic compounds[5]. Most synthetic dyes are not degraded by conventional physical and chemical processes[6,7]. Fungi or their oxidative enzymes can decolorize textile wastewater either by adsorption of dye on fungal mycelium or by oxidative degradation of dye molecule[8]. White rot fungi have emerged as promising organism for treating color from wastewater[9,10].

Decolorization of azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes by white rot fungus *Phanerochaete chrysosporium* have been reported[11,12]. *P. chrysosporium* produces extracellular manganese peroxidase (MnP), which may be responsible for degradation of xenobiotic compounds from wastewater[13], effluent decolorization and bioleaching of kraft pulp[14-16]. MnP oxidizes Mn(II) to Mn(III), which is responsible for oxidation of compound like phenolic compounds[17]. *P. chrysosporium* is also able to produce lignin peroxidases (LiP) and laccases, which are also capable to degrade xenobiotic compounds[18-20]. Several reports[21,22] have shown that lignolytic enzymes are directly involved in degradation process. Zhang *et al*[23] reported high decolorization efficiency of azo dye Orange II using white rot fungus. Azo dyes degradation without formation of aromatic amines using white rot fungi have also been reported[24,25].

This paper reports ability of white rot fungus, *P. chrysosporium,* and its enzymes to decolorize Orange II in liquid systems, besides role of enzymatic activity on color removal.

Materials and Methods

Chemicals

PDB used for liquid fungal culture was purchased from Hi-Media (Bombay), and azo dye Orange II was procured from Nahar Fabrics, Lalru (Punjab) India. Chemicals used for enzyme assay (phenol red, H_2O_2, NaOH) were purchased from Loba - Chemicals (Bombay).

Microorganism and Culture Media

P. chrysosporium (MTCC 4955) culture was obtained from IMTECH, Chandigarh, India. Lyophilized culture was brought in laboratory and stored at 4°C.

To maintain fungal strain on potato dextrose agar (PDA), PDB (20 ¼l) was added to broken vial of pure

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culture and a loopful of inoculum was streaked on PDA plates. Further, strain was maintained on PDA slants and stored at 4°C prior to inoculating into liquid media. For liquid cultures, PDB media was used. PDA plates were incubated statically at 25°C for one week before use. Erlenmeyer flasks (250 ml) containing sterilized liquid dye (100 ml) containing medium were inoculated with 10 mm agar plugs taken from fungal colony growing on PDA plate and incubated in aerobic condition in an incubator shaker at 150 rpm for 7 days.

Decolorization Assay

Effect of pH (4.0-7.0) on biodegradation was studied and pH was maintained using 0.1 N HCl or 0.1N NaOH. Effect of temperature was studied for 24, 26, 28, 30, 32 and 34°C. Different concentrations of dyes (50, 100, 150, 200 and 250 mg/l) were prepared and studied. In all experiments, agitated liquid cultures were grown for 7 days in an incubator shaker. Samples (8 ml) were withdrawn at alternate days, centrifuged at 4000 rpm and supernatant was scanned at 486 nm (λ_{max} for Orange II) for absorbance in a UV-Visible spectrophotometer. All experiments were performed in duplicates. Controls were maintained without dye. Decolorization was calculated as

$$\text{Decolorization } (\%) = \frac{(C_0 - C_e)}{C_0} \text{X } 100$$

where, C_o is initial concentration of dye (mg/l) and C_e is residual dye concentration (mg/l) at different time intervals.

Enzyme Assays

MnP activity[26] was performed. For preparation of inocula, fungi were grown on PDA plates. Erlenmeyer flasks having liquid media were inoculated with mycelia plugs (cut from edge of actively growing colony). Cultures were incubated at 25°C for 7 days in an incubator shaker (150 rpm). Culture was filtered through muslin cloth and final volume was made 50 ml. Filtrate was centrifuged at 7500 rpm for 10 min to obtain crude enzyme for enzyme assay. For preparation of reaction mixture (1000 µl), 0.1 mmol Phenol Red (800 µl) was taken in test tube, crude enzyme (50 µl) was added to it and reaction mixture was incubated at 20°C for 15 min. Reaction started with 0.1 m mol addition of hydrogen peroxide (H_2O_2, 100 µl) in one set of test tube and one

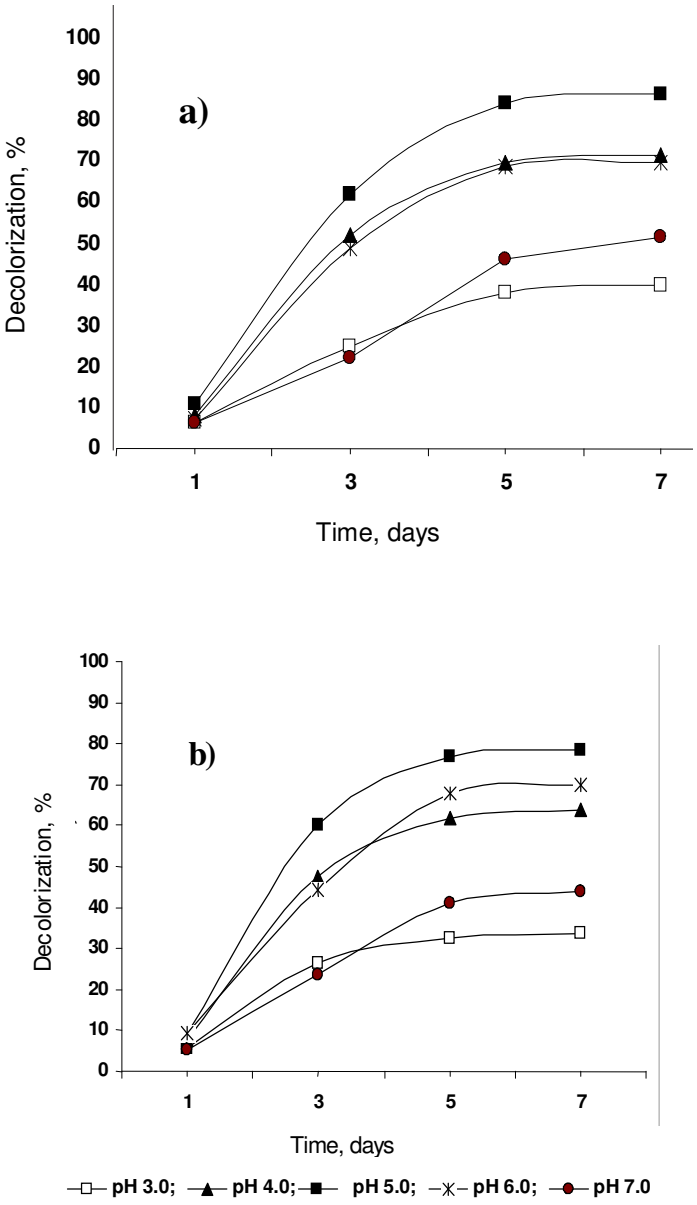

Fig. 1—Using *P. chrysosporium* in liquid medium at 30°C with varying time, effect of pH on decolorization of:
a) Orange II (conc., 50 mg/l);
b) Real wastewater

was kept without H_2O_2. Reaction was terminated with 4.0 M NaOH (50 ¼l). Enzymatic activity was determined spectrophotometrically. One unit of enzyme activity is expressed as the amount of enzyme required to oxidize 1.0 µmol of Phenol Red in one min. Activity in absence of H_2O_2 was subtracted from values obtained in presence of H_2O_2 to establish true peroxide activity. All experiments were carried out in dark. All treatments were run in triplicates.

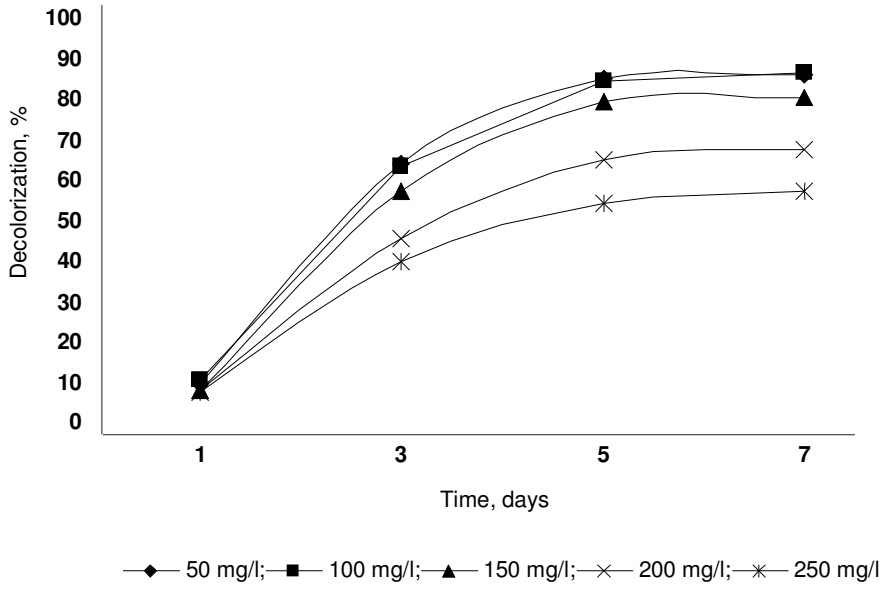

Fig. 2—Effect of initial dye concentration (mg/l) on decolorization of Orange II using *P. chrysosporium* in liquid medium at pH 5.0 and temperature 30°C with varying time intervals

Results and Discussion

Effect of pH on Decolorization of Orange II

Effect of pH (3.0-7.0) was investigated, keeping other parameters constant (temp 30°C and dye conc. 50 mg/l). As pH increased from highly acidic conditions (pH 3 to pH 4.0), decolorization increased from 39.84 to 71.56% (Fig. 1a). Optimum decolorization of dye (86.34%) was found at pH 5.0, with further decrease 69.56% and 51.42% for pH 6.0 and 7.0 respectively. Maximum removal of color was observed at 7th day for all studied pH. Since no significant change in removal of Orange II by studied fungus was observed after 5th day, thus 5th day was optimum decolorization.

Effect of Initial Dye Concentration on Decolorization of Orange II

Effect of varying dye concentration on dye degradation was investigated keeping other operational parameters constant (temp 30°C and pH 5). For 50 and 100 mg/l concentration of dye, removal was almost same but as concentration increased, removal of dye started decrease (Fig. 2). About 85% of dye was removed on 5th day of 100 mg/l of dye.

Effect of Temperature on Dye Removal

To explore temperature effect, experiments were performed at different temperatures (24-34°C) keeping other conditions constant (pH 5.0, dye conc. 50 mg/l). Initially there was an increase in dye degradation rate up to 30°C and afterward dye degradation decreased (Fig. 3a). Maximum degradation was observed between 28-30°C.

Decolorization Experiments with Textile Industry Wastewater

Untreated dark brown effluent from a local textile industry gave following physico-chemical values: total solids, 7.21 g/l; total carbon, 3.16 g/l; COD, 3268 mg/l; and pH 9.0. Maximum degradation (79%) was observed between 28-30°C and pH 5.0 (Fig. 3a). Maximum decolorization was recorded from 3rd to 5th day and afterward little change was recorded. A little decline in decolorization rate was observed when experiments were performed with real textile wastewater, might be due to presence of other pollutants in textile wastewater (Fig. 3b).

Mn-peroxidase Activity

MnP is involved in dye decolorization by *P. chrysosporium*. On, MnP activity was calculated as: 1st day, 43; 3rd day, 137; and 5th day, 249 U/l. After 7 days of incubation, liquid culture showed maximum MnP activity (265 U/l). *P. chrysosporium* has degraded a wide variety of compounds including an extensive number of azo dyes[27-29]. Biodegradation of azo dyes by *P. chrysosporium* is reported[30] to be based on activity MnP. Several products of lignin degradation could act as redox mediators and thus enhance

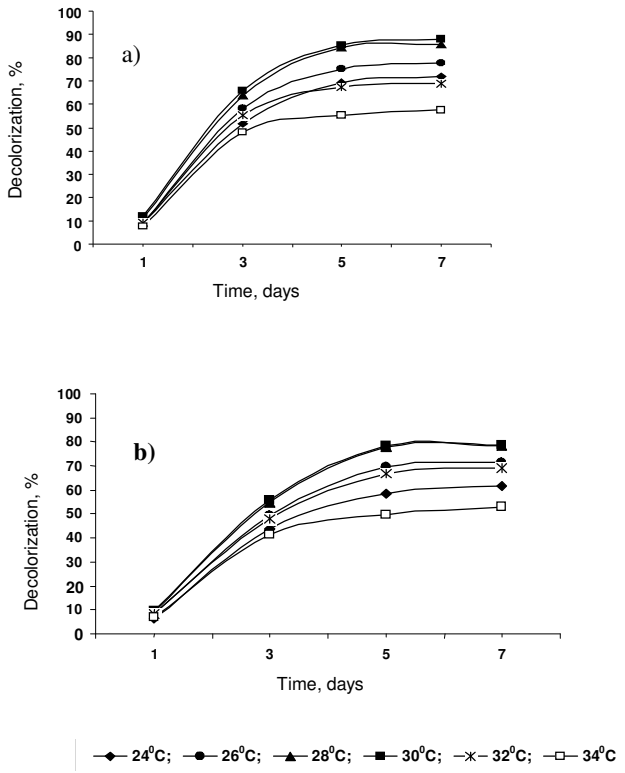

Fig. 3—Using *P. chrysosporium* in liquid medium at pH 5.0 with varying time, effect of temperature on decolorization of: a) Orange II (conc., 50 mg/l); b) Real wastewater

enzymatic base degradation of azo dyes[30]. In present study, degradation of Orange II was found dependent on temperature and pH. Fungus showed maximum degradation in slightly acidic conditions (pH 5.0). In highly acidic conditions, a decrease in decolorization rate may be due to decrease in enzymatic activity when pH changed from optimum levels. Dye concentration (50-100 mg/l), showed little change in decolorization rate, but higher dye concentration had inhibitory effect on fungus thereby reducing color removal. Orange II with three aromatic rings and one sulphonic group, has complex structure and it is very difficult to degrade such dye in higher concentrations. Small structural differences can affect decolorization owing to differences in electron distribution, charge density and steric factors [31]. Activity of MnP in *P. chrysosporium* cultures indicates that enzyme plays an important role in degradation of Orange II. Fungus has an enormous potential for biodegradation of wastewater of a local textile industry.

Conclusions

Temperature, pH and initial dye concentration had a major influence on dye removal by white rot fungus *P. chrysosporium*. Ezymatic activity of selected fungus is possible mechanism involved in removal of dye. Thus any bioprocesses based dye removal system using such type of fungus should be design on the basis of these parameters for successful operation.

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