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SYNTHETIC DYE DECOLOURISATION BY WHITE ROT FUNGUS, *TRAMETES CINGULATA*

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ABSTRACT

Many synthetic dyes present in industrial wastewaters are resistant to degradation by conventional treatments. Decolourisation of four synthetic azodyes by indigenous strain of white rot fungus isolated from outskirts of Mumbai, identified as *Trametes cingulata*, was investigated. The broad-spectrum decolourisation efficiency of the isolate was assessed using chemically different dyes. The fungus showed high decolourisation capacity and was able to decolourise all dyes tested, at different rates. Maximum decolourisation was achieved within four days in a reactor. The isolate was further evaluated for the decolourisation of industrial effluent. Complete decolourisation was achieved within five days of incubation.

INTRODUCTION

Synthetic dyes are extensively used for textile dyeing and other industrial applications. A very large amount of dyestuffs enters the environment through industrial effluents which are toxic and are characterized by presence of high COD, BOD, suspended solids and intense colour (Correia et al. 1994). Furthermore, these coloured molecules are highly conjugated and can be extremely injurious to life (Michaels & Lewis 1985).

Synthetic dyes, classified by their chromophores, have different and stable chemical structures to meet various colouring requirements and often are not degraded and/or removed by conventional physical and chemical processes (Robinson et al. 2001b, Ahn et al. 1999). Moreover, many of these compounds are highly resistant to microbial attack and, therefore, are hardly removed from effluents by conventional biological processes such as activated sludge treatment (Chung & Stevens 1993, Wong & Yuen 1996).

White rot fungi are well known for their outstanding ability to produce extra cellular oxidative enzymes, which are involved in the degradation of lignin in their natural lignocellulosic substrates (Kirk & Farrell 1987). The same unique non-specific mechanisms allow them to degrade lignin and a wide range of pollutants (Barr & Aust 1994). This ability has opened new prospects for the development of biotechnological processes aimed at the degradation of xenobiotic compounds (Field et al. 1993), effluent decolourisation (Palma et al.1997) and biobleaching of kraft pulp (Moreira 1997).

Decolourisation of mono-azo and di-azo dyes has been demonstrated using *Phanerochaete chrysosporium* (Paszczynski et al. 1991), *Pycnoporus cinnabarinus* (Schliephake et al. 1993), *Trametes versicolor* (Swamy & Ramsay 1999), *Bjerkandera adusta* (Heinfling et al. 1998), among others. The main limitations of such an approach are that decolourisation is slow, typically requiring several days. Additionally, the sludge volume increases due to the generation of biomass. The use of enzymes may represent an attractive alternative option for dye decolourisation due to their

biocompatibility and the ease and simplicity of process control (Karam & Nicell 1997).

Enzymes associated with lignin degradation are laccases, lignin peroxidases and manganese peroxidases. While some white rot fungi produce all three classes of enzymes, others produce only one or two of them (Hatakka 1994). Laccases are multicopper enzymes, which catalyse the oxidation of phenolic compounds including a range of dyes with concomitant reduction of oxygen (Eggert et al. 1996). Recent interest in laccase is, in part, a consequence of the findings that the substrate range of laccase can be expanded to include non-phenolic dyes, eventually in the presence of suitable mediators (Bourbonnais & Paice 1990). Recent studies reported the oxidation of mono and diazo-dyes by purified laccases from *Pyricularia oryzae* (Chivukula & Renganathan 1995).

Dyes such as Reactive blue, Reactive orange, Ramazol black and Congo red are the most important and commonly used dyes in textile industries. Here, we have established the role of laccase, from white rot fungus isolated and identified taxonomically in our laboratory as *Trametes cingulata*, to degrade the above dyes and untreated effluent samples collected from textile units from in and around Mumbai.

MATERIALS AND METHODS

The microorganism: The fungus used in this study was *Trametes cingulata* isolated from the suburbs of Mumbai.

Chemicals: Synthetic dyes were obtained from Raymonds Ltd. textile company, and other chemicals used in fungal cultures were purchased from Sigma.

Storage of organism: The fungus was preserved on Potato Dextrose agar plate. New plates were inoculated once a month to ensure viability of the isolates.

Production of enzymes: The medium for cultivation of *Trametes cingulata* contained 4.5% (wt/vol) wheat bran, 1.5% yeast extract, 1% glucose, 0.25% NH_4Cl, 0.05% thiamine dichloride, 0.2% KH_2PO_4, $MgSO_4.7H_2O$, 0.01% $CaCl_2$ and 0.05% KCl. Tap water was used for preparation of the medium, and the pH was adjusted to 5.5 by using NaOH or HCl. Incubation was carried out at 28°C on a rotary shaker (150rpm) in cotton-plugged 250 mL flask containing 100 mL of media. Flasks were inoculated with 1cm square agar pieces from an actively growing fungus on PDA agar. Cultures were harvested after 7 days, filtered and clarified by centrifugation at 8,000 rpm for 20 min to remove the mycelia. The enzyme in the filtrate was assessed and further partially purified.

Purification of laccase enzyme: The mycelium was removed by filtration and the medium was collected. The extracellular proteins were precipitated with ammonium sulphate (up to 95% w/v saturation). The precipitate was dissolved in sodium phosphate buffer at pH 5 and was extensively dialysed against several volumes of the same buffer.

Dialyzate containing laccase was purified on DEAE cellulose column equilibrated with the sodium phosphate dialysis buffer pH 5 and applying a linear salt gradient of 0.0-0.5M NaCl. The fractions were collected at a flow rate of 30mL/hr (Antorini et al. 2002).

Enzyme assay: Laccase activity was determined using 2, 6,-dimethoxyphenol (DMP) as a substrate as described before. The reaction mixture contained 1mM DMP in 50mM sodium malonate (pH 4.5). The formation of 2,2'6,6'-dimethoxyphenoquinone (orange/brown colour) at 30°C was followed spectrophotometrically at 468nm, and laccase activity was calculated from the molar extinction coefficient (E) of 49.6mM cm^{-1} (de Jong et al. 1992).

Microbial treatment of textile dyes and dyeing effluents: *Trametes cingulata* was cultivated as described above and the mycelium was collected by filtration under aseptic conditions and washed thrice with 500mL of sterilized distilled water. A sample of 1g (wet weight) of mycelium was incubated for 8 days with different dyes with concentration ranging from 100mg/L to 1000mg/L. Sterile controls without inoculum were also maintained under the same conditions. Growth of the fungus was inhibited with antibiotics to determine whether the decolourisation was due to metabolic activity of the organism or due to other phenomena.

After 8 days, all incubation mixtures were filtered using 0.45µm pore size filter paper; the decolourisation efficiency was determined spectrophotometrically at the absorption maximum of each dye. Adsorption of dyes to the mycelium was determined by solubilisation of the dyes with water. Adsorbed dye was washed off the mycelium twice with 20mL of water.

Enzymatic treatment of textile dyes in lab bioreactor: Cylindrical reactors with conical bottoms were used for continuous experiments with laccase from *Trametes cingulata*. The reactors had a liquid volume of one litre. They were autoclaved and then filled with laccase immobilized sodium alginate beads (Park et al. 2006). The dye solutions with varying concentrations were added into reactors. The column reactors were kept at room temperature.

An additional reactor with same features as mentioned above was used in order to study decolourisation of dyes in textile wastewater from 4 textile units in the state of Maharashtra, India. The reactor was filled with textile wastewater from the dyeing industries. The pH of the textile wastewater was set between 4-7, the optimal pH range for fungal enzymes. Untreated effluents were also subjected to chemical analysis before pouring in the reactor. The experiment was continued for 5 days. All the reactor experiments were performed at room temperature.

RESULTS AND DISCUSSION

The ability of white rot fungi to decolourise synthetic textile dyes has been extensively studied, particularly with *Phanerochaete chrysosporium* and *Trametes versicolor* (Revankar & Lele 2007). The aim of the current study was to investigate the ability of a poorly studied white rot fungus, *Trametes cingulata*, which was isolated from the outskirts of Mumbai and found to decolourise industrially important synthetic dyes. The role of enzymes of *Trametes cingulata* in decolourising untreated effluent samples from textile units has also been explored here. It was found that DEAE cellulose purified enzyme from *Trametes cingulata* had a laccase activity of 11.5U/mL.

Trametes cingulata was capable of decolourising more than 80% of four commercially used textile dyes when screened with dye concentration in the range of 100mg/L to 1000mg/L and real

Table 1: Chemical analysis of the untreated effluent samples.

Effluent Sample	Chemical Oxygen Demand in mg/L	Dissolved Oxygen in mg/L	pH	Total Suspended Solids in mg/L	Total Dissolved Solids in mg/L
1	600	7.12	10.43	638	2806
2	360	5.30	11.00	1260	6930
3	544	7.23	7.23	726	8684
4	108	7.12	7.12	58	1190

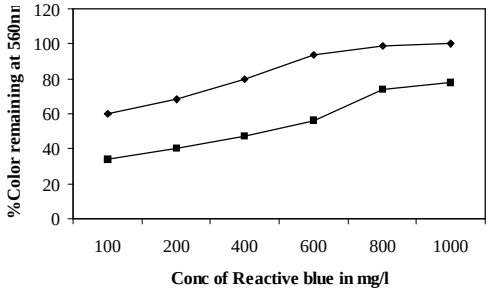

Fig. 1a: Decolourisation of Reactive blue by mycelia of *Trametes cingulata*. Percentage colour remaining on day 1 and on day 8 at different dye concentrations are plotted at 560nm.

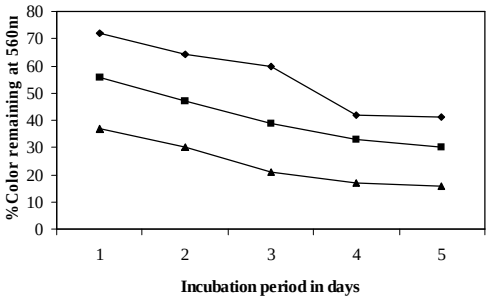

Fig. 1b: Rate of decolourisation of Reactive blue by immobilised enzyme. Percentage colour remaining at 1000mg/L at 600mg/L at 100mg/L of reactive blue after incubation at different time intervals.

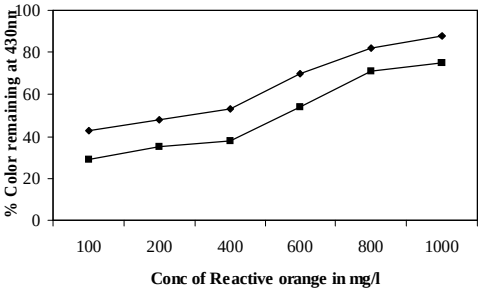

Fig. 2a: Decolourisation of Reactive orange by mycelia of *Trametes cingulata*. Percentage color remaining on day 1 and on day 8 at different dye concentrations are plotted at 430nm.

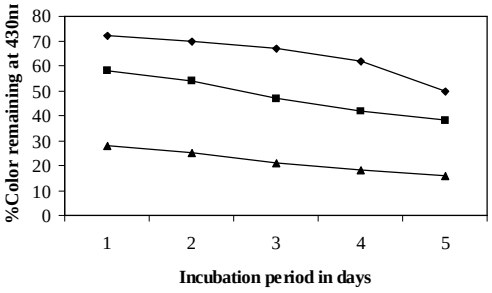

Fig. 2b: Rate of decolorisation of Reactive Orange by immobilised enzyme. Percentage colour remaining at 1000mg/L at 600mg/L at 100mg/L of reactive orange after incubation at different time intervals.

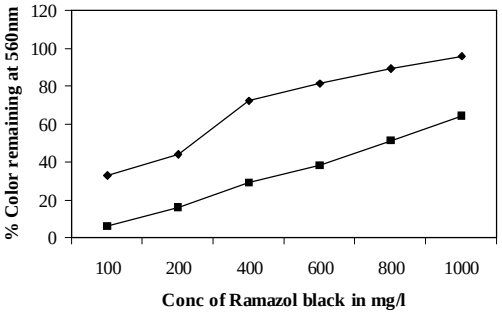

Fig. 3a: Decolorisation of Ramazol black by mycelia of *Trametes cingulata*. Percentage color remaining on day 1 and on day 8 at different dye concentration are plotted at 560nm.

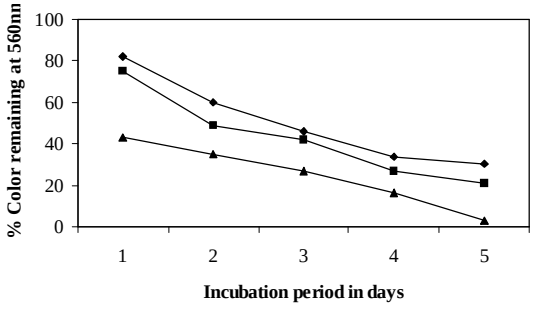

Fig. 3b: Rate of decolorisation of Ramazol black by immobilised enzyme. Percentage colour remaining at 1000mg/L at 600mg/L at 100mg/L of ramazol black after incubation at different time intervals.

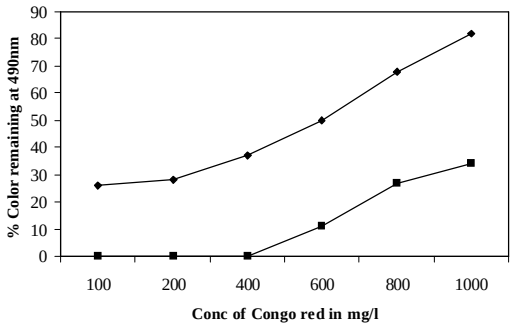

Fig. 4a: Decolorisation of Congo red by mycelia of *Trametes cingulata*. Percentage color remaining on day 1 and on day 8 at different dye concentration are plotted at 490nm.

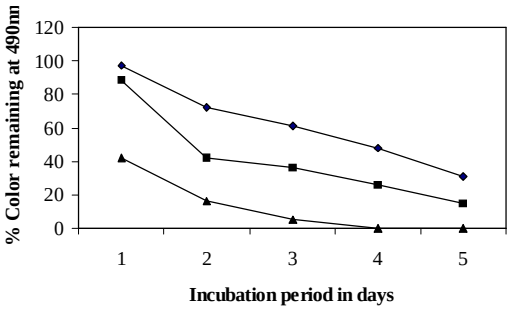

Fig. 4b: Rate of decolorisation of Congo red black by immobilised enzyme. Percentage colour remaining at 1000mg/L at 600mg/L at 100mg/L of congo red after incubation at different time intervals.

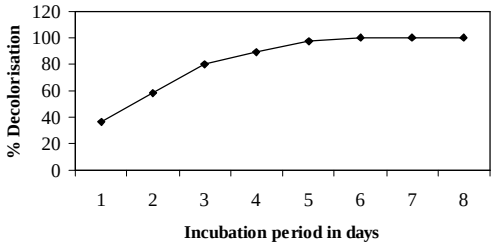

Fig. 5a: Decolourisation of effluent sample by mycelia of *Trametes cingulata*. Percentage decolorisation of effluent sample at different time intervals.

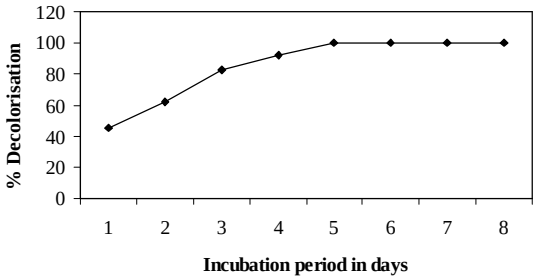

Fig. 5b: Rate of decolourisation of effluent sample by immobilised enzyme. Percentage decolourisation of effluent sample at different time intervals.

textile effluent under stationary incubation condition. *Trametes cingulata* was found to be more efficient in decolourising synthetic dyes within 5 days as compared to other white rot fungus such as *Lintinula edodes* (Boer et al.2004), *Phlebia tremellosa* (Kirby et al. 2000), *Trametes trogii* (Mechichi et al. 2006), *B. adusta* (Robinson et al. 2001a), *Dichomitus squalens, Ischnoderma resinosum* and *Pleurotus calyptrates* (Eichlerova et al. 2005) which require between 7 to 20 days to achieve greater than 80% decolourisation of diverse synthetic dyes with 100mg/L dye concentration. Earlier studies have shown that *Phlebia tremellosa* could decolourise eight synthetic textile dyes (200mg/L) by greater than 70% over a period of 14 days under stationary incubation condition whereas *Lentinula edodes* took 18 days, *Trametes trogii* took 12 days and *Bjerkandera adusta* took 7 days for decolourising 100mg/L concentration of textile dyes.

In this study azo and anthraquinonic reactive dyes such as Reactive blue, Reactive orange, Ramazol black and Congo red were decolourised by immobilized enzymes and mycelium of *Trametes cingulata*. The results obtained by inoculation of mycelia as well as with DEAE cellulose purified immobilised laccase enzyme, following incubation at room temperature (28°C) for 8 and 5 days respectively are shown in Figs. 1-5. The percentage colour remaining of the dye has been plotted as

a function of the time of incubation. It was observed that Reactive blue and Reactive orange showed almost similar pattern of decolourisation of dyes with mycelium as well as with immobilized enzymes, suggesting that extracellular enzyme seem to be mainly responsible for decolourisation of dyes.

Standard solution of dyes in distilled water with varying concentration from 100mg/L to 1000 mg/L were prepared and used for decolourisation study. Incubation of dye solutions with mycelium for 8 days at room temperature (28°C) showed 16% colour remaining with 100mg/L of Reactive blue, 56% with 600mg/L and 78% with 1000mg/L of dye (Fig. 1a). Reactive orange showed 29% colour remaining with 100mg/L, 54% with 600mg/L, and 75% with 1000mg/L following 8 days of incubation (Fig. 2a).

Ramazol black and Congo red were found to be degraded faster than Reactive blue and Reactive orange by both mycelia and immobilized extracellular enzyme in the reactor. Ramazol black showed 6% colour remaining with 100mg/L and 64% with 1000mg/L on day 8 of incubation (Fig. 3a). However, Congo red showed complete decolourisation on day 8 with 100mg/L of dye; whereas for 600mg/L and 1000mg/L of dye 11% and 34% colour remained at the end of 8 days (Fig. 4a).

Continuous decolourisation was also examined with immobilised enzyme in reactor. The extra cellular enzyme was immobilized with sodium alginate beads, which were then packed in laboratory reactor. The reactor was further filled with varying concentrations of dyes and percentage colour remaining was thereafter monitored for subsequent period of 5 days. The extent of decolourisation was found to vary depending upon the concentration of dye.

Among the four reactive dyes Congo red seemed to be degraded faster as it showed 100% decolourisation within four days of incubation (Fig. 4b) followed by Ramazol black, which showed about 97% decolourisation within four days (Fig. 3b). Reactive orange and Reactive blue, both showed 80% decolourisation on day four of incubation (Fig. 2b and Fig. 1b).

Further studies were also performed under similar experimental conditions with four untreated effluent samples, various parameters of which were analysed as shown in Table 1. It was observed that, both mycelia and immobilized enzyme from *Trametes cingulata,* were capable of bringing complete decolourisation of the effluent samples (Fig. 5a and 5b), although the process was found to be slow with effluent samples, which had not been neutralized from their alkaline pH. These samples may contain various dyeing chemicals, COD value, hardness, etc., which directly or indirectly affect the decolourisation process.

Earlier studies with white rot fungi such as *Phanerochaete chrysosporium, Trametes hispida* and *Pleurotus ostreatus* have shown decolourisation of textile dyes or colored effluents. However, most of these evaluations have been done with mycelial suspension cultures.

This is the first independent study, which showed that white rot fungi *Trametes cingulata* isolated from the outskirts of Mumbai is capable of efficiently decolourising a range of industrially important dyes and real wastewater from textile units. Decolourisation of dyes is an important environmental issue and the low cost of laccase production, makes it a promising tool for application in the textile effluent degradation process.

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