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Biological decolourization of textile and paper effluents by *Pleurotus florida* and *Agaricus bisporus* (White-rot basidiomycetes)

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Summary

The extracellular ligninolytic enzymes of white-rot fungi are thought to catalyse the initial steps during the degradation of highly complex compounds like lignin or polycyclic aromatic hydrocarbons. We studied the ability of *Pleurotus florida* isolated from the foothills of the Western Ghats, India to decolourize the three dyestuffs, Reactive Green, Yellow and Blue, which are widely used in the textile industry around Coimbatore, Tamil Nadu, India. The crude culture filtrate of *Pleurotus florida* when incubated with different concentrations of dye decolourized it efficiently on the third day. The highest colour removal was found in the case of Reactive Blue. However, when *Agaricus bisporus* extract was supplemented with *Pleurotus florida* filtrate, the efficiency increased. The dye decolourization was advanced to the second day and the efficiency of dye decolourization of Reactive Yellow was 89% followed by Reactive Green, which was 45% when a dye concentration of 0.5% was used. *Pleurotus florida* filtrate alone and in combination with *Agaricus bisporus* extract reduced the aromatic compounds in textile and paper industry effluents on the first day with >90% efficiency.

Introduction

Colour can be removed from wastewater by chemical and physical methods (Lin & Peng 1994, 1996). These methods are quite expensive and have operational problems (Kapdan & Kargi 2002). White-rot fungi can degrade a wide variety of recalcitrant compounds by their extracellular ligninolytic enzyme systems (Wesenberg *et al*. 2003; Baldrian 2004). There are several advantages of using white-rot fungi for decomposition of recalcitrant compounds (Royer *et al*. 1991; Glenn & Gold 1993). Since the enzymes are extracellular, the substrate diffusion limitation into the cell generally encountered in bacteria, is not observed. Further, the extracellular enzyme system enables white-rot fungi to tolerate high pollutant concentrations (Kapdan & Kargi 2002). White-rot fungi have been used for the decomposition of several recalcitrant compounds in different types of reactors (Schliephake *et al*. 1993; Kapdan *et al*. 2000; Rodrignez Couto *et al*. 2004).

In the light of the aforementioned studies, this study was designed to investigate the effect of *Pleurotus florida*, an isolate obtained from the Western Ghats, Southern India and *Agaricus bisporus*, a locally cultivated edible mushroom on decolourization of textile dye-containing wastewater and paper industry effluent.

Materials and methods

Organism

Pleurotus florida was isolated from the foothills of Nilgiris and maintained in Potato-Dextrose-Agar slants (PDA) at 4 °C. The fungus was transferred to PDA plates and incubated for 7–8 days at 27 °C. Inoculum containing 10^6 spores/ ml was prepared and was transferred to a sterilized 250-ml Erlenmeyer flask containing 10 g wheat bran (obtained from local market) moistened with distilled water (1:1, w/v). The flasks were incubated at 27 °C for 15–20 days.

Crude culture filtrate was obtained by adding 30 ml of distilled water to the flasks and filtering through a muslin cloth. The filtrate was again centrifuged at 10,000 × *g* for 10 min. The supernatant was used as the enzyme source.

Agaricus bisporus was obtained from local market and extracted by taking 10 g and mashing thoroughly in

distilled water, filtered using a muslin cloth and centrifuged at 10000× *g* for 10 min. The supernatant was used as the enzyme source.

Enzyme assay

Extracellular culture fluids were assayed for enzyme activity.

Laccase activity was determined by measuring the oxidation of 3 mM 2,2′-azino bis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) in glycine–HCl buffer (pH 3.0) at 420 nm (Eggert *et al*. 1996).

In some experiments culture supernatant was preincubated with catalase (1000 units ml^{-1}) (E.C.1.11.1.6 from *Aspergillus niger*, Sigma C3515) for 30 min at 30 °C prior to assay in order to remove any endogenous hydrogen peroxide (Pointing *et al*. 2000).

Manganese-independent peroxidase activity was measured by adding hydrogen peroxide (1 mM final concentration) to the laccase assay mixture and subtracting the activity due to laccase alone (Pointing *et al*. 2000).

Lignin peroxidase activity was determined by measuring the production of veratraldehyde from veratryl alcohol at 310 nm in glycine–HCl buffer (pH 3.0) at 30 °C, upon addition of hydrogen peroxide (1 mM concentration) (Kirk *et al*. 1986)

Aryl alcohol oxidase activity was assayed under the same conditions without the addition of hydrogen peroxide. Manganese-dependent peroxidase activity was measured by following the oxidation of phenol red at 431 nm in the presence of 100 μM $MnSO_4{\cdot}5H_2O$ in glycine–HCl buffer (pH 3.0) at 30 °C, upon addition of hydrogen peroxide (0.5 mM final concentration) (Pointing *et al*. 2000).

Tyrosinase activity was measured by the following oxidation of L-DOPA at 475 nm using sodium phosphate buffer (pH 7.0) at 30 °C (Karam & Nicel 1997).

All enzyme assays were carried out using a Beckman DU® 530 u.v.–visible spectrophotometer. Enzyme activities were expressed as units, with one unit defined as that catalyzing the formation of 1μ mol product min^{-1}.

Decolourization studies

Dyestuffs used

Reactive Blue, Reactive Yellow and Reactive Green were a kind gift from 'Together Textile mills', Kanuvai, Coimbatore, Tamil Nadu. These dyes are extensively used in and around textile industries located in Coimbatore. Different concentrations of dye stuff solutions were prepared by dissolving the powdered dye stuff in distilled water effluents.

Textile effluent was collected from Together Textile mills, Kanuvai, Coimbatore, Tamil Nadu. Papermill effluent was collected from Tamil Nadu Paper Mills Limited (TNPL), Erode, Tamil Nadu.

The crude filtrates of *Pleurotus florida* and *Agaricus bisporus* were checked for decolourization of dye stuff as well as textile and paper effluents. The concentrations of dyes used in the present study were 0.1–0.5%. The wavelength scanning was used to identify the λ_{max} of each of the dyes used. They are, for Reactive Blue ($\lambda_{602\ nm}$), Reactive Yellow ($\lambda_{401\ nm}$) Reactive Green ($\lambda_{620\ nm}$), papermill and textile effluents ($\lambda_{220\ nm}$).

Results and discussion

The crude filtrate of *Pleurotus florida* showed activity of laccase (0.20 U/ml), Mn-independent peroxidase (0.012 U/ml), Mn-dependent peroxidase (0.01 U/ml) and lignin peroxidase (0.09 U/ml). Aryl alcohol oxidase activity was found to be negligible. These enzyme have been implicated in dye decolourization as well as for effluent treatment (Graca & Soares 2002; Shin 2004). *Agaricus bisporus* showed tyrosinase activity (1.6 U/ml), which again plays an important role in oxidation of phenolic compounds (Duckworth & Coleman 1970; Karam & Nicel 1997). In the present study the crude filtrate (200 μl) of *Pleurotus florida* decolourized the dye studied to a greater extent on the third day of incubation (Table 1). However, when a combination of *Pleurotus florida* and *Agaricus bisporus* extract were used, the decolourization was more rapid (Table 2). *Pleurotus florida* filtrate when used individ-

Table 1. Treatment of dyes and effluents using *Pleurotus florida* filtrate.

Dye decolourization (%)	Dyes used									Effluent	
	Reactive Blue (%)			Reactive Yellow (%)			Reactive Green (%)			Papermill	Textile
	0.1	0.2	0.5	0.1	0.2	0.5	0.1	0.2	0.5		
Day 1	75	60	45	25	20	10	20	16	10	>90	>90
Day 2	85	80	62	55	50	30	25	20	15		
Day 3	92	91	80	75	60	50	42	25	20		

The experiment was conducted in duplicate. Decolourization of dyestuffs was carried out using 0.1–0.5% (w/v) concentrations. About 0.2 ml of crude culture filtrate of *Pleurotus florida* was used. The extract contained 0.058 mg of protein per ml.

Table 2. Treatment of dyes and effluents using combination of *Pleurotus florida* and *Agaricus bisporus* extracts.

Dye decolourization (%)	Dyes used									Effluents	
	Reactive Blue (%)			Reactive Yellow (%)			Reactive Green (%)			Papermill	Textile
	0.1	0.2	0.5	0.1	0.2	0.5	0.1	0.2	0.5		
Day 1	85	80	62	55	50	30	30	25	20	>90	>90
Day 2	91	90	80	92	90	89	75	60	45		

The experiment was conducted in duplicate. Decolourization of dyestuffs was carried out using 0.1 to 0.5% (w/v) concentrations. 0.2 ml of crude filtrate of *Pleurotus florida* and *Agaricus bisporus* were used (1:1, v/v).

ually or in combination with *Agaricus bisporus* extract completely decolourized both paper and textile effluents within 1 day. The presence of dyes in wastewater is an important environmental problem, which cannot be easily solved. The low cost of *Pleurotus florida* and *Agaricus bisporus* extracts containing various enzymes as a cocktail may prove to be an alternate source of decolourization and an efficient method of treating waste water containing dye stuff as well as efficiently treating textile and paper effluents.

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