Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 98 (2007) 3638–3642
BIORESOURCE TECHNOLOGY

Short Communication

Biodegradation of Reactive blue-25 by *Aspergillus ochraceus* NCIM-1146

G.K. Parshetti, S.D. Kalme, S.S. Gomare, S.P. Govindwar *

Department of Biochemistry, Shivaji University, Kolhapur-416 004, India

Received 18 July 2006; received in revised form 6 November 2006; accepted 8 November 2006
Available online 3 January 2007

Abstract

The present study dealt with the decolorization and degradation of textile dye Reactive blue-25 (0.1 g l^{-1}) by mycelium of *Aspergillus ochraceus* NCIM-1146. Spectrophotometric and visual examinations showed that the decolorization was through fungal adsorption, followed by degradation. Shaking condition was found to be better for complete and faster adsorption (7 h) and decolorization (20 days) of dye Reactive blue-25 (100 mg l^{-1}) as compared to static condition. Presence of glucose in medium showed faster adsorption (4 h) and decolorization of dye from bound (7 days) mycelium. FTIR and GCMS analysis study revealed biodegradation of Reactive blue-25 into two metabolites phthalimide and di-isobutyl phthalate.

Keywords: *Aspergillus ochraceus*; Decolorization; GCMS; Reactive blue-25; Dye degradation

1. Introduction

Presence of different chromophoric groups like azo, triphenyl methane and phthalocyanine are responsible for the structural diversity of dyes (Banat et al., 1996; Ambrosio and Campos-Takaki, 2004; Christie, 2001). In addition to their visual effect and adverse impact in terms of chemical oxygen demand (COD), many synthetic dyes show toxic, carcinogenic and genotoxic effects (Ozfer et al., 2003). The available wastewater treatment systems are unable to remove the recalcitrant dyes completely and other organic residues from such effluents (McMullan et al., 2001). Several developments in recent years have made bioremediation as pollution control tool to decontaminate polluted soil or water. Among the various biological mechanisms of dye decolorization that have been reported in the literature, bioadsorption along with biodegradation seems to have greater potential for industrial application (Conneely et al., 1999). Bacterial degradation of these dyes requires their intracellular uptake while the fungi degrade these by their extracellular enzymes (Wesenberg et al., 2003). In recent years, a number of studies have focused on some fungi, which are able to biodegrade, and biosorb dyes in wastewater including both lignolytic and non-lignolytic fungi (Cha et al., 2001; Ambrosio and Campos-Takaki, 2004; Eichlerová et al., 2006). To date, most of the research concerning bioremediation with these fungi has centered on a single species *Phanerochaete chrysosporium*, which is known to metabolize a wide range of xenobiotic compounds (Cameron et al., 2000). Non-ligninolytic fungi from basidiomycete group, like various *Aspergillus* species have been reported to decolorize various dyes. Strain of *Aspergillus sojae* B10 has shown decolorization of the azo dyes amaranth, congo red and sudan III in nitrogen poor media after 3–5 days of incubation (Ryu and Weon, 1992). Several other wood rotting fungi like *Aspergillus fumigatus* G-2–6 and *Aspergillus oryzae*, capable of decolorizing a wide range of structurally different dyes were isolated and found more effective than *P. chrysosporium* (Knapp et al., 1995). Our earlier report showed effective decolorization

* Corresponding author. Tel.: +91 231 2609152; fax: +91 231 2691533.
E-mail address: spg_biochem@unishivaji.ac.in (S.P. Govindwar).

0960-8524/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2006.11.017

of textile dyes viz. malachite green and cotton blue using 96 h grown mycelium of *Aspergillus ochraceus* NCIM-1146 (Saratale et al., 2006).

Reactive blue-25 is reactive dye based on copper phthalocyanin (Cu PC) as chromophore and a monochlorotriazine as a reactive site. Reactive blue-25 is a reactive dye widely used in the textile industry to color natural fibers. Some of the work has been done in the field of fungal degradation of related pthyalocyanine dyes (Heinfling et al., 1997; Wang and Yu, 1998; Conneely et al., 1999, 2002). Zhou and Zimmermann (1993) have reported the decolorization of Cu PC dyes by actinomycetes. *P. chyrsosporium* was capable of decolorizing the nickel phthalocyanin complex, reactive blue (200 mg l^{-1}) and Cu PC complex reactive dye, reactive violet by 90% in 17 and 20 days, respectively (Heinfling et al., 1997). The main objective of present study was to investigate decolorization and biodegradation of Cu PC based textile dye Reactive blue-25 by *A. ochraceus* NCIM-1146 and identification of its biodegradation products, as well as to monitor extracellular enzymes, viz. laccase, tyrosinase and lignin peroxidase in the culture supernatant during decolorization process.

2. Methods

2.1. Dyes and chemicals

Reactive blue-25 was kind gift from local textile industry, Solapur, India. Yeast extract and glucose were obtained from Hi Media (Mumbai, India). ABTS was purchased from Sigma-Aldrich (Mumbai, India). Catechol, *n*-propanol and all other fine chemicals were purchased from Sisco Research Laboratories (India). All other chemicals used were of the highest purity available and analytical grade.

2.2. Microorganism and culture conditions

A strain of *A. ochraceus* NCIM-1146 was used in this study. The stock culture was maintained on potato–dextrose agar slants at 4 °C. Two fungal disc (8 mm diameter) of 4-day-old culture were inoculated into 250 ml Erlenmeyer flasks having 100 ml of PDB (potato–dextrose broth) medium containing (g l^{-1}) peeled potatoes 200, glucose 20, yeast extract 0.1 and incubated for 96 h at 30 °C at static condition.

2.3. Decolorization experiments: decolorization at static and shaking condition

Ten grams (wet weight) of 96 h grown mycelia of *A. ochraceus* were transferred aseptically into 250 ml Erlenmeyer flasks containing distilled water (100 ml) along with Reactive blue-25 (0.1 g l^{-1}) dye and incubated at 30 °C under static and shaking condition (150 rpm). Two ml of culture broth from each flask was removed at definite time interval and centrifuged at $5000 \times g$ for 15 min to remove suspended biomass. Percent decolorization was determined by measuring the absorbance of culture supernatant at the absorbance maxima of the dye (620 nm for Reactive blue-25). Abiotic controls (without fungi) were always included. To determine the percentage of adsorbed dye, the mycelial mat was homogenized (Remi tissue Homogeniger) in 10 ml methanol. The homogenate was centrifuged at the $5000 \times g$ for 10 min; the pellet was suspended in an additional 5 ml of methanol and recentrifuged. The resultant supernatants were combined and the absorbance of supernatant was measured to calculate the percentage of bound dye (Cripps et al., 1990).

$$\%\text{decolorization/adsorption} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

2.3.1. Decolorization in various media

Various media viz. distilled water + glucose (1%), distilled water + peptone (1%), distilled water + glucose (1%) + peptone (1%) and potassium phosphate buffer (50 mM, pH 7.4) were used for decolorization of Reactive blue-25 (0.1 g l^{-1}). Ten grams of 96 h grown mycelia of *A. ochraceus* were transferred aseptically into 250 ml Erlenmeyer flasks containing various media (100 ml) along with dye. The flasks were kept on shaker (150 rpm) at 30 °C. Decolorization of medium at different time interval was measured as mentioned in Section 2.3.

2.3.2. Effect of pH, temperature and initial dye concentration on decolorization

The pH (3, 5, 7 and 9 at 30 °C) and temperature (20, 30, 40 and 50 °C at pH 5) effects on the decolorization of Reactive blue-25 (0.1 g l^{-1}) was studied in the medium of distilled water + glucose (1%) at 150 rpm up to 7 days of incubation. The effect of various concentration of Reactive blue-25 (0.1–0.5 g l^{-1}) on decolorization performance of *A. ochraceus* was studied in distilled water (150 rpm, 30 °C). Decolorization was measured as mentioned in Section 2.3.

2.4. Enzyme assays

Activities of lignin peroxidase (LiP), laccase and tyrosinase were assayed spectrophotometrically in culture supernatant. Laccase activity was determined in a reaction mixture of 2 ml containing 10% ABTS in 0.1 M acetate buffer (pH 4.9) and increase in the optical density measured at 420 nm (Kalme et al., 2007). Tyrosinase activity was determined in 2 ml reaction mixture containing 0.01% catechol in 0.1 M phosphate buffer (pH 7.4) and measured increase in optical density at 420 nm (Kalme et al., 2007). Lignin peroxidase activity was determined by monitoring the formation of propanaldehyde at 300 nm in 2.5 ml reaction mixture containing 100 mM *n*-propanol, 250 mM tartaric acid, 10 mM H_2O_2 (Jadhav and Govindwar, 2006).