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Research article

Decolorization of synthetic brilliant green carpet industry dye through fungal co-culture technology

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ABSTRACT

Aim of the present study was to evaluate the efficiency of fungal co-culture for the decolorization of synthetic brilliant green carpet industry dye. For this purpose two lignocellulolytic fungi *Pleurotus florida* (PF) and *Rhizoctonia solani* (RS) were employed. The study includes determination of enzyme profiles (laccase and peroxidase), dye decolorization efficiency of co-culture and crude enzyme extracts. Both fungi produced laccase and Mn peroxidase and successfully decolorized solutions of different concentrations (2.0, 4.0, 6.0, & 8.0(w/v) of dye. The co-culture resulted highest 98.54% dye decolorization at 2% (w/v) of dye as compared to monocultures (82.12% with PF and 68.89% with RS) during 12 days of submerged fermentation. The lower levels of dyes were rapidly decolorized, while higher levels in slow order as 87.67% decolorization of 8% dye. The promising achievement of the study was remarkable decolorizing efficiency of co-culture over monocultures. The direct treatment of the mono and co-culture enzyme extracts to dye also influenced remarkable. The highest enzymatic decolorization was through combined (PF and RS) extracts, while lesser by monoculture extracts. Based on the observations and potentiality of co-culture technology; further it can be exploited for the bioremediation of areas contaminated with hazardous environmental pollutants including textile and other industry effluents.

1. Introduction

Discharge of textile dyes causes major health problem to entire ecosystem due to their toxic impacts on receiving waters (Kim et al., 2004; Park et al., 2007). Synthetic dyes are generally very stable to light, temperature, and microbial attack, making them recalcitrant (Pagga and Brown, 1986; Subramonian and Wu, 2014). Furthermore, dye wastewater has a large amount of suspended solids, a broadly fluctuating pH, and is highly colored (Subramonian and Wu, 2014; Wu et al., 2013). However, during last decades researchers focussed on for an effective treatment technology of dyes waste water, but no satisfactory solution has been searched for broad spectrum of dyes degradation. The methodological disadvantages reported were found in physio-chemical remediation techniques and financial disadvantage (Ali and El-Mohamedy, 2012; Asgher and Iqbal, 2013). Microorganisms have enormous dye degrading capabilities for successful bioremediation of textile dyes (Chen, 2006; Asgher et al., 2009, 2012; Oves et al., 2013).

White-rot fungi possess unique property of bioremediation due to the strong machinery of synthesizing extracellular ligninocellulolytic enzymes, which shows low specificity with substrate able for degradation of a wide range of xenobiotic compounds (Barr and Aust, 1994; Scheibner et al., 1997; Pointing, 2001) including textile dyes (Pasti-Grigsby et al., 1992; Paszczynski et al., 1992; Spadaro et al., 1992). The literature evidences number of white-rot fungi producing the lignin-degrading enzymes, lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Fu and Viraraghavan, 2001) which can degrade dyes. Manganese peroxidase (MnP) and lignin peroxidase (LiP) reported as the main enzyme involved in dye decolorization by *Phanerochaete chrysosporium* (Kirby et al., 1995; Chagas and Durrant, 2001), *Pleurotus florida* and *Agaricus bisporus* (Shanmugam et al., 2005). Involvement of enzymatic system of basidiomycetes has a great efficiency for dye bioremediation process. Laccase is documented as the main enzyme involved in dye decolorization by the cultures of *Phlebia tremellosa* (Kirby et al., 2000; Robinson et al., 2001; Kornillowicz-Kowalska and Rybczynska, 2015) and *Pleurotus sajor-caju* (Chagas and Durrant, 2001).

Several microorganisms, including fungi, bacteria, yeasts and

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algae, can decolorize and even completely mineralize azo dyes under certain environmental conditions (Khelifi et al., 2008). Decolorization of dye could also happen through biosorption onto the biomass of either live or dead fungus (Zhang et al., 2015). Most of the studies on the biotreatment of dyes and effluents deal mainly with decolorization by single fungal cultures despite only few reports on mixed cultures (Asgher et al., 2007, 2012). Co-culture is a potential strategy in bioremediation producing high activity enzymes due to the synergistic action (Mai and Morris, 2004; Belenguer et al., 2006). There are so many observed examples for the co-existence of different microorganisms such as forest soils, compost piles and mammalian intestines (Belenguer et al., 2006).

The current study was aimed to evaluate dye decolorization efficiency of two fungi including *Pleurotus florida* and *Rhizoctonia solani* using potent co-culture technology.

2. Material and methods

2.1. Microorganisms

The present study was performed by using two distinct basidiomycete fungi *Pleurotus florida* (PF) and *Rhizoctonia solani* (RS). The PF was procured from the Department of Biotechnology, Mushroom Training & Research Centre (MTRC), Faculty of Science, Veer Bahadur Singh Purvanchal University, Jaunpur (UP), India.

2.2. Carpet industry dye

The synthetic 'brilliant green' carpet dye was used in the form of liquid preparations. The dye was purchased from the local dye market, 'Carpet City' Bhadohi, U.P., India. Absorption maxima (λ_{max}) of dye was determined through scanning by Elico® SL191UV VIS Spectrophotometer USA.

2.3. Maintenance of culture

Pure culture of both *Pleurotus florida* and *Rhizoctonia solani* were grown on potato dextrose agar (PDA) slant and incubated for 7–8 days at 22 °C±2 and maintained at 4 °C through regular and periodic transfer.

2.4. Inoculum preparation

Both fungi were separately grown in Petri dishes containing potato dextrose agar (22 ± 2 °C). Culture plugs of 9 mm diameter were used as inoculums from 7 to 10 day incubated fully grown plate.

2.5. Biomass determination

Total biomass was determined after 10, 15 and 30 days of incubations at 22 ± 2 °C temperature. These cultures were inoculated with equal amount of inoculums. All experiments were performed in triplicates (n = 3). The mycelium was separated from appropriate culture (10, 15, 30 days old) with the help of Whatmann No. 1filter paper. The mycelium was separated and dried at 60 °C in hot air oven and biomass was determined by weighing and expressed in mg/mL.

2.6. Laccase assay

Laccase (EC 1.10.3.2) activity was determined by the oxidation of 2, 2″-azino-bis (3- ethylthiazoline-6-sulfonate), i.e., ABTS at 37 °C. The reaction mixture contained 600 μl enzyme extract, 300 μl (0.1 M) sodium acetate buffer pH 5.0 and 100 μl ABTS solution (1 mM). Oxidation was followed by the increase in absorbance at 420 nm. One unit of enzyme activity was defined as the amount of enzyme oxidizing 1 mmol of ABTS per minute.

2.7. Protein estimation

An aliquot of culture filtrate of both fungi with appropriate dilution was used for estimation of soluble protein content according to the Lowry et al. (1951) using bovine serum albumin (BSA) as protein standard.

2.8. Preparation of crude enzyme extract (CEE)

For preparation of crude enzyme extract, 100 mL of each culture was separately centrifuged at 8000*g* for 10 min in cooling centrifuge (Remi, India). The culture medium was discarded, while the supernatant was mixed with 50 mL of 0.1 M phosphate buffer (pH, 6.8) and used as crude enzyme extracts.

2.9. Mono and co-culture treatment regimen for dye decolorization

For the present study *Pleurotus florida* was cultivated in 250 mL Erlenmeyer flask containing ¼ of original content of potato dextrose broth supplemented with different concentration of textile dye (1, 2 and 3 ppm) in different sets. These preparations were sterilized and inoculated with two mycelial plug. The inoculations for mono-cultures were used independently as separate culture plugs (PF and RS). However, experimental sets for the purpose of co-culture studies were jointly inoculated with the mycelial plugs (PF+RS) of both fungi. Then after these experimental conical flasks were incubated at 28 ± 2 °C in a rotatory shaker incubator (150 rpm). Furthermore samples were filtered to make mycelium free with the help Whatmann paper. Thus obtained supernatant was used for determination of color reduction. Each experimental set was managed in triplicates (n = 3).

2.10. Crude enzyme extract (CEE) treatment regimen for dye decolorization

All decolorization experiments were performed using 150 mL Erlenmeyer conical flasks having different levels of dyes. The reaction was initiated with the treatment of different levels (2, 4, 6, 8 & 10%) of crude enzyme extract in 100 mL carpet dye solution (1 ppm) followed to incubation in the dark at 22 ± 2 °C. Decolorization of different sets of dye concentration were performed in triplicates (n = 3). The samples were taken aseptically by using clean and sterile pipette on different intervals for determination of color reduction decolorizations studies. Dye decolorization was measured on different time intervals (30, 60, 80 and 120 min) and reduction in color was determined.

2.11. Color measurement

Percent dye decolorization was measured spectrophotometrically by measuring absorbance (λ_{max} = 450 nm) of the filtrate reaction mixture.

The percent (%) decolorization was calculated as follows.

$$\text{Decolorization } (\%) = \frac{100\left(Abs_{to} - Abs_{tf}\right)}{Abs_{to}} \quad (1)$$

Where,

Abs_{t0} = Absorbance at initial of reaction mixture.