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Decolourisation of a polymeric dye by selected fungal strains in liquid cultures

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Abstract

Eight basidiomycetes were evaluated for their ability to decolourise a polymeric dye, Poly R-478. Decolourisation experiments were carried out in nitrogen-limited (0.25 mM) and nitrogen-rich (5.2 mM) conditions at a dye concentration of 100 mg/L. Stationary liquid cultures were inoculated with mycelium agar plugs and all fungi formed mycelium mats. The effect of nitrogen on decolourisation was also tested in shaken cultures inoculated with homogenized mycelium, where the biomass grew as mycelium beads. In static cultures, all strains apart from *Polyporus* sp.2 showed better decolourisation in N-rich medium. The greatest removal efficiency was achieved by the fungus *Pleurotus ostreatus* sp.4, attaining a maximum decolourisation of 53% in 18 days. On the other hand, in agitated cultures all strains were more efficient in decolourisation. The higher levels of removal efficiency were exhibited by *Ganoderma australe* in N-rich medium, obtaining decolourisation of about 93% in 18 days.

Keywords: Decolourisation; Poly R-478; White rot fungi; *Ganoderma australe*; Dye removal; Dyestuff degradation

1. Introduction

White rot fungi are unique among eukaryotic and prokaryotic microorganisms, because they possess a very powerful extracellular oxidative enzymatic system, the lignin-degrading enzyme system (LDS). This physiological group of organisms takes part in the global carbon cycle as a result of their ability to degrade lignin, the second most abundant carbon source and widely distributed renewable aromatic polymer in biosphere [1,2]. Their extracellular ligninolytic system involves enzymes, such as manganese peroxidases (MnP), lignin peroxidases (LiP) and laccases (Lac), which are involved not only in the degradation of lignin but also in the degradation of various xenobiotic compounds including dyes, pesticides, PCBs and PAHs [2–7].

Fungal nutrition has repeatedly been shown to be of enormous importance in the effectiveness of fungal decolourisation systems using live mycelium. The ligninolytic enzymes of the white rot fungi are thought to be expressed during secondary metabolism following growth when carbon and/or nitrogen become limiting [1,6]. Most decolourisation studies have been demonstrated using the white rot fungi, *Phanerochaete chrysosporium*, *Bjerkandera adusta*, *Trametes versicolor* or *Phlebia radiata*, which were able to degrade a broad spectrum of dyes [8–11]. *P. chrysosporium* decolourised the dye efficiently in low-nitrogen medium and was subdued in nitrogen-rich (N-rich) cultures, with substantial adsorption of the dyes to the mycelium [12,13]. It is clear that culture conditions affect the expression and the activity of the ligninolytic enzymes by the white rot fungi. Static culture conditions have been reported to be necessary for expression of the ligninolytic enzymes and the agitation of cultures leads to decreased ligninase activity [14,15]. Static cultures of *T. versicolor* and *P. chrysosporium* have led to successful degradation of xenobiotic compounds by both species [16,17]. However, agitated cultures of a wide range of fungi and chromophores showed that agitation of cultures resulted in better decolourisation [18–20].

In this present paper, we have studied the potential of eight strains of fungi to decolourise the polymeric dye, Poly R-478, a lignin model compound in liquid cultures. The purpose was to evaluate the decolourisation ability of the selected filamentous fungus in different conditions. To determine the effect of nitrogen concentration, decolourisation was examined in N-limited (0.25 mM) and N-rich (5.2 mM) cultures. The decolourisation was examined in static and agitated conditions using different inocula (agar plugs and homogenized mycelium, respectively).

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2. Material and methods

2.1. Microorganisms

The following strains of basidiomycetes were used in this study: *Pleurotus ostreatus* sp.3, *P. ostreatus* sp.4, *Pleurotus pulmonarius*, *Ganoderma australe*, *Polyporus ciliatus*, *Polyporus brumalis*, *Polyporus* sp.2 and *Polyporus* sp.3. The two strains of *P. ostreatus* and *P. pulmonarius* were obtained from commercial compost. Fruiting bodies of the other strains were collected from tree stumps or twigs in Greece. The strains were maintained on PDA at 4 °C.

2.2. Growth media

Cultures were maintained on potato dextrose agar (PDA), composed of (g/L): peeled potatoes 200, dextrose 20 and agar 15. Low and high nitrogen basal medium (BM) composed of (g/L): asparagine 0.1 or 2 relatively, glucose 20, KH_2PO_4 0.46, K_2HPO_4 1, $MgSO_4{\cdot}5H_2O$ 0.5 and malt extract broth (MEB) containing (g/L)—malt extract 17 and mycological peptone 3. Media were sterilized by autoclaving at 121 °C for 20 min.

2.3. Dye degradation in static liquid cultures

Four mycelium plugs were inoculated in 50-mL low and high nitrogen BM medium in 125 mL Erlenmeyer flasks. Poly R-478 was added to the growth medium to reach a final concentration 0.1 g/L. The flasks were incubated at 25 °C in darkness without shaking. Uninoculated control flasks were also included. At regular intervals, three flasks were selected randomly and used for analyses. The fluid was separated from biomass by filtration. The filtrate was diluted (1/5) and measured spectrophotometrically at 520 nm. Colour reduction was calculated from the difference of absorbance between samples and the respective abiotic controls at the maximum visible absorbance peak for the dye. A volume of the culture filtrate was also removed in order to measure the pH. Fungal biomass was washed with warm distilled water and in the wash solution the adsorption of the dye was determined. The biomass was dried at 80 °C and gravimetrically determined.

2.4. Dye degradation in agitated liquid cultures

Inocula (1-cm diameter) were cut from grown PDA cultures and transferred in 2% malt extract broth (50 mL in 125 mL Erlenmeyer flasks). The flasks were incubated for 7 days at 25 °C under stationary conditions. The cultures were homogenized using a blender and 5-mL aliquots were added to 45 mL BM media under two different concentrations of nitrogen (2 and 0.1 g/L asparagine) in 125 mL Erlenmeyer flasks. The flasks were agitated at 90 rpm and incubated at 25 °C, in darkness. The decolourisation analyses were determined as described above.

2.5. Statistical analysis

The data were treated with the SPSS statistical package for one-way ANOVA and if any significant difference was observed between the treatment groups, further ranking of the group was performed with the Tukey test. The statistical level of significance for all treatments was 5%. Values are reported as means and standard deviation.

3. Results and discussion

In previous work, these 8 basidiomycetes were preselected among 100 strains which were collected all over Greece and isolated in pure culture (data not shown). These strains were screened using the standard decolourisation method of Poly R-478, due to the correlation of the ligninolytic degradation activity and the decolourisation of the dye [12,21].

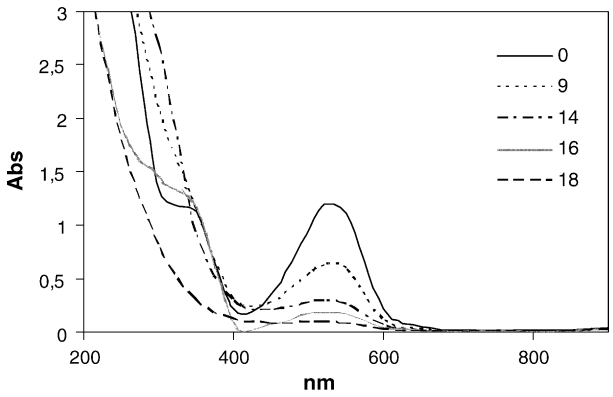

Fig. 1. Absorbance spectrum of Poly R-478 at 0, 9, 14, 16 and 18 days in decolourisation experiments.

Grounded on the growth rates and the decolourisation ability, the fungi were selected for further research. To determine the effects of nitrogen, two different concentrations of asparagine were used. In addition, the decolourisation process was examined in static and agitated cultures.

3.1. Dye degradation in static liquid cultures

Static liquid cultures in BM medium doped with Poly R-478 were conducted with the eight basidiomycetes. Uninoculated controls showed no colour removal. Decolourisation of the dye solution could be attributed to adsorption to the biomass and/or biodegradation. With all statically grown cultures, decrease in dye absorbance was accompanied by a visible adsorption of the dyes to the fungal biomass. This has been previously reported by several static biodecolourisation studies using different fungi such as *T. versicolor* and *P. chrysosporium* [8,13,22,23]. According to Knapp et al. [24], when degradation occurred there was either complete removal of the major visible light peak or a significant spectral change. As it is shown in Fig. 1, the specific peak at 520 nm has disappeared in the period of the experiment.

The decolourisation measurements shown in Table 1 represent the maximum values during the 18 days cultivation. Removal efficiency is expressed as the percentage of the amount of Poly R-478 removed to the initial amount of the dye. Additionally, the removal capability refers to the amount of dye degraded or adsorbed to fungal mycelium to the initial amount of the dye.

All the strains apart from *Polyporus* sp.2, which grew in liquid N-limited medium, showed a lower efficiency in removal of Poly R-478 (∼26%), compared to the N-rich medium (∼44%). The greatest removal efficiency was achieved by the fungus *P. ostreatus* sp.4 in N-rich medium, attaining a maximum decolourisation of 53%. Despite the fact that *P. ostreatus* sp.3 and sp.4 were isolated from two commercial mushrooms, they showed significant differences in the ability to decolourise Poly R-478 and the removal efficiency was approximately halved (from 43 to 23% and from 53 to 28% in 0.25 and 5.2 mM N, respectively).

Comparing the results of the removal efficiency for the static cultures, with the analysis of variance (one-way ANOVA), differences among the N-rich and N-limited media were revealed for the strains *P. ciliatus* and *Polyporus* sp.2 with *p*-values