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Different approaches to improving the textile dye degradation capacity of *Trametes versicolor*

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Abstract

Trametes versicolor pellets were used in a pulsed fluidised bioreactor with laccase production media to decolourise the textile dye Grey Lanaset G. The effect of the enzyme on dye degradation was analysed. In a batch process, degrading the dye with the fungus results in a decolourisation percentage higher than 90% (initial dye concentration 150 mg/L) while the results were lower than 35% using enzymatic degradation. Although most of the decolourisation was initially due to an adsorption process, later on in both phases, the biomass and the culture broth became colourless. In order to check the possibility of improving the degradation capacity in the batch mode operation, after decolourising the initial dye solution, different pulse dye adding strategies were tested. Adding a large pulse resulted in fast enzymatic deactivation while adding a small pulse caused the system to operate below its optimum degradation capacity. There was a close correlation between the amount of laccase produced and the amount of dye degraded. The system worked in a bioreactor for one month without any operational problems. Finally, in the continuous mode the dye degradation has been demonstrated because it is possible to maintain the continuous production of the laccase enzyme.

Keywords: *Trametes versicolor*; Bioreactor; Laccase; Grey Lanaset G; Decolourisation

1. Introduction

Synthetic dyes are increasingly used in the textile and dyeing industries because of their ease and cost effectiveness in relation to synthesis, firmness, high stability in relation to light, temperature, detergent and microbial attack, and the variety of colours available compared with natural dyes.

Textile effluents can damage the environment as they contain dyes with complex and very varied chemical structures. These dyes are visible even in very low concentrations, so they cannot be removed using the common methods. Nowadays, environmental regulations in most countries have made it mandatory to decolourise wastewater prior to discharging it. Currently, textile effluents are mainly treated by physic-chemical methods which are often quite expensive. In addition, these methods do not generally degrade the pollutant, therefore causing an accumulation of the dye as sludge creating a disposal problem. Current available technologies have been reviewed by Robinson et al. [1]. Special attention is given to biological processes because they are cost effective and environmentally friendly. Removing dyes in aerobic conditions is mainly achieved by adsorbing the dyes on bacteria, rather than oxidation. Some anaerobic textile wastewater treatment methods have been developed at a laboratory-scale and have shown to remove colour efficiently [2,3], but the anaerobic treatment of some azo dyes may result in the formation of toxic aromatic amines.

Degrading various classes of dyes using white-rot fungi has been reported. Most work has involved *Phanerochaete chrysosporium*. Goszczynski et al. [4] studied some of the main chemical steps related to degrading azo dyes using ligninolytic enzymes. Spadaro and Renganathan [5], also suggested that lignin peroxidase (LiP) was involved in the decolourisation process of several synthetic textile dyes. Ollikka et al. [6] purified three LiP isoenzymes from *P. chrysosporium* and studied their decolourisation efficiency on several dyes using crude lignin peroxidases and the purified enzymes. *P. chrysosporium* has also

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been used in bioreactors for the dye decolourisation process [7,8].

There have also been studies on using other fungi for dye elimination treatment but they are not as extensive as the studies on *P. chrysosporium.* Some examples are *Funalia trogii* [9], *Pleurotus ostreatus* [10], *Trametes hirsuta* [11], *Lentinus edodes* [12], *Trametes modesta* [13], *Geotrichum* sp. [14] and *Trametes versicolor* [15,16].

The ability of *T. versicolor* to transform and/or mineralise a wide range of organopollutants has also been reported [17]. Some works that use *T. versicolor* in bioreactors can be found in the literature [18–21].

This paper reports on the high capacity of *T. versicolor* pellets to biodegrade the commercial dye Grey Lanaset G. This is a commercial mixture of several metal complex dyes used in a local textile industry. The chemical formula is unavailable, because it is a patented dye; based on its specifications, however, the dye is known to contain cobalt (0.79%) as an organo-metal complex and chromium (2.5%) as a Cr III organo-metal complex. The process was performed in an air pulsed bioreactor under enzyme laccase production conditions.

2. Materials and methods

2.1. Microorganism

T. versicolor was obtained from ATCC #42530. The fungus was maintained on 2% malt agar slants at 25 °C until use. Subcultures were routinely made.

2.2. Culture methods

A mycelia suspension of *T. versicolor* was obtained by inoculation of four 1 cm diameter plugs, from the growing zone of fungi on malt agar, in 150 mL malt extract medium (2%) in a 500 mL Erlenmeyer flask. Flasks were placed in an orbital shaker (135 rpm, $r = 25$ mm) at 25 °C. After 4–5 days a thick mycelial mass was formed, that was ground with an X10/20 (Ystral GmbH) homogenizer. The resulting mycelial suspension was stored in sterilised saline solution (0.85% NaCl) at 4 °C. This suspension was used to produce pellets by inoculating 1 mL of the suspension in 300 mL malt extract medium 2% in a 1 L Erlenmeyer flask. The flasks were incubated in an orbital shaker (135 rpm, $r = 25$ mm) at 25 °C for 5 days. The pellets thus obtained can be used to inoculate the bioreactor or stored in sterilized saline solution (0.8% NaCl) at 4 °C where they will remain active for up to 2 months without loosing their morphology until their use.

2.3. Medium composition

The culture medium contained per liter: 8 g glucose, 1.9 g NH_4Cl, 11 mL of a supplemented medium [22], 1.168 g of 2,2-dimethylsuccinate buffer and 0.15 g dye. The pH of the solution was adjusted to 4.5 with 0.5 M NaOH. It was sterilised at 120 °C for 20 min.

2.4. Chemicals

Grey Lanaset G (a mixture of metal complex dyes) was complimentarily supplied by Ciba (ref. 080173.5). All other chemicals were reagent grade.

2.5. Equipment and operating conditions

A glass air fluidised bioreactor, with a working volume of 500 mL equipped with a pH controller, was used. Air was introduced by means of pulses at the bottom of the reactor. Pulses were generated using an electrovalve controlled by a cyclic timer. Pulse frequency was defined as the inverse of the sum of opening and shutting times of the electrovalve: $F = 1/(t_0 + t_s)$, where F is the frequency, t_0 is the opening time and t_s is the shutting time. In this study t_0 was 1 s, t_s was 5 s and the air flow was 8.2 L/h. This configuration allowed a correct biomass fluidisation. Temperature was maintained at 25 °C, and the pH at 4.5. The bioreactor was inoculated with an amount of pellets equivalent to 1.8 g biomass/L. The biomass was measured as dry cell weight (DCW).

2.6. In vitro biodegradation

Two sources of laccase enzyme were used, laccase from broth culture obtained in our laboratories and commercial purified laccase of *T. versicolor* from Fluka (ref. 53739). Enzymatic biodegradations were carried out in Erlenmeyer flasks which were placed in an orbital shaker (135 rpm, $r = 25$ mm) at 25 °C. In both cases 15 mg of Grey Lanaset G were added to 100 mL of laccase solution.

In order to obtain the broth culture containing enzyme the bioreactor was filled with the medium without dye and it was operated at the same conditions. When the laccase activity was approximately 2500 AU/L, the reactor broth was centrifuged at 10,000 × g at 4 °C for 10 min. The supernatant was filtrated through 0.22 μm filter and the pH was adjusted to 4.5.

2.7. Analytical methods

Glucose was measured with an YSI 2000 enzymatic analyser from Yellow Spring Instruments and Co.

Biomass was determined by centrifugation of the sample. The obtained pellet biomass was resuspended, filtered through a Whatman grade GF/F glass microfiber filter (Whatman Int. Ltd.). Biomass was determined by drying at 105 °C to constant weight.

2.8. Colour determination

Spectrophotometric measurements were carried out at the visible maximum absorbance, 590 nm using UV–vis Cary of Varian at 20 °C.

2.9. Laccase activity

Enzymatic activity was measured using a modified version of the method for the determination of manganese peroxidase