Journal of Hazardous Materials 194 (2011) 297–302

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Production of laccase and decolouration of the textile dye Remazol Brilliant Blue R in temporary immersion bioreactors

Susana Rodríguez-Couto[a,b,*]

[a] CEIT, Unit of Environmental Engineering, Paseo Manuel de Lardizábal 15, 20018 San Sebastian, Spain
[b] IKERBASQUE, Basque Foundation for Science, Alameda de Urquijo 36, 48011 Bilbao, Spain

ARTICLE INFO

Article history:
Received 10 May 2011
Received in revised form 22 July 2011
Accepted 26 July 2011
Available online 5 August 2011

Keywords:
Laccase
Remazol Brilliant Blue R
Sunflower-seed shells
Temporary immersion bioreactor
Trametes pubescens

ABSTRACT

Laccase production by *Trametes pubescens* grown on sunflower-seed shells (SS) under solid-state fermentation (SFF) conditions in temporary immersion bioreactors was studied. Three immersion cycles were considered: 1 min immersed and 9 min non-immersed, 1 min immersed and 30 min non-immersed and 1 min immersed and 60 min non-immersed. The latter led to the highest laccase activities (4000–6000 U l^{-1}). Also, the *in vitro* and *in vivo* decolouration of the recalcitrant textile dye Remazol Brilliant Blue R (RBBR) was assessed. It was found that RBBR (133.33 mg l^{-1}) was efficiently decolourised by *T. pubencens* grown on SS under SSF conditions in temporary immersion bioreactors in five successive batches. The percentage of RBBR decolouration was higher than 55% in 4 h and around 70% in 24 h in all the batches. However, it was found that RBBR decolouration by the crude culture filtrates was more advantageous. Thus, an RBBR decolouration percentage of nearly 80% in 2 h was obtained.

1. Introduction

Reactive anthraquinonic dyes represent the second largest class of textile dyes after azo dyes and are extensively used in the textile industry due to their wide variety of colour shades, easy application and low energy consumption [1]. These dyes are resistant to degradation due to their fused aromatic structure [2]. Also, most of them are toxic, carcinogenic and mutagenic [3]. Therefore, anthraquinonic dyes have to be removed before being discharged into the environment.

Dye removal from wastewater with traditional physical and chemical methods, such as coagulation, adsorption and oxidation with ozone, is costly, generates large volumes of sludge, has limited versatility and usually requires the addition of environmentally hazardous chemical additives [4]. This has impelled the search for more efficient treatment processes. Thus, biological processes are receiving more attention as they are cost-effective and environmentally friendly. In this context, white-rot fungi have proved to be the most efficient microorganisms in degrading synthetic dyes [5]. Their extracellular enzymatic system, which is involved in lignin degradation, consists mainly of oxidative enzymes like lignin peroxidases (LiP; E.C. 1.11.1.14), manganese-dependent peroxidases (MnP; E.C.1.11.1.13) and laccases (EC 1.10.3.2). The latter have aroused recent interest because they use molecular oxygen as a co-substrate instead of hydrogen peroxide as used by peroxidases. In addition, it was shown that in the presence of small molecular weight compounds capable to act as electron transfer mediators laccases were able to oxidise non-phenolic structures [6,7]. The genus *Trametes*, which belongs to the white-rot fungi, is assumed to be one of the main laccase producers. In addition, the genus *Trametes* is probably the most actively investigated in the phylum of Basidiomycota for ligninolytic enzyme formation and application [8]. Also, *Trametes* spp. are known to decolourise different synthetic dyes [9]. Among such genus, *Trametes pubescens* has been described as an efficient laccase producer [10] with high dye-decolourising ability [11,12] and, thus, has been selected to perform this study.

Most studies dealing with ligninolytic enzymes have been performed in submerged conditions despite white-rot fungi grow in nature in solid-state conditions. Solid-state fermentation (SSF) was chosen here for the production of laccase because it mimics the natural environment of the white-rot fungi (wood). In addition, this technique allows the utilisation of diverse agro-industrial wastes as support-substrate, making the process more both economical and ecological [13].

The aim of the present paper was to develop a new, low cost, efficient and relatively simple bioprocess based on the white-rot

* Correspondence address: CEIT, Unit of Environmental Engineering, Paseo Manuel de Lardizábal 15, 20018 San Sebastian, Spain. Tel.: +34 943 212800x2239; fax: +34 943 213076.
E-mail address: srodriguez@ceit.es

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.07.098

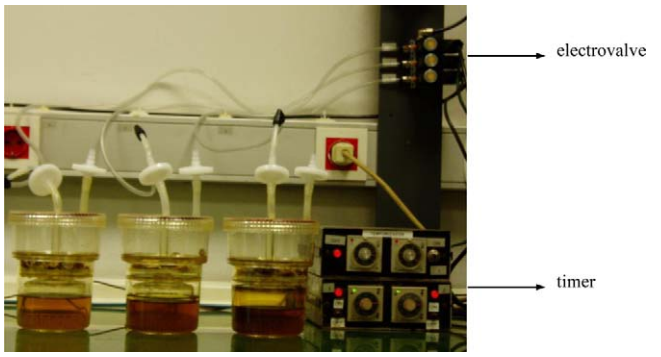


Fig. 1. Photograph of the temporary immersion bioreactors used.

fungus *T. pubescens* to decolourise the recalcitrant anthraquinonic dye Remazol Brilliant Blue R (RBBR).

2. Materials and methods

2.1. Microorganism

T. pubescens MB 89 (CBS 696.94; Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) was obtained from the Institute of Applied Microbiology, University of Natural Resources and Applied Life Sciences (Vienna, Austria) and was maintained on potato dextrose agar (PDA) Petri plates at 4 °C and sub-cultured every three months.

2.2. Support

Sunflower (*Helianthus annuus*) seeds were obtained from a local market and the shells were collected after normal human consumption of the seeds. The sunflower seed shells (SS) were autoclaved at 121 °C for 20 min before use. The chemical composition of the SS according to Demirbas and Akdeniz [14] is 17% lignin, 48.4% cellulose and 34.6% hemicellulose.

2.3. Pre-cultivation of the fungus in Erlenmeyer flasks

The composition of the culture medium was as follows: 10 g l^{-1} glucose, 20 g l^{-1} peptone from casein, 0.9 g l^{-1} $(NH_4)_2SO_4$, 2 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $MgSO_4$ $7H_2O$, 0.1 g l^{-1} $CaCl_2$ $2H_2O$, 0.5 g l^{-1} KCl, 0.5 g l^{-1} thiamine in citrate-phosphate buffer (pH 4.5). The culture medium was sterilised at 121 °C for 20 min. After cooling, the thiamine previously sterilised by filtration (0.22 μm) was added to the culture medium [15].

Fungal cultures were conducted in cotton-plugged Erlenmeyer flasks (250 ml) containing 1.5 g of SS and 20 ml of culture medium [16]. Inoculation was done directly in the Erlenmeyer flasks. Three agar plugs (diameter, 7 mm), taken from a 7-day-old PDA fungal culture, per Erlenmeyer were used as inoculum. The Erlenmeyer flasks were incubated statically under an air atmosphere at 30 °C in darkness for 7 days. After that time the SS colonised by the fungus from two Erlenmeyer flasks were transferred to each bioreactor, this being the starting time for the experiment.

2.4. Bioreactor configuration and operation conditions

The temporary immersion reactor RITA®-System (Cirad, Vitropic, France) (see Fig. 1) was used. It consisted of a lower chamber which contained the culture medium (200 ml) and an upper chamber containing the fungal culture grown on SS. The chambers were separated from each other by means of a sieve. By pumping air (60 l h^{-1}) into the lower chamber, the liquid medium was forced into the upper chamber of the reactor. In this way, temporary immersion of the fungal culture in the culture medium was achieved. Air bubbles passed through the sieve from the lower to the upper chamber, thus aerating the culture.

Three different temporary immersion cycles were tested: 1 min immersed and 9 min non-immersed (immersion frequency 0.1 s^{-1}), 1 min immersed and 30 min non-immersed (immersion frequency 0.032 s^{-1}) and 1 min immersed and 60 min non-immersed (immersion frequency 0.016 s^{-1}). The different immersion cycles were tested simultaneously in different RITA®-System reactors for 44 days. The immersion cycles were automatically performed by means of a timer connected to an electrovalve for each reactor (Fig. 1).

The composition of the culture medium was the same as that used in the pre-cultivation of the fungus but supplemented with 0.5 mM Cu^{2+} to boost laccase production [16]. The bioreactors were kept at room temperature (25 ± 1 °C). Samples were collected once a day, centrifuged (8000 × *g*, 10 min) and analysed. Duplicate experiments were run for comparison and samples were analysed twice. The values in the figures correspond to mean values with a standard deviation lower than 15%.

2.5. Determination of laccase activity

Laccase activity was determined spectrophotometrically as described by Niku-Paavola et al. [17] with ABTS as a substrate. One activity unit was defined as the amount of enzyme that oxidised 1 μmol of ABTS per min. The activities were expressed in U l^{-1}.

2.6. In vitro decolouration of RBBR

In order to assess the degrading capability of the extracellular extract obtained by the above cultures the decolouration of the textile dye RBBR was performed. Laccase was produced as indicated in Section 2.4. Culture broth was collected at the maximum laccase activity, filtered and the supernatant was clarified by centrifugation at 8000 × *g* for 15 min. After that, the supernatant was ultra-filtrated in an Amicon stirred cell apparatus (YM 10 membrane). The resulting concentrated extract was used to perform the *in vitro* decolouration experiments.

2.6.1. Effect of laccase concentration on RBBR decolouration by crude laccase

The effect of laccase concentration (ranging from 50 to 800 U l^{-1}) on RBBR decolouration by crude laccase was studied. The reaction mixture consisted of 25 mM succinic buffer (pH 4.5), RBBR (133.33 mg l^{-1}) and crude laccase in a final volumen of 1.5 ml. All the reactions were incubated at room temperature (25 ± 1 °C), without shaking and in darkness. Samples were taken at time zero and at determined intervals, centrifuged (8000 × *g*, 5 min) and the residual dye concentration was spectrophotometrically measured from 500 to 700 nm and associated with the decrease in the area under the plot. This approach takes into account the conversion of the dye molecules to other compounds absorbing at different wavelengths and then, the ratio of the area under the visible spectrum is always equal or lower than the ratio of the absorbances at the peak.

Dye decolouration was calculated by means of the formula:

$$D = \frac{100\ (A_{ini} - A_{obs})}{A_{ini}}$$

where *D*: decolouration (in %); A_{ini}: area under the curve of the absorption spectrum from 500 to 700 nm at the initial time; A_{obs}: area under the curve of the absorption spectrum from 500 to 700 nm at a determined time.

A control test containing the same amount of a heat-denatured laccase was performed in parallel. The assays were done twice, the experimental error being below 3%.

2.6.2. Effect of initial RBBR concentration on RBBR decolouration by crude laccase

The effect of RBBR concentration (ranging from 66.67 to 533.33 mg l^{-1}) on RBBR decolouration by crude laccase was studied. The reaction mixture consisted of 25 mM succinic buffer (pH 4.5), RBBR and crude laccase (100 U l^{-1}) in a final volume of 1.5 ml. All the reactions were incubated at room temperature (25 ± 1 °C), without shaking and in darkness. Samples were taken at time zero and at determined intervals, centrifuged (8000 × *g*, 5 min) and decolouration was measured as indicated above. A control test containing the same amount of a heat-denatured laccase was performed in parallel. The assays were done twice, the experimental error being below 3%. In addition, the kinetic parameters K_M and V_{max} were determined.

2.7. Decolouration of RBBR in temporary immersion bioreactors

The decolouration of the textile dye RBBR by *T. pubescens* grown on SS in a temporary immersion bioreactor (working volume 200 ml) in five successive batches was performed. RBBR (CI Reactive Blue 19) was selected as a representative of an important class of recalcitrant anthraquinonic dyes. It is the raw material in the production of polymeric dyes and, as a derivative of anthracene, represents an important member of toxic and recalcitrant organopollutants. The dye was purchased from Sigma–Aldrich (St. Louis, MO, USA). The general characteristics of RBBR are indicated in Table 1. A stock solution (0.1%, w/v in distilled water) was stored in the dark at room temperature (25 ± 1 °C). At the end of each batch fresh dye solution was added in order to obtain an absorbance around 1.0–1.2 at the maximum visible wavelength of RBBR. Samples, taken at convenient intervals, were centrifuged (8000 × *g*, 10 min) and the residual dye concentration was spectrophotometrically measured as indicated above.

2.8. Phytotoxicity studies

The toxicity of the original dye (133.33 mg l^{-1}) and the fungal-treated dye after five successive decolouration batches (dilution 1:3, v/v) was assessed by measuring their effect on seeds germination. Although several species have been traditionally used for evaluating phytotoxicity, there are no standardised seed species in use world-wide [18]. It is recommended [19] that at least one monocotyledon and one dicotyledon species are used in these tests. The seeds selected to perform the tests were the monocotyledon *Triticum aestivum* (wheat) and the dicotyledon *Raphanus sativus* (radish) and the Zucconi test was applied [20]. Distilled water was used as a control. Seed germination and root length were measured after 5 days of incubation in the dark. Four replicates of 10 seeds were used for each test.

The germination index (GI) was calculated as follows: GI = GP × La/Lc, where GP is the number of germinated seeds expressed as a percentage of control values, La is the average value of root length in the dye solutions and Lc is the average value of root length in the control.

3. Results and discussion

3.1. Laccase production by T. pubescens in temporary immersion bioreactors

Laccase enzymes were the only enzymes detected in the culture fluid. As shown in Fig. 2 the highest laccase activities were obtained with the lowest immersion frequency tested (0.016 s^{-1}). Thus, from day 14 onwards laccase activities around 4000–6000 U l^{-1} were attained. This might be due to the fungal culture is immersed for less time, cultivation being closer to SSF conditions. It is known that in general white-rot fungi are more stimulated for the production of ligninolytic enzymes when grown under SSF conditions than when grown under submerged fermentation (SmF) ones [21]. In fact, temporary immersion reactors are emerging as alternative methods of submerged fermentation, without the disadvantages of liquid environment [22]. Also, the less immersion frequency might reduce hydrodynamic stress favouring enzyme production [23]. In view of the results obtained, this immersion cycle was used in the subsequent experiments.

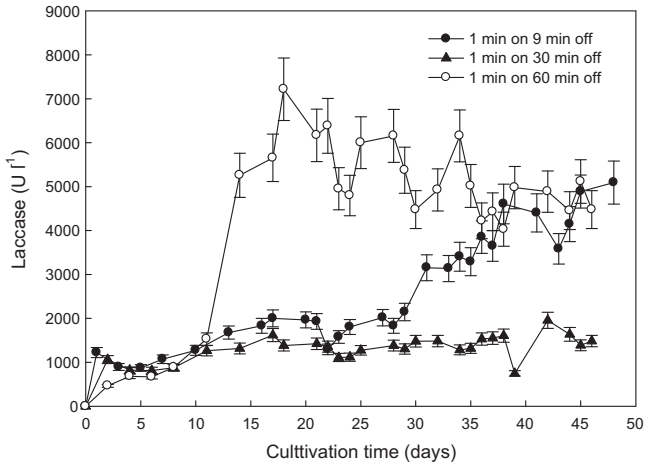


Fig. 2. Laccase production by *T. pubescens* in temporary immersion bioreactors operating at different immersion cycles.

3.2. In vitro decolouration of RBBR by crude laccase from T. pubescens

3.2.1. Effect of laccase concentration on RBBR decolouration by crude laccase

As observed in Fig. 3 the optimum concentration of laccase enzyme for RBBR decolouration was 100 U l^{-1}. Thus, a decolouration percentage of 79.4% in 2 h was obtained. These results are similar to those reported in a previous work [24] in which a percentage of RBBR decolouration around 70% in 2 h by crude laccase

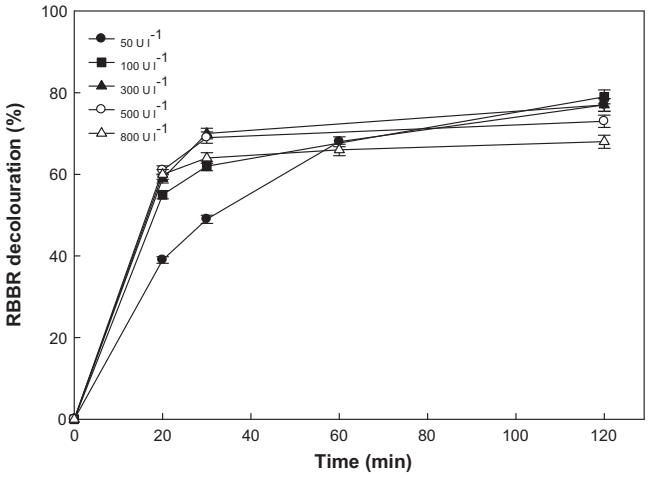
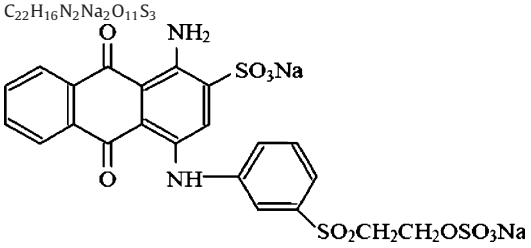


Fig. 3. Effect of laccase concentration on RBBR decolouration (133.33 mg l^{-1}) by crude laccase from *T. pubescens* grown on SS under SSF conditions.

Table 1
General characteristics of the dye Remazol Brilliant Blue R (RBBR).

Properties	Remazol Brilliant Blue R
C.I. name	Reactive Blue 19
C.I. number	61,200
Class	Anthraquinone
Apparent colour	Blue
Molecular weight	626.54 g mol^{-1}
Molecular formula	$C_{22}H_{16}N_2Na_2O_{11}S_3$
Chemical structure	
Maximum absorption wavelength	592 nm
Water solubility	10 g l^{-1}

(500 U l^{-1}) from *T. pubescens* grown on SS under SSF conditions was obtained. However, the percentage of RBBR decolouration obtained was much higher than that reported previously [25] using extracellular liquid (300 U l^{-1} laccase) from *T. pubescens* cultures grown on banana skins under SSF conditions. This might be likely due to the enzymatic complex produced by *T. pubescens* using SS as support-substrate has a higher degrading capability. This underlines the influence of the support on the efficiency of the overall process in these types of cultures. Also, the percentage of RBBR decolouration obtained was much higher and obtained in much less time than that reported by Osma et al. [26] using crude laccase from *Trametes hirsuta* immobilised on alumina pellets (44% in 42 h).

On the other hand, these results differ from those reported by Soares et al. [27], who found that the addition of a redox mediator was necessary for RBBR decolouration by a laccase from an *Aspergillus* strain. The difference in fungal species from which the laccase was obtained might be a reason for this discrepancy [28].

The fact that a laccase activity of 800 U l^{-1} led to a lower decolouration percentage could be attributed to the lack of oxygenation as well as to the increasing concentration of degradation products. Rodríguez-Couto et al. [29] also found this effect on the *in vitro* decolouration of the leather dye Luganil Green (130 mg l^{-1}) by crude laccase from *T. hirsuta*.

3.2.2. Effect of RBBR concentration on RBBR decolouration by crude laccase

It was found that the highest decolouration was obtained for an RBBR concentration of 133.33 mg l^{-1} (Fig. 4). The effect of RBBR concentration on RBBR decolouration was very notorious on the initial decolouration rate. Thus, the initial rate decreased when RBBR concentration increased except for 133.33 mg l^{-1}, which showed the same initial rate than that of 66.67 mg l^{-1} (Fig. 4). After 2 h a percentage of RBBR decolouration of about 78% was obtained for an initial RBBR concentration of 133.33 mg l^{-1}.

The plot profile of initial decolouration rate *versus* substrate (RBBR) concentration (Fig. 5) indicated substrate inhibition for RBBR concentrations higher than 400 mg l^{-1}. Thus, for concentrations up to 400 mg l^{-1} RBBR decolouration followed Michaelis–Menten model and the kinetic constants determined by Lineweaver–Burk plot were K_M 1.065 mM and V_{max} 0.024 mM min^{-1}.

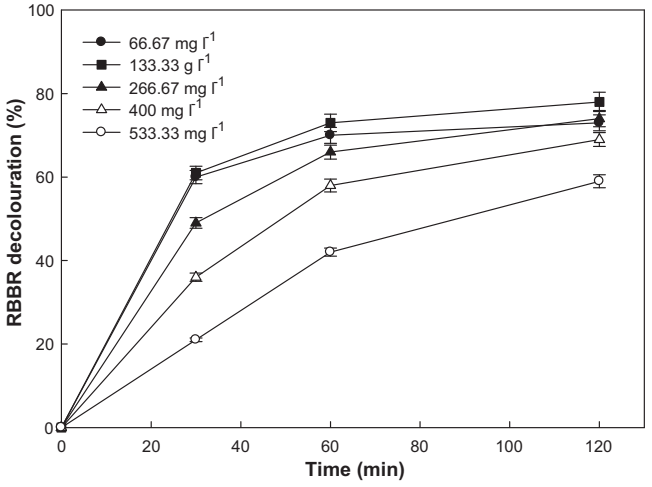


Fig. 4. Effect of RBBR concentration on RBBR decolouration by crude laccase (100 U l^{-1}) from *T. pubescens* grown on SS under SSF conditions.

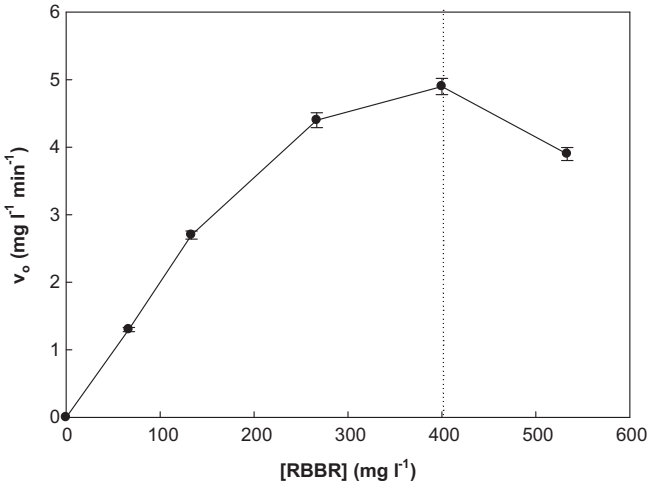


Fig. 5. Initial rate of RBBR decolouration by crude laccase (100 U l^{-1}) *versus* RBBR concentration.

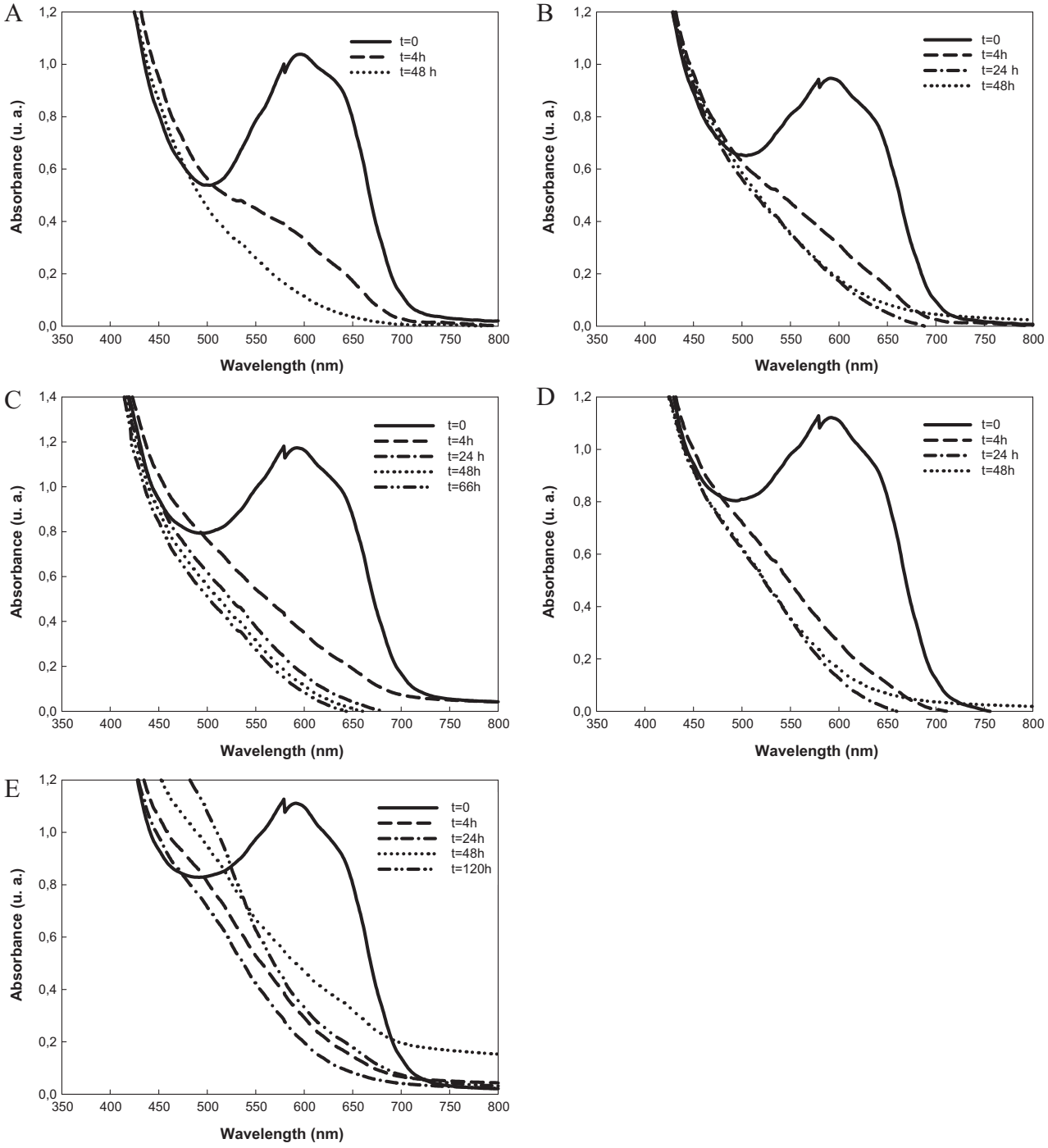


Fig. 6. UV–vis spectra of RBBR decolouration by *T. pubescens* grown on SS under SSF conditions in temporary immersion bioreactors in five successive batches.

3.3. Decolouration of RBBR by T. pubescens grown on SS in a temporary immersion bioreactor

The textile dye RBBR was aseptically added to the temporary immersion bioreactor on day 6 as an aqueous solution in five successive batches. Batches 1, 2 and 4 lasted 48 h whereas batch 3 and batch 5 lasted 66 h and 120 h, respectively. In the first batch the dye was decolourised about 58% in only 4 h and 79% in 48 h (Fig. 6A). In the second batch a decolouration percentage of 55.3% was reached in 4 h and 69.9% in 24 h (Fig. 6B). In the third batch, a decolouration percentage of 57.6% was reached in 4 h, 75.3% in 24 h and 80.5% in 48 h (Fig. 6C). In the fourth batch a decolouration percentage of 64.2% was reached in 4 h and of 77.3% in 24 h (Fig. 6D). In the fifth batch a decolouration percentage of 58.6% was obtained in 4 h and 68.3% in 24 h (Fig. 6E). After 48 h absorbance increased and after 120 h decreased again. This can be due to the release of some dye adsorbed on SS and on fungal biomass and its further degradation by the laccase enzymes secreted by the fungus. However, at the end of each cycle, dye adsorbed on SS and on fungal biomass was negligible. Laccase was the only enzyme involved in the decolouration since there were no other ligninolytic enzymes detected in the culture liquid. Surprisingly, the values obtained are lower than those attained using the crude enzyme (Section 3.2). This is in agreement with the results reported by Zeng et al. [30], who found that RBBR

Table 2
Phytotoxicity of RBBR and fungal-treated RBBR solutions.

	Root elongation (mm)	Germination (%)	GI
Triticum aestivum			
Control	52.6 ± 10.5	92.5 ± 10.0	100
RBBR (133.33 mg l^{-1})	0	0	0
Treated RBBR (1:3)	33.5 ± 9.6	90.0 ± 10.0	61.9
Raphanus sativa			
Control	71.2 ± 10.9	87.5 ± 10.0	100
RBBR (133.33 mg l^{-1})	0	0	0
Treated RBBR (1:3)	5.3 ± 1.3	85 ± 5.0	7.3

decolouration was obtained in shorter time by crude culture filtrates (30 min) than by whole fungal cultures (about 6 days) of the white-rot fungus *Trametes trogii* grown on soybean cake under SSF conditions.

It is worth mention that the culture did not lose its degrading ability after successive dye additions. These results are in agreement with those found by Böhmer et al. [31]. Thus, they performed a successful series of four batch decolouration processes of the textile dyes Levafix Blue and Remazol Brilliant Red using the Rita®-System with *T. versicolor* and *P. chrysosporium* immobilised on both pine wood chips and palm oil fibre.

3.4. Phytotoxicity study

The mean results obtained of root elongation and seed germination together with the germination index for *T. aestivum* and *R. sativa* seeds incubated in distilled water (control), RBBR (133.33 mg l^{-1}) and RBBR transformation products of the 5th batch (1:3, v/v) are shown in Table 2. According to Zucconi et al. [20], values of GI lower than 50% mean high phytotoxicity, values between 50% and 80% moderate phytotoxicity and values over 80% no phytotoxicity. Thus, the parent dye is extremely toxic for both *T. aestivum* and *R. sativa* seeds, since it totally inhibited their germination. The transformation products of RBBR are moderately toxic for *T. aestivum* and highly toxic for *R. sativa* although less toxic than the parent dye. However, laccase was not inactivated by the presence of the dye or its breakdown products.

4. Conclusions

In view of the results obtained, it can be concluded that the white-rot fungus *T. pubescens* grown on SS in temporary immersion bioreactors decolourised the recalcitrant textile dye RBBR in five successive batches with high efficiency without the addition of redox mediators. In addition, no operational problems were detected along cultivation. This makes this system very promising for its scale up as well as for its application to a continuous operation.

On the other hand, laccase enzymes produced by *T. pubescens* grown on SS under SSF conditions in temporary immersion bioreactors hold a great decolouration potential that deserves further research. This is especially interesting since the applicability of whole fungal cultures for decolouration at large scale is difficult.

Acknowledgements

This research was financed by the Spanish Ministry of Education and Science (Project CTM2008-02453/TECNO). Bixent del Barrio is greatly acknowledged for the setting up of the RITA systems.

References

[1] J.R. Aspland, Textile Dyeing and Coloration, Res. Triangle Park, NC, USA, 1997.
[2] I.M. Banat, P. Nigam, D. Singh, R. Marchant, Microbial decolorization of textile-dye containing effluents: a review, Bioresour. Technol. 58 (1996) 217–227.
[3] K. Itoh, Y. Kitade, C. Yatome, A pathway for biodegradation of an anthraquinone dye, C.I. Disperse Red 15, by a yeast strain *Pichia anomala*, B, Environ. Contam. Toxicol. 56 (1996) 413–418.
[4] T. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247–255.
[5] P. Kaushik, A. Malik, Fungal dye decolorization: recent advances and future potential, Environ. Int. 35 (2009) 127–141.
[6] R. Bourbonnais, M.G. Paice, Oxidation of non-phenolic substrates: an expanded role of laccase in lignin biodegradation, FEBS Lett. 267 (1990) 99–102.
[7] H.P. Call, I. Mücke, History, overview and applications of mediated lignolytic systems, especially laccase-mediator systems (LignozymR process), J. Biotechnol. 53 (1997) 163–202.
[8] G.S. Nyanhongo, G. Gübitz, P. Sukyai, C. Leitner, D. Haltrich, R. Ludwig, Oxidoreductases from *Trametes* spp. in biotechnology: a wealth of catalytic activity, Food Technol. Biotechnol. 45 (2007) 250–268.
[9] H. Ali, Biodegradation of synthetic dyes—a review, Water Air Soil. Pollut. 213 (2010) 251–273.
[10] C. Galhaup, H. Wagner, B. Hinterstoisser, D. Haltrich, Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*, Enzyme Microb. Technol. 30 (2002) 529–536.
[11] L. Casieri, G.C. Varese, A. Anastasi, V. Prigione, K. Svobodova, V. Filippello Marchisio, C. Novotny, Decolorization and detoxication of reactive industrial dyes by Immobilized fungi *Trametes pubescens* and *Pleurotus ostreatus*, Folia Microbiol. 53 (2008) 44–52.
[12] P.J. Strong, Fungal remediation of Amarula distillery wastewater, World J. Microbiol. Biotechnol. 26 (2010) 133–144.
[13] A. Pandey, Recent process developments in solid-state fermentation, Process Biochem. 27 (1999) 109–117.
[14] A. Demirbas, F. Akdeniz, Fuel analyses of selected oilseed shells and supercritical fluid extraction in alkali medium, Energ. Convers. Manage. 43 (2002) 1977–1984.
[15] S. Rodríguez Couto, A. Rodríguez, R.R.M. Paterson, N. Lima, J.A. Teixeira, Laccase activity from the fungus *Trametes hirsuta* using an air-lift bioreactor, Lett. Appl. Microbiol. 42 (2006) 612–616.
[16] S. Rodríguez Couto, J. Osma, J.L. Toca Herrera, Removal of synthetic dyes by an eco-friendly strategy, Eng. Life Sci. 9 (2009) 116–123.
[17] M.L. Niku-Paavola, L. Raaska, M. Itävaara, Detection of white-rot fungi by a non-toxic stain, Mycol. Res. 94 (1990) 27–31.
[18] P.R. Warman, Evaluation of seed germination and growth tests for assessing compost maturity, Compost Sci. Util. 7 (1999) 33–37.
[19] OECD, Guideline of the OECD for testing chemical products – terrestrial plants. Growth Test–Method #208, Paris, France, 1984.
[20] F. Zucconi, A. Monaco, M. Forte, M. De Bertoldi, Phytotoxins during the stabilization of organic matter, in: J.K.R. Gasser (Ed.), Composting of Agricultural and Other Wastes, Elsevier, London, 1985, pp. 73–85.
[21] S. Mazumder, S.K. Basu, M. Mukherjee, Laccase production in solid-state and submerged fermentation by *Pleurotus ostreatus*, Eng. Life Sci. 9 (2009) 45–52.
[22] E.B. Gueguim Kana, J.K. Oloke, A. Lateef, R.H. Azanfack Kenfack, A. Adeyemi, Implementation details of computerized temporary immersion bioreactor (TIB): a fermentation case of *Pleurotus pulmonarius*, Biotechnol. Biotec. Equip. 24 (2010) 2149–2153.
[23] T. Jamroz, B. Sencio, P. Głuszcz, S. Ledakowicz, Effect of process conditions on the efficiency of laccase biosynthesis, Chem. Process Eng. 29 (2008) 293–303.
[24] K. Enayatzamir, F. Tabandeh, B. Yakhchali, H.A. Alikhani, S. Rodríguez-Couto, Assessment of the joint effect of laccase and cellobiose dehydrogenase on the decolouration of different synthetic dyes, J. Hazard. Mater. 169 (2009) 176–181.
[25] J.F. Osma, J.L. Toca-Herrera, S. Rodríguez-Couto, Banana skin: a novel waste for laccase production by *Trametes pubescens* under solid-state conditions. Application to synthetic dye decolouration, Dyes Pigments 75 (2007) 32–37.
[26] J.F. Osma, J.L. Toca-Herrera, S. Rodríguez Couto, Biodegradation of a simulated textile effluent by immobilised-coated laccase in laboratory-scale reactors, Appl. Catal. A – Gen. 373 (2010) 147–153.
[27] G.M.B. Soares, M.T. Pessoa de Amorim, M. Costa-Ferreira, Use of laccase together with redox mediators to decolourize Remazol Brilliant Blue R, J. Biotechnol. 89 (2001) 123–129.
[28] M. Guo, F. Lu, M. Liu, T. Li, J. Pu, N. Wang, P. Liang, C. Zhang, Purification of recombinant laccase from *Trametes versicolor* in *Pichia methanolica* and its use for the decolorization of anthraquinone dye, Biotechnol. Lett. 30 (2008) 2091–2096.
[29] S. Rodríguez Couto, D. Hofer, Mª.Á. Sanromán, G.M. Gübitz, Production of laccase by *Trametes hirsuta* grown in an immersion bioreactor and its application in the decolorization of dyes from a leather factory, Eng. Life Sci. 4 (2004) 233–238.
[30] X. Zeng, Y. Cai, X. Liao, X. Zeng, W. Li, D. Zhang, Decolorization of synthetic dyes by crude laccase from a newly isolated *Trametes trogii* strain cultivated on solid agro-industrial residue, J. Hazard. Mater. 187 (2011) 517–525.
[31] U. Boehmer, S.H. Suhardi, T. Bley, Decolorizing reactive textile dyes with white-rot fungi by temporary immersion cultivation, Eng. Life Sci. 6 (2006) 417–420.