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ORIGINAL PAPER

Decolourisation of mushroom farm wastewater by *Pleurotus ostreatus*

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Abstract Mushroom production on coffee pulp as substrate generates an intense black residual liquid, which requires suitable treatment. In the present study, *Pleurotus ostreatus* growth in wastewater from mushroom farm was evaluated as a potential biological treatment process for decolourisation as well as to obtain biomass (liquid inoculum). Culture medium components affecting mycelial growth were determined, evaluating colour removal. Laccase activity was monitored during the process. *P. ostreatus* was able to grow in non diluted WCP. Highest biomass yield was obtained when glucose (10 g/l) was added. The addition of this carbon source was necessary for efficient decolourisation. Agitation of the culture improved biodegradation of WCP as well as fungal biomass production. Laccase and manganese-independent peroxidase activities were detected during fungal treatment of the WCP by *P. ostreatus* CCEBI 3024. The laccase enzyme showed good correlation with colour loss. Both wastewater colour and pollution load (as chemical oxygen demand) decreased more than 50% after 10 days of culture. Phenols were reduced by 92%.

Keywords White-rot fungi · *P. ostreatus* · Decolourisation · Fungal growth · Wastewater

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Introduction

In Latin-America the production of edible mushroom is generally carried out on agricultural waste as substrate. Among them, coffee pulp is a substrate of choice (Martínez-Carrera 2000). The cultivation of mushroom on coffee pulp has been developed in order to ensure its suitability towards local conditions. Advantages of such a process include recycling of the pulp, the rapid and easy production of a food rich in protein and low in fat and an end-product that can be used as an organic fertilizer or as cattle feed (Bermúdez et al. 2001).

However the technology generates a wastewater with intense black colour. Until now, due attention has not been paid to the study of the effluent produced, mainly because of the small scale production units which involve quantities that are low and where contamination is not yet a serious issue. Nevertheless, considering the rapid proliferation of similar production units and the increase in volume of commercial mushroom, the problems related to the

process must be eliminated or reduced. In addition, problems related to colour of different wastewaters discharged into natural water course, have attracted wide interest. Apart from the esthetically undesirable aspect, this situation often is related to the presence of toxic or recalcitrant compounds that increase the negative environmental impact on the receiving bodies.

Anaerobic degradation of wastewater from pasteurized coffee pulp (WCP) has been studied. It had been shown that its organic load (as COD) decreased to acceptable levels, but its dark colour remained (Bello and Sánchez 1997). The use of wood-degrading white-rot fungi could be an alternative biological process for the treatment of WCP, because these organisms are known to have a multi-enzymatic extracellular system that favors the degradation of complex structures present in coloured effluents (Coulibaly et al. 2003).

Pleurotus ostreatus is the third most important cultivated mushroom for food purposes and it is also a well studied white-rot fungus. The genus *Pleurotus* is often associated with the bioconversion of agricultural wastes into valuable food products through the use of their ligninolytic enzymes for biodegradation of organo-pollutants, xenobiotics and industrial contaminants (Cohen et al. 2002)

The use of WCP as a substrate in submerged fermentation to produce mushroom inoculum will reduce its negative impact on the environment, and will allow not only the production of a secondary inoculum but a reduction of substrate contamination as well. Mycelial culture on solids is the common method of inoculum preparation for the production of edible mushroom; however, the use of a liquid medium also offers some advantages (Nieto and Sánchez 1997).

In this study, the growth of *Pleurotus ostreatus* on WCP was assayed under different conditions and its effect on bioremediation parameters (colour, COD and phenols) was evaluated.

Materials and methods

Effluent and analytical methods

WCP was collected from the mushroom farm at the Center for Industrial Biotechnology, (CEBI, Universidad de Oriente, Santiago de Cuba). The sample was filtered through a gauze to eliminate solid materials, centrifuged at 3,000 rpm for 5 min and stored in plastic flasks at 4°C. Composition of effluent was determined and the results are presented in Table 1.

Analyses of pH, DQO, DBO_5, nitrogen, total solids and luminance for colour measurement, were carried out in accordance with methods proposed by APHA (1998).

The calculation of colour removal was carried out as described by Rodriguez et al. (2003). The hue was designated as the degree of brightness by luminance. This parameter is determined from the light transmission characteristics of the filtered sample by means of a spectrophotometer. Transmittance values corresponding to ten wavelengths (narrow spectral band) are summarized and then multiplied by the appropriate factors (0.10). The calculation of colour removal was carried out following equations:

$$A = 2 - \text{Log } L \quad (1)$$

$$R = (A_i - A_f)/A_i \quad (2)$$

where:

L = luminance values,
R = colour removed,
A = absorbance (initial or final) obtained from the values of luminance according to Eq. 1,
Log = logarithm

Phenol concentration was quantified using the Folin-Denis method (Maestro et al. 1991). Residual

Table 1 Composition of the wastewater from the edible mushroom farm (WCP)

pH	6.4 ± 0.6
Colour (dil. 1:10)	50.9 ± 3.1
Apparent colour	Black
COD (g/l)	59.4 ± 3.3
BOD_5 (g/l)	31.5 ± 3.1
Total solids (g/l)	7.90 ± 0.90
Total phenol (g/l)	0.26 ± 0.05
Carbohydrates (g/l)	1.15 ± 0.19
Reducing sugar (mg/l)	1.94 ± 0.12
Ammoniacal nitrogen (mg/l)	12.50 ± 0.97
Phosphorous (mg/l)	2.95 ± 0.10

glucose and carbohydrates present in the culture medium were determined as described by Miller (1959) and Dubois et al. (1956), respectively. Biomass was calculated as dry weight (APHA 1998). Biomass/substrate yield ($Y_{X/S}$) is the quotient of biomass over reduced sugar consumed.

Laccase activity was measured by the oxidation of guaiacol at 460 nm in a reaction mixture at 30°C, containing guaiacol 10 mmol/l and 50 mmol/l of phosphate buffer, pH 6.0 (Palmieri et al. 2000). Enzymatic activity is defined as the quantity of enzyme that produces an increase of absorbance of 1 unit/min.

Microorganism and preparation of inoculum

Stock cultures of *Pleurotus ostreatus f*.sp.(CCEBI 3024) were maintained at 4°C on potato-dextrose agar (Difco Laboratories). *P. ostreatus* mycelia were adapted to grow on WCP in agar plates containing potato dextrose broth and WCP (20%). Cell extracts were prepared from *P. ostreatus* cultures grown for 7 days on plates. Mycelium from each plate was scraped off and added to an Erlenmeyer flask containing 50 ml of NaCl (1%), and then homogenized. Aliquots of the homogenate were used to inoculate erlenmeyer flasks with liquid media (10% v/v). The inoculum represented approximately 0.02 g/l in dry weight.

Culture conditions

The BM medium used was a modification of basidiomycetes rich medium described by Martínez et al. (1994), which included the following components (g/l): yeast extract 0.5, L-asparagine 0.65, KH_2PO_4 1.0, KCl 0.5 and $MgSO_4 \cdot 7H_2O$ 0.5. One milliliter of micro-element solution (1000×) was added. This solution contained (mg/l): boric acid 500, $CuSO_4 \cdot 5H_2O$ 40, KI 100, $FeCl_2 \cdot 6H_2O$ 200, $MnSO_4 \cdot 4H_2O$ 400, $ZnSO_4 \cdot 7H_2O$ 400 and $NaMbO_4 \cdot 2H_2O$ 200. The pH of BM was adjusted to 6.

Different concentrations (5, 10, 20 g/l) of glucose were evaluated. Two other carbon sources were assayed, besides glucose: sucrose and glycerol. Each one was added at a concentration of 20 g/l in BM.

A second medium termed simple medium (SM) was comprised of (g/l): glucose 20, peptone 5, yeast extract 2, KH_2PO_4 2 and $MgSO_4 \cdot 7H_2O$ 0.5, at the same pH as BM.

WCP was used at a final concentration of either 50% or 100% in both media. To that, BM or SM or some of its components were added directly. Wastewater (WCP) at a concentration of 50% without addition of external nutrient was also tested as substrate for fungal growth. Culture media were compared in order to produce biomass, laccase enzyme and to reduce colour.

All Erlenmeyer flasks were kept at 28°C and stirred at 120 rev/min (except where stated) for 10 days in the dark. Experiments were performed using five replicates for each set of conditions.

Decolourization test

Optimized conditions obtained from experiments described above, were used to evaluate the potential of *Pleurotus* for WCP bioremediation. Erlenmeyer flasks with 150 ml of medium (WCP 100%) were inoculated as previously described. The treatment was carried out over a 15 day period and COD, phenol and colour removal were determined. Besides laccase activity, manganese-independent or versatile peroxidase (VP), manganese peroxidase (MnP), lignin peroxidase (LiP) and aryl-alcohol oxidase (AAO) were also assayed.

MnP, LiP, AAO and VP were determined by standard methods according to Heifling et al. (1998), Tien and Kirk (1984), Guillén et al. (1992) and Camarero et al. (1999), respectively. Enzyme units were defined as 1 μmol of substrate oxidized per minute, under the assay conditions described.

Results and discussion

WCP is the wastewater from edible mushroom farms obtained when coffee pulp is pasteurized during the substrate preparation process. This effluent has high pollution characteristics, particularly an intense dark colour, a significant COD as well as presence of phenols. Disposal into the environment without degradation or detoxification, may result in serious environmental pollution (Table 1). Soluble organic

compounds from coffee pulp are responsible for the pollutant effect of WCP, similar to other wastewaters from depulping or washing of coffee in processing units. However, in the present case, the high temperatures employed in this operation probably facilitate the liberation and diffusion of components present in the pulp (Bello and Sánchez 1997). Oligomeric and polymeric phenolic compounds (hydroxycinnamic acids and condensed tannins) give this wastewater its characteristic black colour with recalcitrant properties (Ramírez and Clifford 2000).

The BOD/COD relation of WCP, higher than 0.5 suggests the use of a biological treatment for its bioremediation. However high-molecular-weight compounds are not easily degraded by bacteria, and thus coloured compounds pass through biological treatment systems largely undegraded. Besides, phenols present in the wastewaters have antibacterial effects, thereby limiting conventional treatment by aerobic-anaerobic processes (Scalbert 1991).

Previously studies have reported that *P. ostreatus* is capable of decolourizing wastewater with high polyphenol content (Fountoulajkis et al. 2002; Rodríguez et al. 2003). Nevertheless the removal capacities of white-rot fungi varied with the culture conditions (Kim et al. 1996). With relation to the latter, WCP is limited in nutrients such as simple carbon, nitrogen and phosphorous sources (Table 1).

Effect of carbon source and its concentration

The presence of glucose in the culture media resulted not only in highest biomass production but also in greatest colour removal capacity (Tables 2 and 4). Increasing the glucose concentration in the medium resulted in a seven-fold increase in the decolourising ability of *P. ostreatus*, although the enzymatic activity that was monitored (laccase) showed no significant changes (Table 2). This behavior is also appreciated in values shown in Table 4 where the sole addition of glucose (20 g/l) to WCP produced similar effects. These results suggest that glucose-induced enzymes play a key role in colour removal.

White-rot mushroom are able to degrade recalcitrant compounds (often associated with colour) through a cometabolic process that requires an alternative source of carbon (Aust and Benson 1993). Other authors have recognized that critical quantities of glucoses between 1 and 5 g/l are necessary to maintain the decolourization capacity of white-rot fungi (Zhang et al. 1999). On the other hand, it is known that glucose oxidase (GO) is produced on glucose-rich media together with AAO by such organisms (Ander and Marzullo 1997). Studies are underway to correlate enzymatic activities of GO and AAO with the production of H_2O_2 and their inter-relation with ligninolytic enzymes.

Table 2 Influence of glucose concentration on biomass, laccase activity and colour reduction

Glucose concentration (g/l)	Biomass (g/l)	Laccase activity (U/ml)	Colour removal (%)
WCP + 5	1.42 [c]	3.18 [a]	8.1 [c]
WCP + 10	4.37 [b]	2.19 [a]	23.3 [b]
WCP + 20	8.26 [a]	1.88 [a]	57.1 [a]

WCP diluted 50% (v:v) was supplemented with basidiomycete medium (BM). Values in the same column with the same letters are not statistically different at the level of 5%

As early as the 70's Green et al. (1977) proposed that regulation of lignin polymerization may occur through the action of GO. Later, Leonowickz et al. (1999, 2001) reported on the cooperation between laccase (LAC) and GO in a proposed enzymatic system that appeared to work during lignocellulose transformation. In this system LAC oxidizes lignin-derived radicals to quinones, which can serve as hydrogen acceptors for GO. Once reduced, radicals and quinines prevent spontaneous repolymerization, counteracting poisonous levels of quinines in the medium enabling laccase to continue its function. GO produces H_2O_2, which serves as a co-substrate for peroxidase activities along with other enzymes that participate in lignin breakdown. Similar mechanisms could take place when considering the transformation of phenolic compounds present in WCP which are responsible for its dark colour. Decolourization is therefore affected by glucose addition to the medium.

Pleurotus spp. produces MnP, VP and laccase, but not LiP. Laccase has been correlated with the decolourization of wastewaters by such fungi (Kissi et al. 2001; Dias et al. 2004). It was therefore chosen to monitor these enzymatic activities as representative of ligninolytic activity in the present work.

Biomass dry weight increased with higher concentrations of glucose (Table 2), and reached values

Table 3 Effects of different carbon source on biomass production and decolourization

Carbon source	Biomass (g/l)	Laccase activity (U/ml)	Colour removal (%)
WCP + BM	3.15 [c]	1.12 [c]	15 [c]
WCP + Glucose + BM	13.27 [a]	2.34 [a]	66 [a]
WCP + Sucrose + BM	6.16 [b]	1.77 [b]	50 [b]
WCP + Glycerol + BM	10.77 [ab]	2.19 [ab]	66 [a]

Values in the same column with the same letters are not statistically different at the level of 5%. BM (basidiomycete medium). WCP diluted 50% (v:v)

of 8.26 g/l at 20 g/l glucose. These results were similar to those obtained by Guillén et al. (1998) on synthetic medium (8.6 g/l) but after 16 days of culture. The best $Y_{X/S}$ was achieved at 10 g/l of glucose with a value of 0.68; compared with 0.28 and 0.41 obtained for 5 g/l and 20 g/l glucose, respectively. However, the highest concentration of glucose is necessary in order to improve decolourisation (57.1%).

Different carbon sources were utilized to supplement 50% diluted WCP in order to investigate their effect on colour removal and mushroom growth, as well as on laccase activity. No significant differences were found with regards to the decrease of WCP colour between cultures grown in the presence of glucose or glycerol (Table 3); therefore, either could be used as carbon source to help in WCP decolourization and to obtain liquid inoculum. Similar results were obtained by Kissi et al. (2001) and Fountoulajkis et al. (2002) for olive mill wastewater biodegradation when using glycerol or glucose as an additional substrate. Addition of sucrose resulted in low biomass production coupled with non optimal decolourization (Table 3).

Effect of culture medium composition

Poor growth was observed when using WCP alone (diluted 50%) as this wastewater lacks nutrients. Nevertheless the fungus was capable of using some components from WCP to grow to 0.26 g/l biomass and also to activate its laccase enzyme(s) for detoxification, showing 15% colour removal (Table 4).

Table 4 Effects of media composition on growth and decolorizing ability of *Pleurotus ostreatus* CCEBI 3024

Medium composition	Biomass (g/l)	Laccase activity (U/ml)	Colour removal (%)
WCP (50%)	0.26 [d]	1.07 [d]	15.0 [d]
WCP (50%) + glu	3.15 [c]	1.27 [d]	58.0 [c]
BM	7.30 [b]	0.14 [e]	–
SM	6.90 [b]	0.19 [e]	–
WCP (50%) + BM	13.27 [a]	2.19 [c]	66.0 [b]
WCP (50%) + glu + microelements	11.80 [a]	2.30 [c]	60.0 [c]
WCP (50%) + SM	8.20 [b]	8.53 [b]	74.6 [a]
WCP (100%) + SM	14.60 [a]	12.70 [a]	68.6 [ab]

BM, basidiomycete medium which contained 20 g/l of glucose (glu); SM, Simple medium. Similar letters in the same column indicate no statistical difference. WCP dilution v:v is shown in parenthesis. The microelements are described in BM composition

WCP contains anti-physiological compounds e.g. polyphenols such as tannins (Field and Lettinga 1987) that could affect biomass concentration but stimulate laccase activities at least fifteen times higher than the activities observed in control media. The highest laccase activity was detected in WCP (100%) supplemented with SM and highest biomass production was also observed. Laccase activity in fungal cultures can be increased by the addition of different aromatic compounds to the media (Marques de Souza et al. 2004) or by different industrial effluents that contain them (Dahiya et al. 2001; Dias et al. 2004). Moreover, Carbajo et al. (2002) showed that hydrolysable tannins such as tannic acid could act as inducers of laccase activity due to the enhancement of expression of laccase genes. Tannic acid is the tannin most widely distributed in nature. Tannins are also the second most abundant group of plant polyphenols, after lignin. Therefore WCP can be considered as a cheap laccase inducer, an enzyme with many potential applications (Riva 2006).

Although the best colour removal value was measured in SM medium with WCP diluted at 50%, no significant differences were found between both WCP concentrations (50 and 100%) (Table 4).

The two culture media tested, BM and SM are similar in composition. However, BM contains only yeast extract as complex nitrogen source whereas SM contains both peptone and yeast extract. Therefore BM could be considered limited in nitrogen. In

addition, BM contains a number of microelements that are absent in SM such as copper and manganese which are known as effective inducers of ligninolytic enzymes (Palmieri et al. 2000). In WCP supplemented with BM medium components, high biomass production was obtained but with low colour removal. However, significant colour removal was observed in the presence of SM medium (Table 4). This could be linked to the presence of peptone in the SM medium. Indeed, Dahiya et al. (2001) showed that peptone had a positive effect on wastewater decolourization. SM therefore constitutes the best alternative for the dual objective: biomass production to use as mushroom inoculum as well as wastewater biodegradation. Undiluted WCP can be treated by *P. ostreatus*. In the culture medium, mycelial growth is obtained in pellet form, typical of the submerged culture with agitation, but colour adsorption was not observed in the mycelium (data not shown).

Effect of agitation

The effect of agitation on biodegradation resulting from *P. ostreatus* (10 days in 50% WCP/SM medium) was studied. The decolourization and COD removal increased in agitated culture (120 rev/min) as shown in Table 5. Biomass was greater in agitated culture as compared to static culture. COD removal and decolourization can therefore be attributed to increased amounts of dissolved O_2 that favoured mushroom growth and then its metabolic processes.

Table 5 Effect of agitation (120 rev/min) on growth of *P. ostreatus* in WCP and reduction of COD and colour

Treatment	Biomass (g/l)	Colour removal (%)	COD removal (%)
WCP (50%) + SM agitated	8.20	74.6	71.6
WCP (50%) + SM static	2.45	49.7	49.2

SM, Simple medium. WCP dilution v:v is shown in parenthesis. The values are the mean of five values

Also, diffusion of nutrients, substrates and products (enzymes) as a result of agitation was augmented. Yesilada et al. (1998, 2003) described the same effects on decolourization of olive mill wastewaters and dyes.

Decolourisation assay

To obtain more information about *P. ostreatus* ligninolytic enzymes implicated in WCP decolourization, several enzyme activities were assayed using the optimized conditions: non diluted wastewater, glucose 20 g/l and SM components in agitated culture (Fig. 1).

WCP decolourization was associated with fungal growth and colour decreased by 77.8% after 12 days of culture. *P. ostreatus* did not need carbon or nitrogen-limiting conditions to induce the ligninolytic activities as was the case in other studies (Martinez et al. 1994; Kim et al. 1996). Of the five enzyme

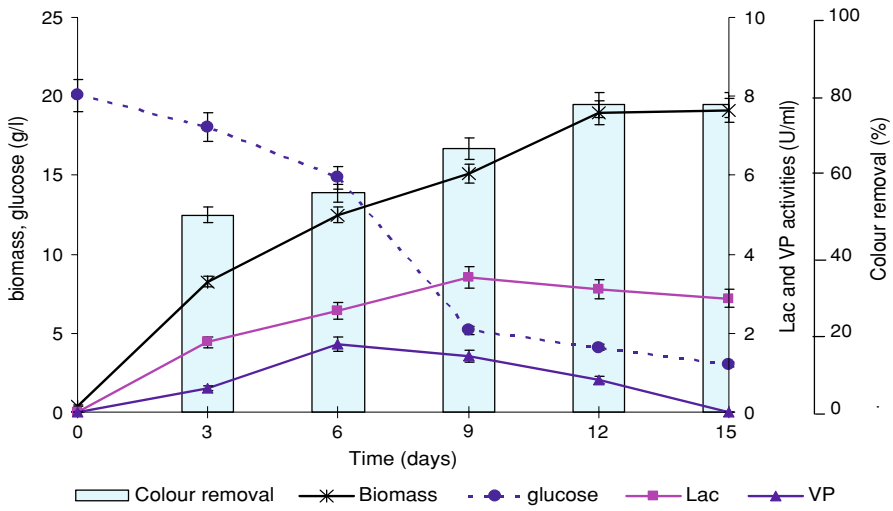

Fig. 1 Effect of *P. ostreatus* growth on bioremediation of non diluted WCP. Error bars indicate standard deviation from mean values

activities assayed in these extracts, only LAC and VP were detected. Laccase activity seemed to be correlated ($R^2 = 0.97$, $P < 0.05$) with wastewater decolourization in the growth phase. VP was first reported when a peptone based medium was used (Martinez et al. 1996). The presence of peptone in SM therefore favoured VP induction. The maximum value of this enzymatic activity was detected on the nineth day and then it diminished to undetectable levels at the end of the culture period.

Pleurotus ostreatus was able to reduce the pollution generated by WCP, removing the organic load (78.9%) and the colour (77.8%) in the residual liquid, over a period of 15 days. The COD removal values are similar to those reported by other authors using other treatments in wastewaters rich in polyphenols (Fountoulajkis et al. 2002; Coulibaly et al. 2003). However, little is known to date about colour removal in wastewater from coffee pulp. Fungal treatment resulted in high polyphenol removal (92%).

Conclusions

Pleurotus ostreatus is, therefore, able to grow using WCP as medium and to reduce notably colour, phenol content and COD, thus proving to be a good agent for the effective treatment of this wastewater.

References

American Public Health Association (1998) Standard methods for the examination of water and wastewater, 20th edn. Washington, DC, USA, p 1124

Ander P, Marzullo L (1997) Sugar oxidoreductases and veratryl alcohol oxidase as related to lignin degradation. J Biotechnol 53:115–131

Aust SD, Benson J (1993) The fungus among us-use of white rot fungi to biodegrade environmental pollutants. Environ Health Perspect 101:232–233

Bello R, Sánchez JE (1997) Anaerobic filter treatment of wastewater from mushroom cultivation on coffee pulp. World J Microbiol Biotechnol 13:51–55

Bermúdez RC, García N, Gross P, Serrano M (2001) Cultivation of *Pleurotus* on agricultural substrates in Cuba. Micología Aplicada Internacional 13(1):25–29

Camarero S, Sarkar S, Ruiz-Dueñas FJ, Martínez MJ, Martínez AT (1999) Description of a versatile peroxidase involved in natural degradation of lignin that has both Mn-peroxidase and lignin-peroxidase substrate binding sites. J Biol Chem 274:10324–10330

Carbajo JM, Junca H, Terron MC, Gonzalez T, Yague S, Zapico E, Gonzalez AE (2002) Tannic acid induces transcription of laccase gene cglccl in the white-rot fungus *Coriolopsis gallica*. Can J Microbiol 48(12):1041–1047

Cohen R, Persky L, Hadar Y (2002) Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. Appl Microbiol Biotechnol 58:582–594

Coulibaly L, Gourene G, Agathos (2003) Utilization of fungi for biotreatment of raw wastewaters: review. Afr J Biotechnol 2(12):620–630

Dahiya J, Singh D, Nigam P (2001) Decolourisation of synthetic and spentwash melanoidins using the white-rot fungus *Phanerochaete chrysosporium* JAG-40. Bioresour Technol 78(1):95–98

Dias A, Bezerra R, Pereira N (2004) Activity and elution profile of laccase during biological decolorization and dephenolization of olive mill wastewater. Bioresour Technol 92:7–13

Dubois M, Guilles K, Hamilton J, Roberts P, Smith F (1956) Colourimetric determination of sugars and related substances. Anal Chem 28:350–356

Field J, Lettinga G (1987) The effect of oxidative coloration on the methanogenic toxicity and anaerobic biodegradability of phenols. Biol Waste 29:161–179

Fountoulajkis M, Dokianakis M, Kornaros M, Aggelis G, Lyberatos G (2002) Removal of phenolics in olive mill wastewaters using the white-rot fungus *Pleurotus ostreatus*. Water Res 36:4735–4744

Green TR (1977) Significance of glucose oxidase in lignin degradation. Nature 268(5615):78–80

Guillén F, Martínez AT, Martínez MJ (1992) Substrate specificity and properties of the aryl-alcohol oxidase from the ligninolytic fungus *Pleurotus eryngii*. Eur J Biochem 209:603–611

Guillén G, Márquez F, Sánchez J (1998) Producción de biomasa y enzimas ligninolíticas por *Pleurotus ostreatus* en cultivo sumergido. Rev Iberoam Micol 15:302–306

Heinfling A, Martínez MJ, Martínez AT, Bergbauer M, Szewzyk U (1998) Purification and characterization of peroxidases from the dye-decolorizing fungus *Bjerkandera adusta*. FEMS Microbiol Lett 165:43–50

Kim B, Ryu S, Shin K (1996) Effect of culture parameters on the decolorization of Remazol brilliant blue R by *Pleurotus ostreatus*. J Microbiol 34:101–104

Kissi M, Mountandar M, Asshobei O, Gargiulo E, Palmieri G, Giardina P, Sannia G (2001) Roles of two white-rot basidiomycete fungi in decolorisation and detoxification of olive mill waste water. Appl Microbiol Biotechnol 57:221–226

Leonowicz A, Cho N, Luterek J, Wilkolazka A, Wotjas M, Matus A, Hofritchter M, Wesenberg D, Rogalski J (2001) Fungal laccase: properties and activity on lignin. J Basic Microbiol 41:185–227

Leonowicz A, Rogalski J, Jaszek M, Luterek J, Wasilewska M, Malarczyk E, Ginalska G, Fink-Boots M, Cho N (1999) Cooperation of fungal laccase and glucose 1-oxidase in transformation of Bjorkman lignin and some phenolic compounds. Holzforschung 53:376–380

Maestro D, Borja R, Martin A, Fiesta JA, Mendoza J (1991) Biodegradación de los compuestos fenólicos presentes en el alpechín. Fasc 42:271–276

Marques de Souza C, Tychanowicz G, Farani D, Peralta R (2004) Production of laccase isoforms by *Pleurotus*

pulmonarius in response to presence of phenolic and aromatic compounds. J Basic Microbiol 44:129–136

Martínez AT, Camarero S, Guillén F, Gutiérrez A, Muñoz C, Varela M, Martínez E, Barrosa J, Ruel K, Pelayo M (1994) Progress in biopulping of non woody materials: chemical, enzymatic and ultrastructural aspects of wheat–straw delignification with ligninolytic fungi from the genus *Pleurotus*. FEMS Microbiol Rev 13:265–284

Martínez MJ, Ruiz-Dueñas FJ, Guillén F, Martínez AT (1996) Purification and catalytic properties of two manganese–peroxidase isoenzymes from *Pleurotus eryngii*. Eur J Biochem 237:424–432

Martínez-Carrera D (2000) Mushroom biotechnology in tropical America. Int J Mushroom Sci 3:9–20

Miller G (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem 31:426–428

Nieto-López C, Sánchez-Vazquez J (1997) Mycelial growth of *Pleurotus* and *Auricularia* in agroindustrial effluents. Micología Neotropical Aplicada 10:47–56

Palmieri G, Giardina P, Bianco C, Fontanella B, Sannia G (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. Appl Environ Microbiol 66:920–924

Ramírez J, Clifford M (2000) Coffee pulp polyphenols: an overview. In: Sera T, Soccol CR, Pandey A, Roussos S (eds) Coffee biotechnology and quality. Kluwer, Dordrecht, pp 471–488

Riva S (2006) Laccases: blue enzymes for green chemistry. Trends Biotechnol 24(5):219–226

Rodríguez S, Fernández M, Bermúdez RC, Morris H (2003) Tratamiento de efluentes industriales coloreados con *Pleurotus* spp. Rev Iberoam Micol 20:164–168

Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30:875–883

Tien M, Kirk TK (1984) Lignin-degrading enzyme from *Phanerochaete chyrsosporium*: purification, characterization and catalytic properties of a unique H_2O_2-requiring oxygenase. PNAS-Biol Sci 81(8):2280–2284

Yesilada O, Asma D, Cing S (2003) Decolorization of textile dyes by fungal pellets. Process Biochem 38:933–938

Yesilada O, Sik S, Sam M (1998) Biodegradation of olive oil mill wastewater by *Coriolus versicolur and Funalia trogi:* effects of agitation, initial COD concentration, inoculum size and immobilization. World J Microbiol Biotechnol 14:37–42

Zhang F, Knapp J, Tapley K (1999) Decolourisation of cotton bleaching effluent with wood rotting fungus. Water Res 33(4):919–928