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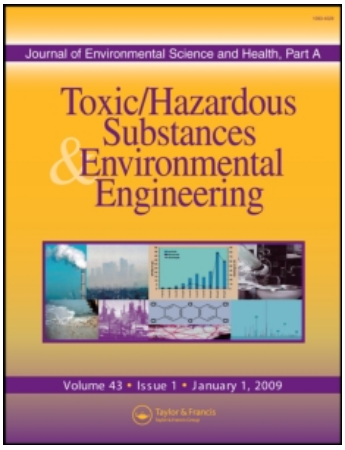

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Application of 2_{III}^{7-3} fractional factorial experimental design to enhance enzymatic activities of *Pleurotus ostreatus* with high concentrations of polychlorinated biphenyls

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Application of 2_{III}^{7-3} fractional factorial experimental design to enhance enzymatic activities of *Pleurotus ostreatus* with high concentrations of polychlorinated biphenyls

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A 2_{III}^{7-3} fractional factorial experimental design was used to establish 16 culture media, with and without PCBs to enhance the activities of laccase (Lac), manganese peroxidase (MnP), and versatile peroxidase (VP) produced by the white rot fungus *Pleurotus ostreatus.* The culture was added to 10,000 mg L^{-1} of transformer oil, containing 71% of the identified Arochlor 1242. The culture conditions were established with eight variables at two values (levels); pH (4 and 6), agitation (100 and 200 rpm), $CuSO_4$ (150 and 250 mg L^{-1}), $MnSO_4$ (50 and 200 mg L^{-1}), Tween 80 (13 and 3500 mg L^{-1}), wheat straw (0 and 2.5 g L^{-1}), sugarcane bagasse (0 and 2.5 g L^{-1}),and Arochlor 1242 (0 and 7100 mg L^{-1}) at 4, 8, 12, 16 and 20 days old culture. Laccase activity was enhanced at a high value of pH and low value of agitation ($P < 0.001$) and correlated positively ($R^2 = 0.9$; $\alpha = 0.05$) with the removal of polychlorinated biphenyls (PCBs). VP activity was enhanced 27-fold with PCBs, Tween 80 and pH. The MnP activity was increased 1.2-fold with PCBs. The fractional factorial experimental design methodology allowed us to determine the *P. ostreatus* culture media conditions to enhance Lac and VP activities for efficient removal of Arochlor 1242 (one of the most recalcitrant organochloride pollutants). The factors that shown the greatest effect on Lac activity were: pH, agitation and high concentrations of Arochlor 1242.

Keywords: Polychlorinated biphenyls, versatile peroxidase, manganese peroxidase, laccase, *Pleurotus ostreatus,* arochlor, transformer oil.

Introduction

PCBs can be degraded under anaerobic and aerobic conditions,[1] and ligninolytic fungi can degrade relatively high concentrations of PCBs under solid and liquid conditions as demonstrated in our previous work.[2,3] Ligninolytic fungi degrade these toxic compounds by producing enzymes namely; Lac, MnP, lignin peroxidase (LiP), and VP. Biodegradation of organic compounds can be improved by increasing the enzymatic activities, mainly by changing the nutritional and/or environmental conditions of the fungal culture, through the use of experimental designs. Macedo et al.;[4] Alam et al.[5]; Manera et al.[6] and Seker et al.[7] employed a fractional factorial design to optimize the culture medium, without contaminants, for the production of enzymes from different microorganisms.

Prasad et al.[8] evaluated the effect of combined factors (pH, glucose, wheat bran, urea, inoculum, yeast extract, 2,5-xylidine [inducer], and KH_2PO_4), in the absence of organic pollutants on *P. ostreatus* Lac activity. Rodríguez et al.[9] studied the transformation of benzo(a)pyrene by Lac and VP in a glucose peptone yeast extract medium with fixed conditions of $MnSO_4$ and $CuSO_4$, however, under these conditions, changes in the response variables do not allow to know the real effect of working under several conditions at the same time.

In addition, the fractional designs are an effective methodology that greatly reduce the number of experiments and expressed using the notation l^{k-p}, where l is

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the number of levels of each factor investigated, k is the number of factors or variables analyzed, and p represents the factors generated (e.g., in a 2_{III}^{7-3} fractional design, l = 2, k = 7, and p = 3). As a result the design does not provide full resolution of a full factorial design, which means that certain interaction effects are confounded with (i.e., identical to) other effects.[10] The 2_{III}^{7-3} fractional factorial experimental design is 1/7 of a two-level, seven factors in the factorial design, that requires only 16 runs rather than the 128 runs required for the full 2^7 factorial design. The levels of each factor are commonly coded as +1 (for the high value of the variable) and −1 (for the low value of the variable).

In the present study, instead of using these coded values the actual value of each variable was provided and a fractional factorial experiment was generated from a full factorial experiment by choosing an alias structure that determined which effect was confounded by another. The aim of this study was to determine culture conditions to enhance Lac, MnP, and VP activities of *P. ostreatus* for PCBS removal using 2_{III}^{7-3} fractional factorial experimental design with and without PCBs.

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Materials and methods

Strain

White rot fungus, *P. ostreatus* (Jacq:Fr) Kummer (ATCC 38540) was cultured at 28°C on potato dextrose agar plates, and sub-cultured every month.

Identification of the arochlor

PCBs were extracted from 10 mL of Transformer oil (donated by the DEBISA Company, México) with four volumes of 40 mL of hexane-acetone (10:6, v v^{-1}). The extracted organic phase was dissolved in 1.5 mL of concentrated H_2SO_4, washed twice with distilled water, and then the organic phase was transferred to vials with anhydrous sodium sulfate to dry the samples. Samples were filtered through a 0.22-μm Millipore membrane and purified through a florisil cartridge.[11]

Arochlor was identified by gas chromatograph (Agilent model 6890N), equipped with a 5973 mass selective detector and an Agilent HP-5MS capillary column. The conditions used were: oven temperature 280°C and helium was used as the carrier gas with a constant flow rate of 1.9 mL min^{-1}. The injector was kept at constant pressure and temperature (250°C), with a detector temperature of 270°C. The injection volume was 1 μL in the splitless mode. The internal and calibration standards were: 1,3,5-trichlorobenzene and Arochlor 1242, respectively.

Table 1. Coded and actual values of the factors for 2_{III}^{7-3} fractional factorial experimental design.

Factor	*Low (−1)*	*high (+1)*
$CuSO_4$ (mg $L^{.1}$)	150	250
$MnSO_4$ (mg L^{-1})	50	200
Wheat straw (g L^{-1})	0	2.5
Sugarcane bagasse (g L^{-1})	0	2.5
pH	4	6
Agitation (rpm)	100	200
Tween 80 (mg L^{-1})	13	3,500

PCBs (mg L^{-1}) = 0 and 10,000.

Experimental design, 2_{III}^{7-3} factorial experimental design to determine the effect of culture parameters on enzyme activities

In this work, seven variables; $MnSO_4$, $CuSO_4$, Tween 80, agitation, pH, wheat straw, sugarcane and bagasse with and without PCBs; were tested at two values (Table 1). Table 2 shows the 16 culture media established in the 2_{III}^{7-3}fractional factorial design[12] with and without PCBs, therefore a total of 32 media cultures were established. The Lac, MnP, and VP activities of *P. ostreatus* were measured at 4, 8, 12, 16, and 20 days for each culture condition.

The model for the enzyme activities (response variables), is described as follows:

$$\hat{y} = u + \beta_N X_k + \varepsilon \quad (1)$$

where:
$\hat{y}$ = Response variable (calculated)
u = mean of the response variable
X_k = nominal variable
β_n = coefficients of the X_k variable
ε = Standard error

Culture conditions

Fungus was grown in 125-mL Erlenmeyer flasks, containing 40 mL of minimum medium: 5 g L^{-1} dextrose and 5 g L^{-1} yeast extract[13] and cultured in an orbital shaker at 100 and 200 rpm, and pH 4 and 6 (adjusted prior to sterilization). Mycelia, grown in agar plates were mixed for 30 s in a sterilized blender cup containing 15 mL of sterilized water; then 1 mL of homogenate was inoculated into each flask, cultures were pre-incubated at 28°C in darkness for 7 days. Then 1% of prepared inoculums were added to each sterilized Erlenmeyer flask containing the 16 formulations, prepared according to the established conditions (Table 2). Then, 16 media were amended with 7100 mg L^{-1} of PCBs and 16 were set without them and incubated for 4, 8, 12, 16, and 20 days. Enzyme analyses were performed in aliquots of 1 ml centrifuged at 13,960 rpm for 20 min at 4°C. An abiotic control (sterilized mycelium) was established.

Table 2. Matrix for the 2_{III}^{7-3}fractional factorial experimental design.

Treatment	*CuSO4*	*MnSO4*	*Wheat straw*	*Sugarcane bagasse*	*pH*	*Agitation*	*Tween 80*
1	−1	−1	−1	−1	−1	−1	−1
2	+1	−1	−1	−1	+1	−1	+1
3	−1	+1	−1	−1	+1	+1	−1
4	+1	+1	−1	−1	−1	+1	+1
5	−1	−1	+1	−1	+1	+1	+1
6	+1	−1	+1	−1	−1	+1	−1
7	−1	+1	+1	−1	−1	−1	+1
8	+1	+1	+1	−1	+1	−1	−1
9	−1	−1	−1	+1	−1	+1	+1
10	+1	−1	−1	+1	+1	+1	−1
11	−1	+1	−1	+1	+1	−1	+1
12	+1	+1	−1	+1	−1	−1	−1
13	−1	−1	+1	+1	+1	−1	−1
14	+1	−1	+1	+1	−1	−1	+1
15	−1	+1	+1	+1	−1	+1	−1
16	+1	+1	+1	+1	+1	+1	+1

Coded values of variables were obtained by algebraic multiplication, as follows; pH = ± ($CuSO_4$ × $MnSO_4$ × Wheat straw); Agitation = ± ($MnSO_4$ × Wheat straw × Sugarcane); Tween 80= ± ($CuSO_4$ × Wheat straw × Sugarcane bagasse).

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Enzyme activity determination

Enzyme activities were analyzed in the supernatants. Lac activity was determined by oxidation of 2,2'-azinobis, 3-ethylbenzothiazoline-6-sulphonate (ABTS) at 436 nm (ε 29300 $M^{-1}cm^{-1}$). Enzyme extract (100 μL) and ABTS (0.5 mM final concentration) were appropriately diluted in a 60 mM acetate buffer (pH 4.5).[14] MnP activity was assayed by the formation of Mn^{3+}-malonate complexes.[15] The reaction mixture contained 50 mM malonate buffer (pH 4.5), 1 mM $MnSO_4$, 0.1 mM H_2O_2, and 100 μL enzyme extract, and absorbance was measured at 270 nm (ε 11590 $M^{-1}cm^{-1}$). VP activity was monitored by the formation of Mn^{3+}-tartrate complexes at 238 nm (ε 500 $M^{-1}cm^{-1}$) during the oxidation of 0.1 mM Mn^{2+} ($MnSO_4$) in 0.1 M sodium tartrate buffer (pH 5), 0.1 mM H_2O_2, and 100 μL enzyme extract.[16] The VP and MnP activities were determined by subtracting the values in the absence of $H_2O_2$from the values in the presence of H_2O_2. One unit of enzyme activity was defined as the amount of enzyme that transformed 1 μmol $min^{-1}$of substrate; enzyme activities were reported as specific activities (U mg $protein^{-1}$).

Arochlor determination in culture media

After 20 days of incubation, the cultures with increased enzymatic activities in the presence of the PCBs were selected for Arochlor determination (Table 3), according to the following procedure: the whole culture was homogenized with 40 mL hexane-acetone 3 times and filtered through Whatman No. 2 filter paper to separate the biomass and broth. Arochlor was extracted from the liquid fraction using the technique described previously. Extractions were performed in duplicate. Arochlor was quantified in a gas chromatograph (Agilent model 6890N), equipped with a 5973 mass selective detector and an Agilent HP-5MS capillary column as above described.

Statistical analysis

The difference in values of enzyme activities in the culture media was determined using the General Linear Model Procedure (GLM) and Least Significant Difference (LSD) test to establish the best medium ($\alpha \leq 0.05$). Correlations between variables were calculated with linear regression analysis using the statistical software SAS (V.8; SAS Institute Inc., Cary, NY).

Results

Arochlor identification from transformer oil

The transformer oil registered 71% of Arochlor 1242 and its chemical composition was: 44% tri-, 38% tetra-, 13% di-, 4% penta-, and 1% mono-chlorinated biphenyls.

Table 3. PCBs removal by *Pleurotus ostreatus* at 20 d of culture in selected media.

Medium	*Enzyme*	*Increase (fold)*	*PCBs removal (%)*
1	VP	27.1	19.2 ± 2.2
2	MnP	1.2	0
5	Lac	9.75	50.7 ± 2.1
16	Lac	10.3, 9.3	0

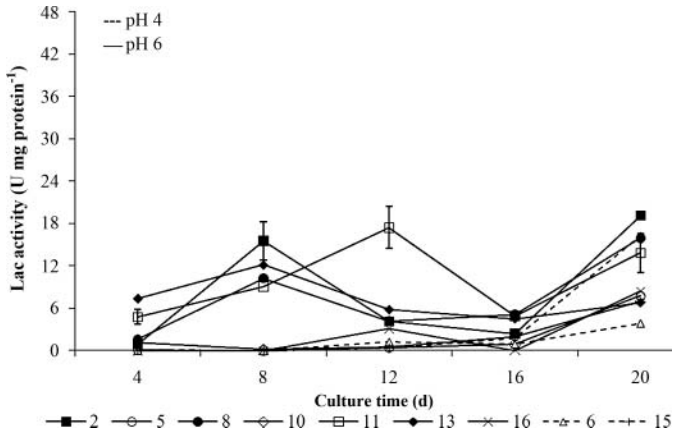

Fig. 1. Lac activity by *P. ostreatus*, in media culture with 7100 mg/L Arochlor 1242.

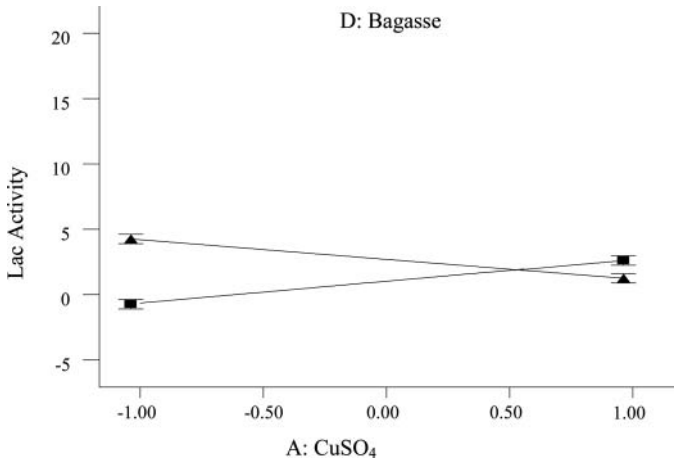

Fig. 2. Interaction between $CuSO_4$ and bagasse. ■ without bagasse; ▲ 2.5 g/L bagasse.

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Enzymatic activities

Lac activity

Lac activity with PCBs was affected by pH and agitation ($P < 0.001$), the activity increased at pH 6 and 100 rpm (Fig. 1). The highest activities with PCBs were registered in medium 2 at 8 and 20 days (15.5 and 19.1 U mg of protein^{-1}), and medium 11 at 12 days (17.4 U mg of protein^{-1}). At pH 6 7 of 8 media, showed activity, while at pH 4, only 2 media showed the enzyme activity. Equation 2 expresses the model for Lac activity obtained by regression analysis.

$$\begin{aligned}\mathrm{Lac_{PCBs}} = {} & +2.13 - 0.406\,\mathrm{CuSO_4} + 0.65\,\mathrm{MnSO_4} \\ & -0.27\ \mathrm{straw} + 0.911\ \mathrm{bagasse} + 1.85\,\mathrm{pH} \\ & -1.45\ \mathrm{Agitation} + 0.518\ \mathrm{Tween} - 0.42\,\mathrm{CuSO_4} \\ & \times \mathrm{MnSO_4} + 0.696\,\mathrm{CuSO_4} * \mathrm{straw} - 1.59\,\mathrm{CuSO_4} \\ & \times \mathrm{bagasse} - 0.58\,\mathrm{CuSO_4} * \mathrm{pH} + 0.95\,\mathrm{CuSO_4} * \mathrm{Agitation} \\ & - 0.43\,\mathrm{CuSO_4} * \mathrm{Tween} + 0.79\,\mathrm{MnSO_4} * \mathrm{bagasse} \end{aligned} \quad (2)$$

Laccase activity with PCBs, was enhanced at high value of pH, low speed of a gitation and interactions between low values of $CuSO_4$ with amended bagasse (Equation 2 and Fig. 2). All media that produced Lac reached their highest production in 20 days old culture, except media 11 and 13 in which maximum activity was observed in 12- and 8-day old-cultures (19.1 and 12.2 U mg of protein^{-1}, respectively). Media 1, 3, 4, 7, 9, 12 and 14 were not suitable for Lac production. In some media enzyme inhibition was observed, e.g., media 12 and 6 at 8 days, media 15 and 10 at 12 days.

The Lac activity without PCBs showed (Fig. 3) the highest activities in medium 8, at 4 days (7.1 U mg of protein^{-1}), 2 at 8 days (47.48 U mg of protein^{-1}), 15 at 16 days (8.9 U mg of protein^{-1}) and 6 at 20 days (18.6 U mg of protein^{-1}). Equation 3 shows the model obtained by regression analysis:

$$\begin{aligned}\mathrm{Lac} = {} & +1.325 + 0.057\,\mathrm{CuSO_4} + 0.188\,\mathrm{MnSO_4} \\ & + 0.34\ \mathrm{straw} - 0.025\ \mathrm{bagasse} + 1.22\,\mathrm{pH} \\ & - 0.98\ \mathrm{Agitation} - 0.36\ \mathrm{Tween} + 0.313\,\mathrm{CuSO_4} \\ & \times \mathrm{MnSO_4} + 0.18\,\mathrm{CuSO_4} * \mathrm{straw} \\ & - 1.076\,\mathrm{CuSO_4} * \mathrm{bagasse} - 0.376\,\mathrm{CuSO_4} * \mathrm{Tween} \\ & - 0.32\,\mathrm{MnSO_4} * \mathrm{bagasse} - 0.39\,\mathrm{CuSO_4} * \mathrm{MnSO_4} * \mathrm{bagasse} \end{aligned} \quad (3)$$

All variables were significant and also the interactions between them, as shown in Equation 3 and Figure 2, where the interaction between low value of $CuSO_4$ and bagasse presented an increase in Lac activity.

Lac activity was higher (ranging from 2.3- to 22-fold) in media without Arochlor than in media with Arochlor, medium 2 at 4 day and medium 15 at 12-day-old culture. In 20-day-old culture, however, the enzymatic activity was increased 9.3 (medium 16) and 9.7 (medium 5) folds with PCBs.

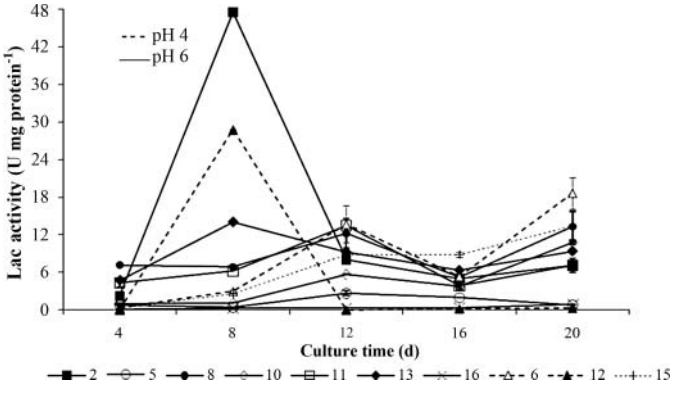

Fig. 3. Lac activity by *P. ostreatus*, in media culture without Arochlor 1242.

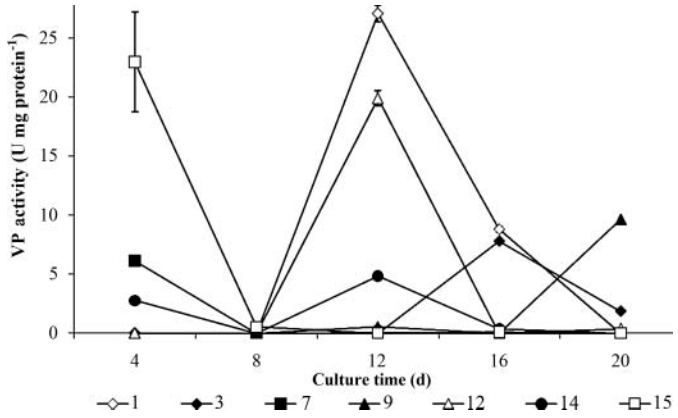

Fig. 4. VP activity by *P. ostreatus,* in media culture with 7100 mg/L Arochlor 1242.

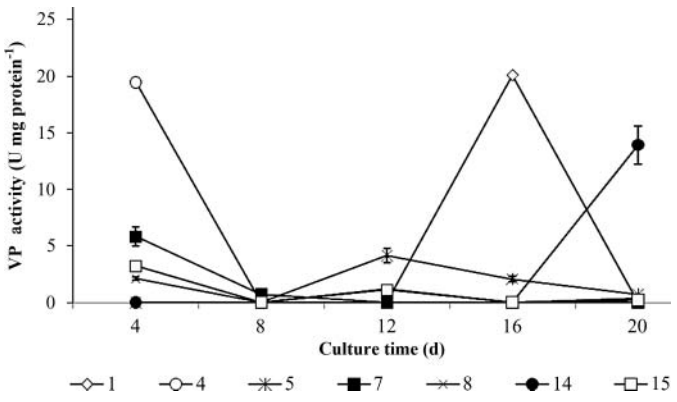

Fig. 5. VP activity by *P. ostreatus,* in media culture without Arochlor 1242.

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VP activity

The VP activity was favored by pH 4 and surfactant concentration 13 mg L^{-1} ($P < 0.001$). Figure 4 shows the VP activities in the different media at the five culture times. The highest production of VP was observed in the medium 1 in 12-day-old culture (27.1 U mg of protein^{-1}). In the media 2, 4, 5, 6, 8, 10, 11, 13 and 16 no activity was observed or was negligible. In medium 15 the maximum activity at 4 days of culture was 23 U mg of protein^{-1} and the media 1, 12 and 14 at 12 days with 27.1, 19.9 and 4.8 U mg of protein^{-1}, respectively. Medium 3 at 16 days (7.8 U mg of protein^{-1}) and finally the medium 9 at 20 days showed the maximum VP activity (9.6 U mg of protein^{-1}). The model obtained by regression analysis for VP with PCBs is given in Equation 4.

$$VP_{PCBs} = 1.46 - 1.18\,pH_5 - 0.86\,Tween \qquad (4)$$

In culture without Arochlor , the highest VP activities were observed in media 4 at 4 days (19.4 U mg of protein^{-1}) and 1 at 16 days (20.1 U mg of protein^{-1}), and the culture conditions did not affect VP activity. VP activity was higher in cultures with Arochlor at 12 days of incubation than in cultures without Arochlor (Figs. 4 and 5), an increase in the VP activity in the media with Arochlor ranged from 7.2- to 7.8-fold when compared to media without Arochlor. Also the presence of Arochlor caused an early enzyme production in the case of medium 1. Under conditions studied, the high concentration of surfactant (3500 mg L^{-1}) inhibited the activity of the peroxidase.

MnP activity

In the presence of 7100 mg L^{-1} of Arochlor 1242 the MnP activity was increased in media containing 250 mg L^{-1} $CuSO_4$, 3500 mg L^{-1} of Tween 80 and 2.5 g L^{-1} of sugarcane bagasse and the highest enzyme activities were registered in the media 16 at 8 days (1.3 U mg of protein^{-1}) and 2 at 12 days (1.2 U mg of protein^{-1}). The low MnP activity in diverse media indicated that peroxidase activity was not favored under the tested conditions; MnP activities in the absence of Arochlor were observed only in media 14 and 3 with 12.4 and 5.4 U mg of protein^{-1}, respectively, at 20 days (Fig. 6). The culture conditions had no effect on enzyme activity. MnP activity in medium without Arochlor was higher (23.2- and 97.3-fold) compared to the culture with Arochlor.

PCB removal by P. ostreatus on selected media

The PCBs removed by *P. ostreatus* after 20 days and the correlation with the increase in enzyme activity presented in Table 3. Although Lac activity was similarly increased in media 5 and 16 but only medium 5 showed maximum Arochlor removal.

Discussion

Many studies have attempted to increase the enzymatic activities by altering the nutritional and environmental conditions of fungal cultures; e.g., Cu for Lac induction,[17–19]

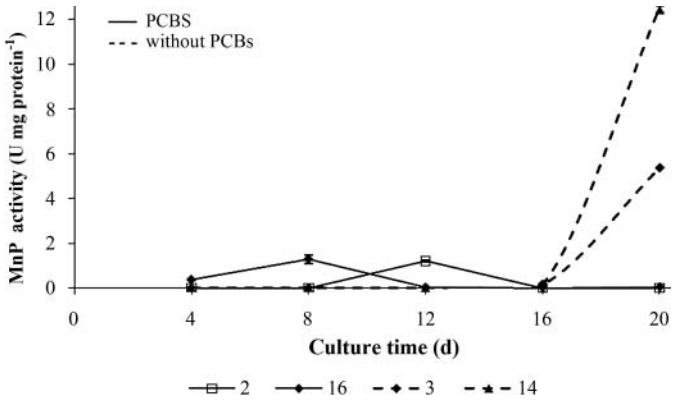

Fig. 6. MnP activity by *P. ostreatus*, in the different media culture.

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Mn for MnP induction;[20] pH in the culture medium to change the electrostatic properties of protein surfaces, which affects the active center and enzyme stability,[21–23] and the rate of agitation to impact oxygenation,[24] because O_2 acts as an electron acceptor in redox reactions. Also different carbon and nitrogen sources have been used as nutrients for fungal growth[25,26] and they can be supplied through lignocellulosic substrates.[2] Some of these ligniocellulosic materials are wheat straw and sugarcane bagasse, because they have a relatively high amount of carbohydrates[27] (between 40% and 70%). Lignocellulose can also serve as a physical support for fungal growth,[28] they might adsorb hydrophobic compounds and reduce direct contact with microorganisms that inhibit their growth. Surfactants can be added to culture media to enhance the availability of PCBs to microorganisms, since these compounds are hydrophobic.[3,29]

Lac activity

Lac activity from *P. ostreatus* depended on the presence of PCBs and the formulation of the culture medium, which was established in the $2_{III}{}^{7-3}$, fractional factorial experimental design. Enzyme activity was higher in cultures without Arochlor 1242 than with it. Lac activity increased in medium with $MnSO_4$ and bagasse, that might be due to the high reduction potential of Mn. Lac is affected not only by Mn but by transition metals such as Cu and Mn.[30,31] Maximum Lac activity was obtained in medium 2, mainly by interaction with bagasse, which contains high amounts of carbohydrates (about 70%), that enhance the ligninolytic enzymes production. In addition, Lac activity was increased in medium with $CuSO_4$ (250 mg L^{-1}), agitation (100 rpm), and pH (6). The highest laccase activity occurred with bagasse and low concentration of $CuSO_4$, it is known that Cu is a Lac inducer, which could explain this increase.[19]

In other studies, Jaszek et al.[32] reported that the addition of paraquat (1,1'-dimethyl-4,4' bipyridinium dichloride hydrate, methyl viologen dichloride) to liquid cultures of *Trametes versicolor* and *Abortiporus biennis* stimulated Lac activity and also Pozdnyakova et al.[33] observed the induction of Lac activity of *P. ostreatus* by the polycyclic aromatic hydrocarbons (i.e., naphthalene, anthracene, phenanthrene, pyrene and benzo[a]pyrene).

Enzymatic activities changed during the culture, with maximum values at 8, 12 and 20 days, both with and without PCBs. This behavior of Lac activity can be explained by protease production, which may inactivate the Lac enzyme; it was demonstrated that proteases presents in the broth culture degraded laccase isoenzymes from *P. ostreatus*.[19] The presence of Arochlor decreased the enzyme activity, but after 20 days of culture the activity was similar to that in medium without PCBs.

Also the pH is a very important parameter for Lac activity during fungal cultivation; for example, maximum Lac yield is obtained in liquid *P. ostreatus* culture at pH 5.5.[34] According to Rubilar et al.[35] the optimum pH in terms of Lac activity is pH 6, with a range from 4 to 7.

VP activity

Other studies demonstrated that for VP the optimum pH ranges from 3 to 5.[36] Moreover, the activity of this peroxidase also depends on the culture medium and the fungus strain, as demonstrated by Ruiz-Dueñas et al.[37] who reported that VP activity of *P. eryngii* in glucose–peptone medium was inhibited by the addition of 25 μM Mn^{2+}. In our case, the highest activity (medium 1, 27 U mg protein^{-1}) for *P. ostreatus* was at 50 mg L^{-1} $MnSO_4$ (331 μM) in the presence of PCBs. These results suggest that the effect of Mn on VP activity differs between *Pleurotus* spp., and that *P. ostreatus* tolerates higher Mn concentrations than *P. eryngii*, even in presence of 7100 mg L^{-1} of Arochlor.

Our results indicate that in some media the presence of Arochlor 1242 increased VP activity (media 1 and 12). Rodríguez et al.[9] also reported that 100 μM 2–4 dichlorophenol and benzo(a)pyrene stimulated VP and Lac activities in liquid cultures of *Pleurotus* spp. In this study the activity was increased in media with 7100 mg L^{-1} of Arochlor 1242, which is the highest concentration of pollutant reported until now. And in media with 3500 mg L^{-1} Tween 80 (i.e., media 4 and 11), the surfactant repressed peroxidase activity if the medium contained PCBs.

MnP activity

The low MnP activity in the diverse media indicates that peroxidase activity was not favored under the tested conditions. Although, *P. ostreatus* is not good producer of MnP, the presence of Cu^{2+} enhanced MnP activity in media with PCBs; some metals[38] stabilize enzyme activities but this is the first report that Cu influences on MnP activity. Also, 3500 mg l^{-1} Tween 80 enhanced MnP activity, probably because the surfactant acts as a co-oxidant for the MnP system through production of peroxyl radicals.[39] These peroxyl radicals allow the oxidation of non-phenolic compounds, thus Hirai et al.[40] observed oxidative dechlorination of methoxychlor in the presence of MnP–Tween 80. In our case, however, there was no correlation between MnP activity and PCB degradation in the presence of Tween 80.

Also the MnP activity was enhanced by the sugarcane bagasse, as reported by Sen et al.[41] who observed that sugarcane bagasse improved MnP activity of *P. ostreatus* in liquid and semi-solid cultures. This lignocellulosic substrate has high carbohydrate content,[27] and may be used as a secondary carbon source for fungal growth. Moreover several lignocellulosic materials, such as sugarcane bagasse, contain p-coumaric, ferulic, and vanillic compounds,[42] and other components that were released into the media

during sterilization increased the content of soluble carbohydrates, aromatic compounds, and microelements, which may induce the biosynthesis of ligninolytic enzymes.[43]

PCB removal in the selected culture media

The data shown in Table 3 suggests that VP and Lac enzyme activities are involved in the biodegradation of PCBs by *P. ostreatus*. The presence of PCBs in media 1 and 5 induced VP and Lac activities, respectively; induction of Lac activity by xenobiotics was previously reported.[32,33] Furthermore, Keum and Li[22] reported that hydroxyl PCB was degraded by purified Lac from *T. versicolor* and *P. ostreatus.* Although the mechanism of PCB degradation was unclear, *P. ostreatus* is a good biodegrader of this pollutant. Moeder et al.[44] studied a culture of *P. ostreatus* grown on straw in solid state fermentation with concentrations of PCBs up to 2000 mg kg^{-1}, but the enzyme activity was not evaluated. Rodriguez et al.[9] reported benzo(a)pyrene and 2,4-dichorophenol degradation in liquid culture with Lac and VP from *Pleurotus* spp.

Conclusions

This experimental design allowed us to identify culture media parameters that enhanced enzyme activity for Arochlor degradation. Lac activity of *P. ostreatus* was increased by Arochlor 1242, pH, agitation, and time; in medium 5 where Lac increased 10-folds; the concentration of Arochlor 1242 was reduced to 51%.

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