



Biodegradation of anthracene and different PAHs by a yellow laccase from *Leucoagaricus gongylophorus*

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Received: 30 June 2018 / Accepted: 8 January 2019 / Published online: 1 February 2019
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Abstract

Laccases produced by *Leucoagaricus gongylophorus* act in lignocellulose degradation and detoxification processes. Therefore, the use of *L. gongylophorus* laccase (Lac1Lg) was proposed in this work for degradation of anthracene and others polycyclic aromatic hydrocarbons without the use of mediators. Degradation reactions were performed in buffer aqueous solution with 10 ppm of anthracene and other PAHs, Tween-20 in 0.25% v/v and a laccase preparation of 50 U. The optimum condition (pH 6.0 and 30 °C) was determined by response surface methodology with an excellent coefficient of determination (R^2) of 0.97 and an adjusted coefficient of determination (R^2_{adj}) of 0.93. In addition, the employment of the mediator ABTS decreased the anthracene biodegradation from $44 \pm 1\%$ to $30 \pm 1\%$. This optimum pH of 6.0 suggests that the reaction occurs by a hydrogen atom transfer mechanism. Additionally, in 24 h Lac1Lg biodegraded $72 \pm 1\%$ anthracene, $40 \pm 3\%$ fluorene and $25 \pm 3\%$ phenanthrene. The yellow laccase from *L. gongylophorus* biodegraded anthracene and produced anthrone and anthraquinone, which are interesting compounds for industrial applications. Moreover, this enzyme also biodegraded the PAHs phenanthrene and fluorene justifying the study of Lac1Lg for bioremediation of these compounds in the environment.

Keywords Polycyclic aromatic hydrocarbons · Organic pollutant · Fluorene · Phenanthrene · Anthraquinone · Anthrone

Introduction

The fungus *Leucoagaricus gongylophorus* lives in a mutualistic relationship with cutting ants of the *Atta* and *Acromyrmex* genera. Several studies involving this microorganism have

been conducted to understand its role and the importance of its enzymes to ants (Aylward et al. 2015). Some of these studies suggest that the produced enzymes, including laccases, act in the lignocellulose degradation and in detoxification processes, oxidizing compounds originated from the metabolism of plants (Aylward et al. 2013; Licht et al. 2014).

Laccases (1.10.3.2 ECF, *p*-diphenol: oxygen oxidoreductase) are a class of enzymes that oxidizes aromatic and non-aromatic compounds (Filazzola et al. 1999). Unlike other ligninolytic enzymes such as manganese peroxidase (MnP) and lignin peroxidase (LiP), which require hydrogen peroxide to exert their activity, laccases catalyze the substrates oxidation with oxygen as electron acceptor (de la Rubia et al. 2002). Because of this property, laccases are enzymes with high potential for biotechnological applications, in which they can be employed as biocatalysts in organic synthesis and in biodegradation processes (Kudanga et al. 2017; Riva 2006).

Laccases contain four copper atoms and a maximum absorption at 600 nm responsible for the intense blue color of these enzymes (Madhavi and Lele 2009). Blue laccases from different microorganisms have been employed for biodegradation of *polycyclic aromatic hydrocarbons* (PAHs), such as CotA from

Responsible editor: Gerald Thouand

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11356-019-04197-z>) contains supplementary material, which is available to authorized users.

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Bacillus subtilis, CueO from *Escherichia coli* (Zeng et al. 2016), and laccase from *Trametes villosa* (Prasetyo et al. 2016).

However, there are laccases that do not absorb at 600 nm and are called yellow or yellow/white laccases (Leontievsky et al. 1997). Blue laccases usually require the use of chemical mediators to catalyze reactions in non-phenolic compounds, differently from yellow laccases that shows modifications in the type I copper and perform reactions directly in this type of substrates (Bourbonnais and Paice 1992; Huang et al. 2011).

Yellow laccases have been studied without mediators for different applications, including the decolorization of the dyes Reactive Black 5, Remazol Brilliant Blue R, Reactive Red 195, Reactive Yellow 145 (Marim et al. 2016), the transformation of toluene and substituted derivatives to the corresponding benzaldehydes (Sharma et al. 2016) and the oxidation of ethyl ferulate and ferulic acid (Aljawish et al. 2014).

PAHs are a class of organic compounds composed by two or more fused aromatic rings, which can be formed during the thermal decomposition of organic molecules and their subsequent recombination (Bamforth and Singleton 2005). The burn of fossil fuels like coal and oil, and volcanic eruptions are examples of natural sources of PAHs. However, most of these compounds are produced by human activity, as the burning of diesel, wood, plastic, plant material, trash, and lubricating oil (Haritash and Kaushik 2009). Given the highly recalcitrant and toxic nature of these substances, PAHs are considered worrying and widespread pollutants (Ghosal et al. 2016; Brewster et al. 2018).

The activity of a laccase is intrinsically related to the pH and temperature of the reaction medium (Xu 1997). Response surface methodology (RSM) is an effective set of statistical and mathematical tools for optimizing processes in a faster and more economical approach when compared to the classical one-variable-at-a-time method (Myers et al. 2009). In this context, RSM is a useful method to evaluate how the variables pH and temperature affect the efficiency of the laccase from *L. gongylophorus* on PAHs biodegradation and to determine how the combination of these variables allow the highest degradation result.

The use of isolated laccases and microorganisms for PAHs degradation has been reported in several studies. However, the degradation depended on mediator molecules like 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) or 1-hydroxybenzotriazole (HBT) in most of the literature, reducing the reaction efficiency and increasing the process cost (Chang et al. 2016).

A monomeric yellow laccase from the fungus *L. gongylophorus* (Lac1Lg) was identified and characterized in a previous work and was able to oxidize wide non-phenolic substrate (anthracene) in the absence of exogenous mediators, despite having a very closed catalytic site. Therefore, showing potential for future biotechnological processes (Ike et al. 2015).

In this work, we proposed the use of Lac1Lg for anthracene biodegradation using RSM for the determination of the optimized pH and temperature of the reaction. Exploring, in addition, the biodegradation of different PAHs such as fluorene, fluoranthene, phenanthrene, and pyrene to expand the application scope of this recently discovered enzyme.

Materials and methods

Fungus culture

L. gongylophorus strain FF-2006 was isolated from a *Atta sexdens rubropilosa* nest and was maintained in a solid medium of 2% malt extract, 0.5% bacteriological peptone, 0.2% yeast extract and 2% agar (Bicalho 2011).

Laccase preparation

The laccase preparation was performed as carefully described by Ike et al. (2015). The mycelium was cultivated in modified Tien and Kirk medium without dimethyl succinate at 25 °C for 11 days, and then lyophilized (Tien and Kirk 1988). Subsequently, the enzymatic extract was dialyzed and applied in ionic exchange chromatography with a hydroxyapatite column (CHTTM ceramic hydroxyapatite type I, 1.5 cm × 12 cm, Bio-Rad). The elution was performed with 400 mM sodium phosphate pH 5.8 with 0.0075 mM CaCl₂ (solvent B) in a linear gradient mode (0–100%B). The fractions with laccase activity were dialyzed with a 50 mM sodium phosphate solution pH 7.0 and concentrated ten times with a Amicon Ultra-15 (Millipore) ultrafiltration system.

Determination of laccase activity

The laccase activity was measured by the oxidation of syringaldazine as described by Ike et al. (2015). The assay contained 10 μL of enzymatic solution and 10 μM of syringaldazine in a total volume of 0.5 mL of 20 mM aqueous sodium acetate buffer (pH 6.0). The determination of the oxidation reaction was carried out at 525 nm with the extinction coefficient of 65,000 M⁻¹ cm⁻¹. One unit of laccase activity was defined as the amount of enzyme necessary to oxidize 1 μmol of substrate per minute (Jordaan 2005; Wolfenden and Willson 1982).

PAHs degradation

The degradation of PAHs by the partially purified laccase was initially evaluated using anthracene. Anthracene-stock solutions were prepared at a concentration of 1000 ppm using DMSO as solvent. Degradation reactions were performed in aqueous buffer (in the evaluated pH) containing anthracene at

a final concentration of 10 ppm, laccase preparation (50 U) and 0.25% v/v of the detergent Tween-20 (Sigma). The final volume of the reactions was 5 mL. For the pHs 5 and 6, 20 mM of sodium citrate aqueous buffer solution was employed, whereas 20 mM sodium phosphate buffer was used for reactions at pH 7. The reactions were kept under controlled temperature and magnetic stirring (100 rpm). The pH and temperature of the reaction medium were defined as described in the [Experimental Design](#) section. After 24 h, the residual PAH was recovered by three liquid-liquid extractions with an equal volume of ethyl acetate (5 mL). The organic phase was dried with anhydrous sodium sulfate, filtered, and then evaporated under reduced pressure at 30 °C. The product was then dissolved in 1 mL of acetonitrile. The temperature and pH that allowed the highest anthracene degradation was also applied to evaluate the degradation of fluorene, fluoranthene, phenanthrene and pyrene in the same reactional conditions.

Experimental design

A 2² central composite rotatable design was used to analyze the main effects and interactions of the variables pH and temperature on the residual concentration of anthracene. Based on preliminary experiments, the independent variables and their levels were selected as shown in [Table 1](#). A total of 11 experiments were carried out, consisting of 4 factorial experiments, 4 axial experiments, and 3 replicates of the center point. All the experiments were carried out at random to minimize systematic errors.

Quantification of PAHs biodegradation

PAHs were quantitatively analyzed by a validated method employing a Shimadzu high pressure liquid chromatographic system composed of an LC-20AT pump, a DGU-20A5 degasser, a SIL-20AHT sampler, a SPD-M20A UV-VIS detector, a CTO-20A column oven, and a CBM-20A controller. The separation was performed using a reverse phase C18 column (Shim-pack CLC-ODS column, 25 cm × 4.6 mm × 5 μm). For elution, it was employed a linear concentration gradient of 40–95% water/acetonitrile from 0 to 30 min using a flow rate of 1.0 mL min⁻¹. Standard curves were produced with standard solutions of 10, 20, 30, 40, 50, and 60 mg L⁻¹ for each analyte, i.e., anthracene (357 nm), fluorene (299 nm), fluoranthene (286 nm), phenanthrene (250 nm) and pyrene

(334 nm). Chromatograms and analytical curves are presented in [Electronic Supplementary Material-1, Fig. S1–10](#).

Method recovery

Experiments for determination of the method recovery were performed in the same biodegradation reactional conditions (pH 6.0, 30 °C) without the enzyme. Therefore, a method recovery was estimated for anthracene, phenanthrene, fluoranthene, fluorene and pyrene.

Metabolite identification

The metabolites were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) in a Shimadzu GC2010 plus with a mass selective detector (Shimadzu MS2010 plus) in electron ionization mode (EI, 70 eV). The GC-MS (equipped with a 30 m × 0.25 mm × 0.25 μm J&W Scientific DB5 column) conditions were oven temperature starting at 90 °C for 4 min, increased to 280 °C at 6 °C min⁻¹, and held for 6 min; injector and interface temperature was maintained at 250 °C; splitless 1 μL injection; helium as carrier gas at a constant flow of 0.75 mL min⁻¹; and 40 min of analysis time. The employed scan mode was 40–500 m/z.

Statistical analysis

Multiple regression analysis was used to fit a second-order polynomial equation (Eq.1) to the experimental data collected from the 2² central composite rotatable design by the least-squares method (Calado and Montgomery 2003).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_1 \beta_2 X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \tag{1}$$

where *Y* represents the predicted response, β₀ is the model intercept, β₁ and β₂ are the linear coefficients, β₁β₂ are the interaction coefficient, β₁₁ and β₂₂ are the quadratic coefficients, and ε corresponds to the model residue (Calado and Montgomery 2003).

The statistical significance of the independent variables on the response was determined by the evaluation of the *p* value and Fisher’s test with 95% significance level obtained from the analysis of variance (ANOVA). The fit of the model was

Table 1 Uncoded and coded levels of the independent variables evaluated in the study of anthracene degradation

Independent variables	Symbol	Levels				
		− α (− 1.41)	− 1	0	+ 1	+ α (+ 1.41)
pH	X ₁	4,6	5	6	7	7,4
Temperature (°C)	X ₂	16	20	30	40	44

expressed by the coefficient of determination (R^2) and the adjusted coefficient of determination (R^2_{adj}). The three-dimensional surface plot was produced using the fitted model to illustrate the relationship between the independent variables and the response, aiming the determination of the optimal conditions of pH and temperature which result in the highest anthracene degradation. All calculations and graphics were obtained by the Statistica software (Statsoft version 7.0, USA) (Calado and Montgomery 2003; Montgomery 1991).

Results

Method recovery

A method validation was carried out without enzyme and a residual anthracene of $50.1 \pm 1.5 \text{ mg L}^{-1}$ was determined, showing that the employed methodology presented a method recovery of 100.2% with a standard deviation of 3% for anthracene.

Validation experiments were also performed for the others PAHs, including phenanthrene ($47.4 \pm 1.0 \text{ mg L}^{-1}$, 94.8% recovery and 2.0% precision); fluorene ($42.2 \pm 1.4 \text{ mg L}^{-1}$, 84.4% recovery and 2.8% precision); fluoranthene ($40.1 \pm 1.2 \text{ mg L}^{-1}$, 80.2% recovery and 2.4% precision); and pyrene ($40.3 \pm 1.5 \text{ mg L}^{-1}$, 80.6% recovery and 3.0% precision). Therefore, the determination method employed in this study was adequate.

Optimum pH and temperature

A factorial design was employed to determine the best conditions of pH and temperature for carrying out the anthracene degradation experiments. Table 2 shows the coded (in parenthesis), the uncoded independent variables (pH and

temperature) and the experimental values of the response variable, residual anthracene concentration. Multiple regression analysis was used to fit Eq. 1 to the experimental data aiming to describe the relation between the dependent variables and the independent variables. The statistical significance of the model fit was determined by analysis of variance (ANOVA) at a confidence level of 95% (p value < 0.05), and the results were presented in Table 3. The significance of each term was evaluated by the F -test and p value, in which the corresponding terms are more significant if the absolute F value becomes bigger and the p value becomes smaller.

The quadratic term of temperature (X_2^2) had the greatest influence on anthracene degradation, and the quadratic term of pH (X_1^2) was the second most important factor, as observed in Table 3. In addition, the linear term temperature (X_2) and the interaction between pH and temperature (X_1X_2) were also significant (p value < 0.05). The regression equation obtained for the residual anthracene concentration without non-significant terms was given in Eq. 2:

$$\text{Residual anthracene}(\%) = 315.33 + 0.05X_1^2 - 88.59X_2 + 8.15X_2^2 - 0.22X_1X_2 \quad (2)$$

The model adequacy was evaluated by the coefficient of determination (R^2) and the adjusted coefficient of determination (R^2_{adj}) whose values were 0.9672 and 0.9344, respectively. Showing that the fitted model could explain above 93% of the total variability within the range of this study and that the experimental values could be significantly predicted by the model. A three-dimensional surface was generated based on Eq. 2 and was shown in Fig. 1. The response surface plot shows that the lowest residual concentration of anthracene was observed at pH 6 and 30 °C of temperature.

Table 2 Independent variables of the 2^2 central composite rotatable design and the experimental results for the residual anthracene after biodegradation

Run	Independent variables		Response variable
	Temperature (°C)	pH	Residual anthracene (%)
1	20 (−1)	5 (−1)	38.9
2	20 (−1)	7 (+1)	45.7
3	40 (+1)	5 (−1)	40.4
4	40 (+1)	7 (+1)	38.1
5	16 (−1.4)	6 (0)	40.3
6	44 (+1.4)	6 (0)	37.0
7	30 (0)	4.6 (−1.4)	39.7
8	30 (0)	7.4 (+1.4)	50.0
9	30 (0)	6 (0)	28.1
10	30 (0)	6 (0)	28.7
11	30 (0)	6 (0)	27.9
Exp. with ABTS mediator ^a	30 (0)	6 (0)	35.1

^a Experiment carried out in the optimum reactional conditions with 1.0 mM of ABTS

Table 3 Analysis of variance (ANOVA) results for the residual anthracene after biodegradation

Source of variation ^a	Sum of square	df	Mean square	F value	p value
X ₁	14.4908	1	14.4908	4.7758	0.080568 ^d
X ₁ ²	139.1836	1	139.1836	45.8710	0.001067 ^c
X ₂	45.4409	1	45.4409	14.9761	0.011762 ^b
X ₂ ²	367.2706	1	367.2706	121.0421	0.000108 ^c
X ₁ X ₂	20.7025	1	20.7025	6.8230	0.047550 ^b
Error	15.1712	5	3.0342		
Total SS	504.63	10			
R ²			0.9672		
R ² _{adj.}			0.9344		

^a X₁, temperature (°C); X₂, pH

^b Significant at 5% probability (p < 0.05)

^c Significant at 1% probability (p < 0.01)

^d Non-significant

It is important to note that a method validation was carried out and a residual anthracene of 50.1 ± 1.5 mg was determined, showing that the employed methodology presented a method recovery of 100.2% with a standard deviation of 3.0%. Therefore, the used method for anthracene determination in this study was adequate.

An additional experiment with the addition of the mediator ABTS (1.0 mM) was carried out at 30 °C and pH 6.0, in which a residual anthracene of 35.1 ± 0.5 mg mL⁻¹ (29.8 ± 1.0% biodegradation) was determined. Showing that the process efficiency is reduced by the addition of this mediator, since a residual anthracene of 28.2 ± 0.4 mg mL⁻¹ (43.6 ± 0.8% biodegradation) was obtained without ABTS in the same reactional conditions.

The biodegradation of anthracene resulted in the formation of anthrone and mainly anthraquinone (Fig. 2). Chromatograms

and spectra are available in Electronic Supplementary Material 2 and 3, Fig. S11–15. Showing that the laccase from *L. gongylophorus* was able to oxidize anthracene and produce valuable products for future applications.

Biodegradation of others PAHs

Biodegradation reactions with fluorene, phenanthrene, fluoranthene, and pyrene were performed in the optimized conditions of anthracene biodegradation, pH 6.0 and 30 °C. It is important to emphasize that these conditions not necessarily correspond to the optimal conditions for degradation of others PAHs.

After 24 h of reaction, anthracene and fluorene were the compounds more susceptible to oxidation by this laccase, showing 71.8 ± 0.6% and 41.7 ± 2.6% of degradation, respectively. Moreover, phenanthrene was also degraded (25.3 ± 2.6%). Unfortunately, metabolites were not observed probably because of their aqueous solubility and low concentration. Pyrene and fluoranthene biodegradation was not significant, since the residual concentrations of these PAHs in the biodegradation experiments were similar to those determined for the method recovery (Table 4, Fig. 3).

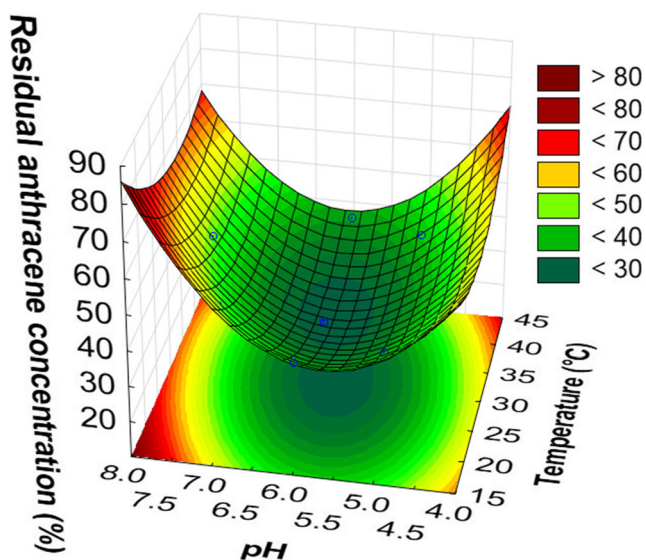


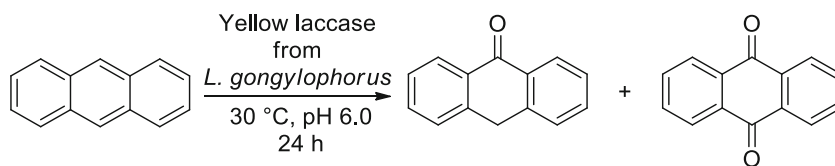
Fig. 1 Response surface plot showing the effect of reaction pH and temperature on the residual anthracene concentration. The blue circles correspond to the experimental results

Discussion

The major product formed by the oxidation of anthracene was anthraquinone in a reaction whose mechanism is not fully known (Ike et al. 2015). However, the oxidation of PAHs by laccases may occur by the removal of an electron from the substrate, generating a highly reactive free radical (Jeon et al. 2012).

Two mechanisms have been suggested for the electron withdraw of the reaction. In the electron transfer mechanism,

Fig. 2 Reaction of anthracene catalyzed by the yellow laccase from *L. gongylophorus*



the electron is withdraw from a π bond, whereas in the hydrogen atom transfer (HAT), the electron withdraw would occur by the elimination of a hydrogen atom (Canas and Camarero 2010).

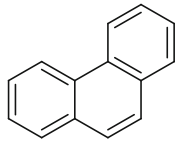
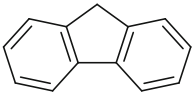
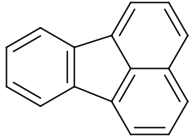
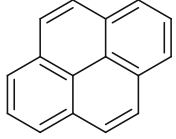
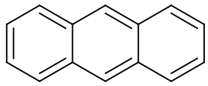
The optimum pH for laccases reported in the literature is pH 4.0, for nonphenolic substrates like ABTS and, pH 6.0 for phenolic substrates such as syringaldazine. Lac1Lg presented in a previous study an optimum pH of 6.0 and 3.0 for syringaldazine and ABTS, respectively (Ike et al. 2015).

The increased efficiency in pH 6.0 determined in our study for anthracene was the same determined for syringaldazine

and can be explained by a HAT mechanism, since an alkaline reaction medium would promote the proton abstraction (Canas and Camarero 2010; Farnet et al. 2009; Han et al. 2004; Munusamy et al. 2008).

The optimum temperature for anthracene biodegradation was 30 °C, which was the same determined for syringaldazine consumption, whereas the optimized temperature for ABTS was 50 °C (Ike et al. 2015), showing that Lac1Lg can catalyze PAHs reaction in mild temperature, property that can be important in future applications.

Table 4 Degradation by yellow laccase from *L. gongylophorus*

Compound	P.I. (eV)	Residual concentration (mg mL ⁻¹)	Method Recovery (mg mL ⁻¹)	Degradation (%)
 Phenanthrene	8.19	35.4±1.3	47.4±1.0	25.3±2.6
 Fluorene	9.91	24.6±1.3	42.2±1.4	41.7±2.6
 Fluoranthene	7.90	39.6±2.5	40.1±1.2	NO
 Pyrene	7.50	39.9±1.3	40.3±1.5	NO
 Anthracene	7.43	14.1±0.3	50.1±1.5	71.8±0.6

NO, biodegradation was not observed

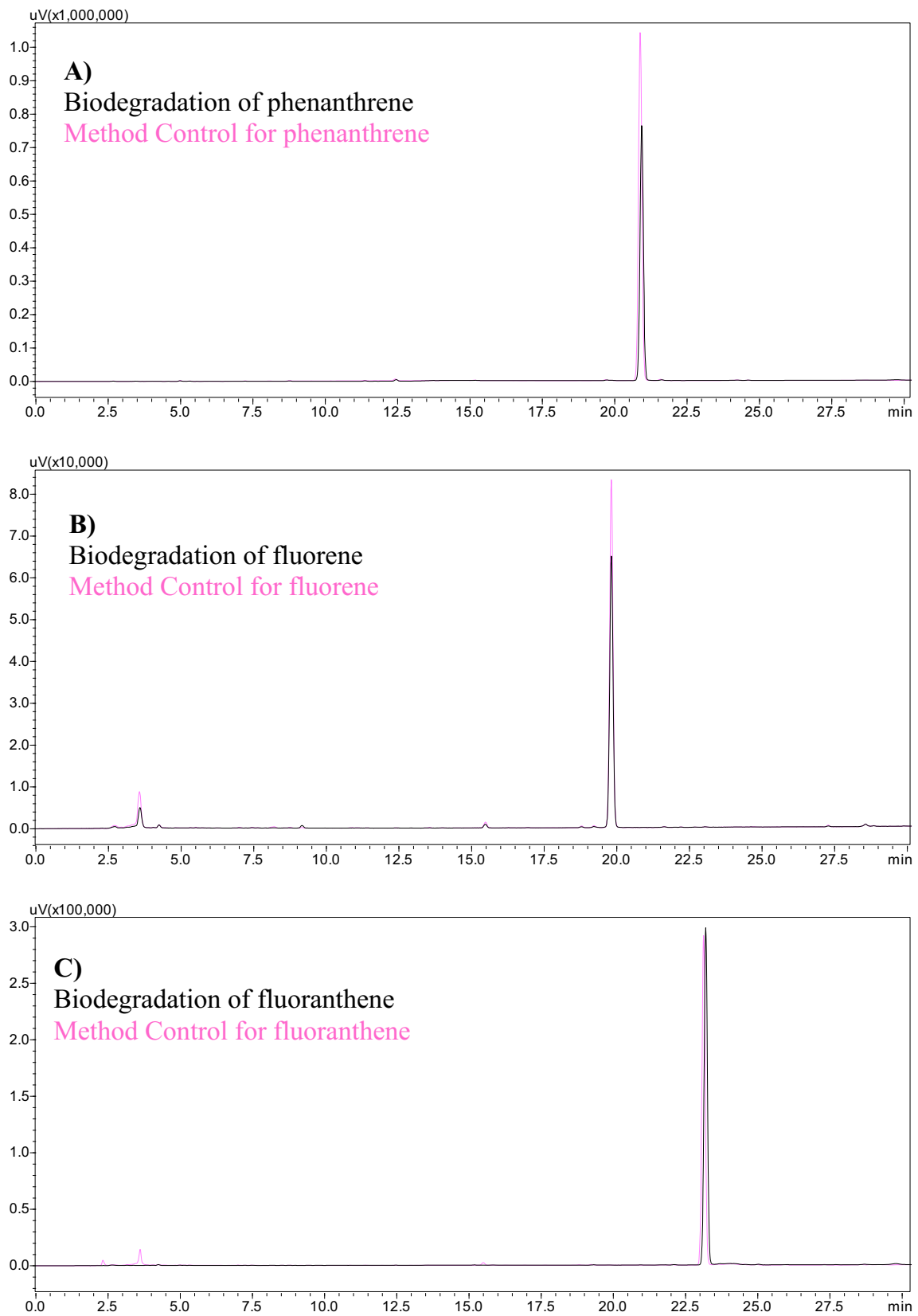


Fig. 3 Biodegradation (30 °C, pH 6.0, 24 h) and control HPLC-UV chromatograms of **a** phenanthrene, **b** fluorene, and **c** fluoranthene

The addition of the mediator ABTS in the reaction decreased the anthracene biodegradation ($29.8 \pm 1.0\%$ biodegradation and $43.6 \pm 0.8\%$ biodegradation with and without mediator, respectively), probably because the use of this compound enabled secondary reactions which reduced the process efficiency.

The biotransformation of anthracene to anthraquinone was reported in the literature by different laccase-producer fungi, such as *Pycnoporus sanguineus* H1 (Li et al. 2014b), *Armillaria* sp. F022 (Hadibarata et al. 2013) and *Polyporus* sp. S133 (Hadibarata et al. 2012), showing that this laccase-catalyzed reaction is important in PAHs biodegradation.

The biotransformation of anthracene by laccases can be an important approach to produce anthraquinone. Therefore, studies were described with mediators, i.e., by laccase from *Trametes versicolor* (Li et al. 2014a) or without mediators such as by the yellow laccase from *Pleurotus ostreatus* D1 (Pozdnyakova et al. 2006) and from *L. gongylophorus*, as reported in our study, aiming an efficient anthracene biotransformation.

Some studies relate the oxidation capacity of enzymes to the ionization potential (IP) of the approached PAH. In one of them, a lignin peroxidase of the fungus *Phanaerochaete crysosporium* was capable of oxidizing benzo[a]anthracene, pyrene, anthracene, benzo[a]pyrene, and perylene, whose IP values are 7.54, 7.50, 7.43, 7.06, and 7.23 eV. Whereas phenanthrene, benzo[c]phenanthrene, chrysene, and benzo[e]anthracene, whose PI values are 8.19, 7.93, 7.8, 7.62 eV were not oxidized (Hammel et al. 1986).

Similarly, the laccase-producer fungus *Marasmius quercophilus* was able to oxidize anthracene and benzo[a]pyrene, which PIs are smaller than 7.5, and was not able to oxidize naphthalene (IP = 8.10 eV) and phenanthrene (IP = 8.19 eV) (Farnet et al. 2009).

Based on these studies, it would be expected that the degradation of PAHs depended on their IP, so that the employed enzyme could oxidize compounds up to a certain IP maximum. However, this relationship was not observed for the Laccase from *L. gongylophorus*, which was not able to oxidize pyrene or fluoranthene that present low value of PI among the tested compounds, but oxidized phenanthrene which presents the highest value of PI. The observed behavior may be explained by a steric impediment, since pyrene and fluoranthene present a higher molecular weight than anthracene, phenanthrene and fluorene. However, further studies should be provided to justify these observations.

Anthracene, fluorene and phenanthrene are included on a list of the The National Waste Minimization Program of the U. S. Environmental Protection Agency, which focuses on reducing the production and use of 31 Priority Chemicals that are considered persistent, bioaccumulative, and toxic (EPA 2016).

Our results showed that the laccase from *L. gongylophorus* has potential for biocatalytic processes in organic synthesis or

bioremediation, since it was able to oxidize these three compounds in mild conditions of pH and temperature, without the addition of mediators.

Conclusion

The yellow laccase from *L. gongylophorus* biodegraded anthracene without mediators and produced anthrone and anthraquinone, which are interesting compounds for industrial applications. The optimum conditions were pH 6.0 and 30 °C determined by the response surface method, which presented an excellent coefficient of determination (R^2) of 0.97 and an adjusted coefficient of determination (R^2_{adj}) of 0.93. Moreover, the employment of the mediator ABTS decreased the process efficiency. This enzyme also biodegraded the PAHs phenanthrene and fluorene justifying the study of this laccase from *L. gongylophorus* for future bioremediation of these compounds, since they are listed as priority pollutants.

Funding information PTLI and WGB (grant no. 141656/2014-0) thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their scholarships, respectively. The authors also thank CNPq (grant no. 558062/2009-1) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant no. 2012/19934-0) for financial support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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