

The influence of Tween 80 on laccase production by *Pleurotus sajor-caju* and the efficiency of crude enzyme broth in the removal of bisphenol-A

*Influência do Tween 80 na produção de lacases de *Pleurotus sajor-caju* e eficiência do caldo enzimático bruto na remoção de bisfenol-A*

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ABSTRACT: Bisphenol-A is currently considered an environmental pollutant, capable of interfering in the endocrine system of organisms and causing alterations in its development and reproductive system. An alternative method to the chemical treatment of this pollutant has been the use of oxidative enzymes, especially laccases produced by fungi. In order to reduce production costs, agro-industrial waste can be used in the culture medium composition. Nonionic surfactants, which are only slightly toxic to biological membranes, can be applied, as well as Tween 80, to facilitate the excretion of these enzymes into the culture medium. The objectives of this work were: a) characterize the immersion water of banana straw used in the formulation of the culture medium; b) evaluate laccase production by *Pleurotus sajor-caju* in culture medium with and without addition of Tween 80, through shaken flasks; c) evaluate the efficiency of the crude enzyme broth in degrading bisphenol-A. The shaken flasks were incubated at 30°C for 12 days. The immersion water had a C:N ratio of 13.8, ash percentage of 28.6%, and pH close to neutrality. The addition of Tween 80 on the culture medium (7.5%, m/v) yielded laccase activity and productivity values equal to 3,016.47 U L⁻¹ and 502.7 U L⁻¹ day⁻¹, respectively. These values were 50 and 33.5 times higher than those obtained in the culture medium without addition of Tween 80 for laccase activity and productivity, respectively. The crude enzyme broth degraded 100% of bisphenol-A after 48 hours, regardless of concentration (500, 750 and 1,000 mg L⁻¹).

KEYWORDS: nonionic surfactant; enzymatic activity; endocrine disruptor.

RESUMO: O bisfenol-A é considerado um poluente ambiental capaz de interferir no sistema endócrino dos organismos, ocasionando alterações em seu desenvolvimento e sistema de reprodução. Um método alternativo ao tratamento químico desse tipo de poluente tem sido a utilização de enzimas oxidativas, especialmente as lacases, produzidas por fungos. A fim de diminuir custos de produção, resíduos agroindustriais podem compor o meio de cultivo. Assim, surfactantes não iônicos e pouco tóxicos para as membranas biológicas, como o Tween 80, podem ser utilizados para facilitar a excreção dessas enzimas para o meio de cultivo. Os objetivos deste trabalho foram: caracterizar quimicamente o resíduo água de imersão de palha de bananeira, usado na formulação do meio de cultivo; avaliar a produção de lacase por *Pleurotus sajor-caju* em meio de cultivo líquido (frascos Erlenmeyer) com e sem adição de Tween 80; e avaliar a eficiência do caldo enzimático bruto em degradar bisfenol-A. Os frascos foram incubados a 30°C, por 12 dias. A água de imersão apresentou relação C:N 13,8, percentual de cinzas 28,6% e pH próximo da neutralidade. O cultivo adicionado de Tween 80 (7,5%, m/v) propiciou valores de atividade e produtividade em lacase iguais a 3.016,47 U L⁻¹ e 502,7 U L⁻¹ dia⁻¹, respectivamente. Esses valores são 50 e 33,5 vezes maiores que os obtidos no cultivo sem adição de Tween 80, para atividade e produtividade em lacase, respectivamente. O caldo enzimático bruto degradou 100% do bisfenol-A após 48 horas, independentemente da concentração (500, 750 e 1.000 mg L⁻¹).

PALAVRAS-CHAVE: surfactante não iônico; atividade enzimática; interferente endócrino.

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INTRODUCTION

In recent years, one of the issues that has come to attention of the scientific community is the presence of endocrine disrupters in the environment. These compounds have the ability to interfere in the endocrine system of organisms, causing adverse implications for development, reproduction, neurological and immune system alterations (BERNARDO et al., 2015; SIFAKIS et al., 2017; JALAL et al., 2018).

Bisphenol-A is classified as an endocrine interfering compound, produced and used on large scale worldwide. In fact, it is considered as one of the most widely used synthetic compounds on the planet (JALAL et al., 2018). It is largely used in its monomeric form for the production of epoxy resins and polycarbonate plastics, plasticizers, dental sealants, food in cans which are internally coated with polymer film, eye-glass lenses, bottles, mineral water bottles, water supply pipes, adhesives, etc. (BERNARDO et al., 2015). As a precaution, and considering their toxic potential, some countries, including Brazil, have prohibited the importation and manufacture of bottles containing bisphenol-A, due to the greater exposure and susceptibility of the individuals who use this product. This prohibition has been in force in Brazil since January 2012 and was made through Resolution of the Collegiate Board (RDC) no. 41/2011 (BRASIL, 2011). Bisphenol-A is still permitted for other applications. However, legislation establishes the maximum specific migration threshold for this substance for food, defined based on the results of toxicological studies (FAO/WHO, 2010).

Chemical and biological methods are proposed in literature for the removal of bisphenol-A from the environment. Included in the proposed chemical methods are photocatalytic degradation (WANG et al., 2016), and advanced oxidative processes (POA) (JIANG et al., 2014), among others. According to FREITAS et al. (2017), these methods present some disadvantages in relation to biological degradation, such as high cost and the formation of toxic compounds. Thus, it is believed that biological degradation would be a less costly, safer and, according to green chemistry definitions, an environmentally correct method.

Fungi of the *Pleurotus* genus are commonly cited in literature as capable of degrading bisphenol-A (LIBARDI Jr. et al., 2012; MACELLARO et al., 2014; CHANG; CHANG, 2016; FREITAS et al., 2017). Fungi of this genus are known to be good producers of lignocellulosic enzymes such as laccases, manganese peroxidases and lignin peroxidases, all capable of degrading bisphenol-A (HIRANO et al., 2000; SONOKI et al., 2011). However, among them, laccase (EC 1.10.3.2), generally produced in greater concentration in relation to the others, was chosen to be studied for degradation processes (LIBARDI Jr. et al., 2012; MELO, 2015; FREITAS et al., 2017). Laccases are highly reactive and selective (FREITAS et al., 2017), and have the function of catalyzing the oxidation

of several aromatic compounds by reducing the molecular oxygen to water through a multi-copper enzyme system, resulting in oxidation of the hydrogen of the substrate and, consequently, in its degradation (BALDRIAN, 2006).

The production of lignocellulosic enzymes by basidiomycete fungi occurs in both submerged and solid-state culture (BONATTI et al., 2004; ORLANDELLI et al., 2012; MELO, 2015). Submerged cultivation presents some advantages in relation to solid-state cultivation, such as greater control of cultivation conditions, shorter production time, among others. A determinant and intrinsic factor for the production of enzymes by fungi is the composition of culture medium (BELLETINI et al., 2016). In biotechnological processes, agro-industrial wastes are commonly used in the composition of the culture medium in order to reduce production costs (BONATTI et al., 2004, LIBARDI Jr. et al., 2012; MELO, 2015; BILAL; ASGHER, 2016). At the same time, disposal of this type of waste in the environment is reduced, while adding value to it. Santa Catarina stands out in the national scenario as one of the largest producers of bananas and, consequently, as a generator of large quantities of banana straw waste, among others. Santa Catarina, together with the states of São Paulo, Bahia and Minas Gerais, represents 51% of Brazilian production (EPAGRI/CEPA, 2016).

Compounds classified as inducers may also form part of the composition of the culture medium. Non-ionic surfactants and very slightly toxic surfactants for biological membranes, such as Tween 80, can be added to the culture medium in order to stimulate the release of these enzymes into the culture medium (ELSAIED et al., 2012; EL-BATAL et al., 2015). These compounds have been used to increase the viability of the reactions between the enzymes and their respective substrates (POZDNYAKOVA, 2004), as well as to alter the structure of the cell membrane, thus facilitating the passage of the enzymes to the culture medium (UREK; PAZARLIOGLU, 2007).

Based on that, the objectives of this study were to characterize the residue immersion water of banana straw used in the culture medium composition and to evaluate the influence of Tween 80 on laccase production by *Pleurotus sajor-caju*, taking into account the removal of the endocrine interfering compound bisphenol-A.

MATERIALS AND METHODS

Characterization of immersion water

Banana straw, supplied by the Típicus Alimentos company, located in Garuva, Santa Catarina, Brazil, was dried at 60°C, shredded into 2 to 5 cm particles and immersed in water, in the proportion of 1:2, banana straw:water (m/v), for 12 hours. Then, the excess water was drained, in the process called banana straw immersion water. Part was reserved for

submerged cultivation and to the determination of pH, and part was concentrated in rotovapor (40°C) and lyophilized, in order to dry it and subsequently determine its chemical composition. All analyses were performed in triplicate and the results presented in terms of mean replicate values.

A Mettler Toledo pHmeter was used to determine the hydrogen potential. The ash percentage was calculated by the mass of the sample after incineration, according to the Association of Official Analytical Chemists (AOAC, 1984). The organic carbon and organic matter contents were determined according to the methodology proposed by KIEHL (1985). The organic carbon content was obtained by applying the correction factor 1.8 to the organic matter content. The total nitrogen content was obtained using the Kjeldahl method, according to the methodology proposed by AOAC (1984). The crude protein content was obtained by applying the correction factor 4.38 to the percentage of total nitrogen (BRENE, 1990). The monosaccharide composition was determined by adding to 2 mg of lyophilized sample 1 mL of 1 M trifluoroacetic acid. The mixture was incubated at 100°C for 12 hours, and the pH was adjusted to 9 (WOLFROM; THOMPSON, 1963a; ALBERSHEIM et al., 1967; GORIN et al., 1996). The acetylation, according to the method proposed by WOLFROM; THOMPSON (1963b), was performed with acetic acid, followed by filtration and drying in rotavapor. The addition of 4 mL of methanol and drying in rotavapor was repeated three times. Following, 300 µL of pyridine and 300 µL of acetic anhydride were added and, after 12 hours, 2 mL of chloroform and 1 mL of 5% CuSO₄ too. The monosaccharides were analyzed using a Varian Saturn 2000 R-3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer with DB-225 fused silica capillary column (25 m x 0.25 mm), and ultra-pure helium, at the flow rate of 1 mL min⁻¹ as the drag gas. The injection temperature was 50°C, with the heating ramp of 40°C min⁻¹ until the constant temperature reached 220°C.

Production of the crude enzyme broth by *Pleurotus sajor-caju*

The species *Pleurotus sajor-caju* obtained from the Collection of Basidiomycetes Cultures from the University of São Paulo under the code CCB 019 was used. The lineage was maintained in Petri dishes containing TDA medium (1 L of wheat extract, 20 g of dextrose and 15 g of agar) (FURLAN et al., 1997), under refrigeration at 4°C, with maintenance performed every three months.

As culture medium, 10 g L⁻¹ glucose, 5.4 mM dibasic ammonium tartrate and 150 µM CuSO₄, dissolved in banana straw immersion water, were used, as described by CESA et al. (2013). In order to evaluate the influence of the Tween 80 compound on laccase production by *Pleurotus sajor-caju*, the culture was performed without and with addition of Tween 80 (7.5% w/v). Both assays were performed in triplicate.

The assays were carried out in 500 mL Erlenmeyer flasks, containing 100 mL of culture medium (previously sterilized — 121°C for 15 minutes), inoculated with two 12-mm diameter agar discs containing fungal mycelium. The flasks were incubated at 30°C with reciprocal shaking of 105 min⁻¹ for 12 days, and the samples were withdrawn every two days and filtered in Whatman no. 1 paper. The biomass was then dried to a constant mass (60°C) and the obtained value divided by the volume of the sample. The result was defined as cell concentration (expressed in g L⁻¹). The filtrate was used to quantify enzymatic activity and glucose concentration. The glucose concentration was determined using enzymatic-colorimetric kit, following the principle described by TRINDER (1969). The activity of the laccase enzyme was determined by varying the absorbance at 420 nm in a spectrophotometer (Shimadzu, UV-160), produced by the oxidation of ABTS (2,2'-azino-bis (3-ethylbenzothiazolino-6-sulfonic acid) (Sigma-Aldrich, St. Louis, Missouri, United States) (BUSWELL et al., 1995), by the crude enzyme broth. A unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 µmol of the ABTS substrate per minute using the molar extinction coefficient of 36,000 M⁻¹ cm⁻¹.

Removal of bisphenol-A by crude enzyme broth

The ratio 1:1 crude enzyme broth (332.22 U L⁻¹):bisphenol-A (95% purity; Sigma-Aldrich, St. Louis, Missouri, United States), totaling 10 mL reaction volume, was used. The initial concentrations of bisphenol-A evaluated were 500, 750 and 1,000 mg L⁻¹. The mixtures were incubated at room temperature, and extractions of bisphenol-A were performed at 0, 24, 48 and 72 hours. All assays were performed in triplicate.

The extraction and quantification of bisphenol-A were carried out as follows: one drop of concentrated acetic acid was added to 2 mL of the sample and shaken. To the mixture, 2 mL of ethyl ether was added and shaken, and the supernatant was analyzed for the concentration of bisphenol-A by gas chromatography (model 6890, Agilent) with flame ionization detector (FID). The HP-5 column (5% phenylmethylsiloxane) was maintained at 290°C during the injection and then programmed for heating at 140°C min⁻¹ at 310°C for 10 minutes. Bisphenol-A was identified according to the retention time. Concentrations of the compound were calculated based on the calibration curve established with the external standard of bisphenol-A.

Methodology used in kinetic calculations

Maximum laccase activity (ΔL_{max}) was determined according to the Equation 1:

$$\Delta L_{max} = L_{max} - L_o \text{ (U L}^{-1}\text{)} \quad (1)$$

In which:

L_{\max} and L_0 : the laccase activities at the process time (defined as the one in which the laccase activity was maximal) and at the beginning of the process, respectively.

Global productivities in laccase (QL) and biomass (XQ) were determined according to the Equations 2 and 3:

$$QL = \frac{L_{\max} - L_0}{t} \text{ (U L}^{-1} \text{ day}^{-1}\text{)} \quad (2)$$

$$XQ = \frac{X - X_0}{t} \text{ (g L}^{-1} \text{ day}^{-1}\text{)} \quad (3)$$

In which:

t : the process time;

X and X_0 : the biomass concentrations in the process time and at the beginning of the process, respectively.

The specific rates of cell growth (μ) and laccase production (μ_p) were calculated using the Le Duy Zargic method (1973), according to the Equations 4 and 5

$$\mu = \frac{1}{X} \frac{dX}{dt} \quad (4)$$

$$\mu_p = \frac{1}{P} \frac{dP}{dt} \quad (5)$$

The Equations 6, 7, 8 and 9 refer to the kinetic model of a fermentative process proposed by LEUDEKING; PIRET (1959) and were used for the analysis of the laccase production by *P. sajor-caju*.

$$\mu = \alpha\mu_p + \beta \quad (6)$$

$$\frac{1}{X} \frac{dX}{dt} = \alpha \frac{1}{X} \frac{dP}{dt} \quad (7)$$

$$\frac{1}{X} r_x = \alpha \frac{1}{X} r_p + \beta \quad (8)$$

$$r_x = \alpha r_p + \beta X \quad (9)$$

In order to define the form of laccase production by *P. sajor-caju*, the Gaden (BAILEY; OLLIS, 1986) classification was used, according to the Equation 10, for synthesis associated with growth, Equation 11, for partially associated synthesis, and Equation 12, for dissociated growth synthesis.

$$\mu_p = \alpha\mu \quad (10)$$

$$\mu_p = \alpha\mu + \beta \quad (11)$$

$$\mu_p = \beta \quad (12)$$

Statistical analyses

The Dixon Q test, with 95% confidence level, was used to evaluate the rejection of deviant values (RORABACHER, 1991) for the calculation of the means. In order to evaluate the existence of statistically significant differences between the means of the results, the Tukey test was used, with the confidence level of 95%, and the Origin 8.0 PRO program. The latter was also used for the preparation of the kinetic curves and the graph of the percent removal of bisphenol-A.

RESULTS AND DISCUSSION

The chemical composition of the banana straw immersion water is presented in Table 1. It can be observed that the highest percentages of sugars found in the immersion water were glucose (31.6%) and galactose (31.5%). These sugars, released by the banana straw in the water together with the supplementation of the culture medium with 10 g L⁻¹ glucose, represent the soluble carbon source most readily used by *P. sajor-caju*. Furthermore, the fungus can use organic carbon (39.1%) from the organic matter content (70.4 ± 1.55) found in the immersion water. Regarding the mean crude protein and total nitrogen content, values of 12.4 ± 0.84 and 2.84 ± 0.13%, respectively, were found. Fungi, non-nitrogen-fixing chemoorganotrophic organisms, require the supply of carbon and nitrogen containing compounds by the culture medium in order to promote their development. The nitrogen and carbon compounds commonly found in culture media are ammonium sulfate, yeast extract, soy peptone, glucose, sucrose, maltose, etc. (BELLETINI et al., 2016). The carbon/nitrogen ration was equal to 13.8. The ash content was equal to 28.6 ± 0.56%. The determination of the ash content indicates de richness

Table 1. Chemical composition of immersion water of banana straw.

Parameter	Mean value ± standard error
Glucose	31.60 ± 0.00%
Galactose	31.50 ± 0.00%
Xylose	25.10 ± 0.00%
Mannose	11.80 ± 0.00%
Organic carbon	39.10 ± 0.20%
Organic matter	70.40 ± 1.55%
Protein	12.45 ± 0.84%
Total nitrogen	2.84 ± 0.13%
C/N ratio	13.83 ± 0.51%
Ash	28.60 ± 0.14%
pH	6.05 ± 0.35%

of the sample in terms of mineral elements, which are essential for the synthesis of enzymes, vitamins, proteins, nucleotides, etc. (ORLANDELLI et al., 2012; BELLETINI et al., 2016). The pH value found in this work is close to neutrality (around 6.0), thus avoiding costs with pH correction. Studies indicate pH values close to neutrality as ideal for laccase production by basidiomycete fungi (MELO, 2015). Therefore, it is possible to suggest the use of banana straw immersion water in the formulation of new culture media for the production of fungi enzymes.

MELO (2015) studied the influence of immersion water of banana straw on the composition of culture media for the production of lignocellulolytic enzymes by *P. sajor-caju*. The best result in terms of laccase activity ($2,416.5 \pm 35.5 \text{ U L}^{-1}$) was obtained using culture medium called OXI (banana peel powder 60 g L^{-1} , ammonium tartrate $5,4 \text{ mM}$, $150 \mu\text{M CuSO}_4$ and 10 g L^{-1} glucose, dissolved in banana straw immersion water). This value of laccase activity is 22 times higher than that obtained by the same author using the culture medium called Manachini Solution, defined in literature for the production of lignocellulolytic enzymes by basidiomycete fungi (MANACHINI et al., 1987).

Compounds classified as inducers, such as Tween 80 surfactant, may also be added to the culture medium. Figs. 1 and 2 show variations in glucose concentrations, biomass, laccase activity and pH as a function of time (days) of *P. sajor-caju*

cultivation, with and without addition of Tween 80 (7.5% m/v), respectively, to the culture medium. In the absence of Tween 80 (Fig. 1), the highest laccase activity (60 U L^{-1}) was observed in four days of cultivation, decreasing after this period. In four days of cultivation, the global productivity in laccase was $15 \text{ U L}^{-1} \text{ day}^{-1}$. The highest concentration of biomass was observed around eight days of cultivation (6 g L^{-1}), leading to a biomass productivity equal to $0.75 \text{ g L}^{-1} \text{ day}^{-1}$. The pH value, initially at 6.0, throughout the crop drops to the range of 4 to 5. CONFORTIN (2006) observed similar behavior in experiments with *P. sajor-caju* in submerged culture, as well as ELSAYED et al. (2012) in experiments with *Pleurotus ostreatus*. According to CONFORTIN (2006), the decrease in the pH value may be indicative of the existence of acid metabolism generating acid condition.

In the presence of Tween 80 (Fig. 2), the highest concentration of biomass (8.8 g L^{-1}) was observed around 10 days of cultivation, with biomass productivity equal to $0.88 \text{ g L}^{-1} \text{ day}^{-1}$; higher than the one seen in the culture without addition of Tween 80. After 10 days of culture, stationary cell growth phase and residual glucose concentration were reported approximately 8 g L^{-1} (percentage of glucose consumption equal to 37.3%). According to PARENTI et al. (2013), the main reason for reaching the stationary growth phase does not seem to be the exhaustion of the carbon source. The initial pH value (approximately 6.0) drops along the culture

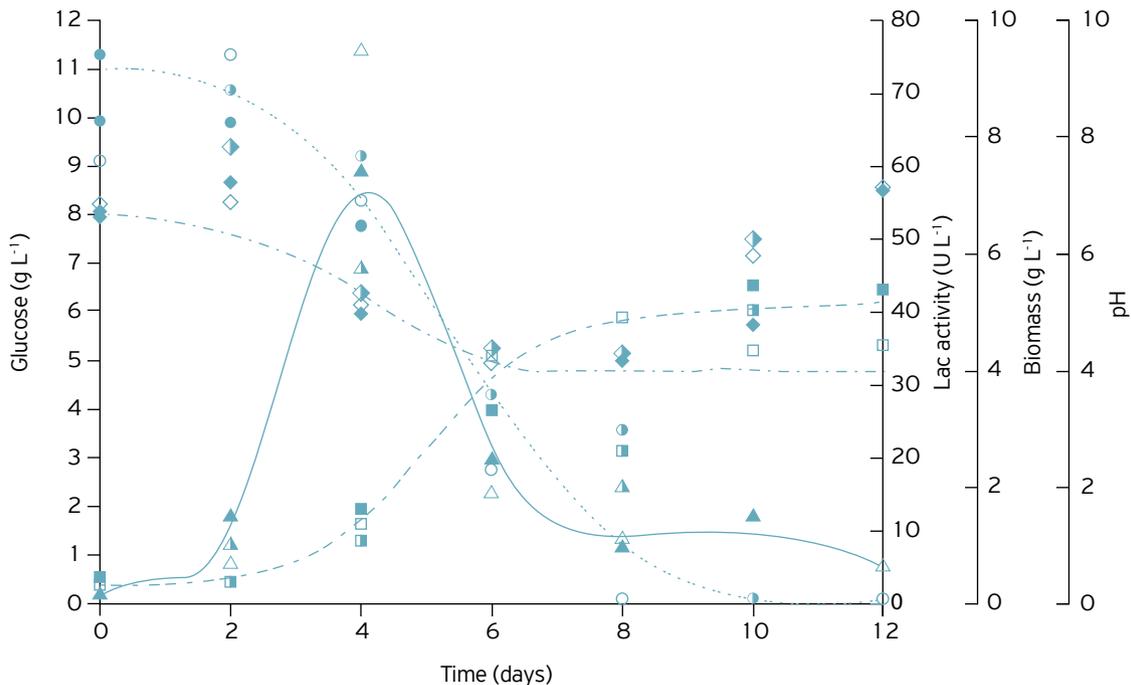


Figure 1. Profiles of biomass growth (dashed line, -■-, -□-), glucose consumption (dotted line, -●-, -○-), laccase activity (continuous line, -▲-, -△-) and pH (dash dot line, -◆-, -◇-) with time (days), in the absence of Tween 80. The open and closed symbols represent data from duplicates.

to around 4, like the one observed in the culture without addition of Tween 80.

ZHANG; CHEUNG (2011) observed an increase of 51.3% in the biomass concentration of *Pleurotus tuber-regium*, as well as an increase in the rate of glucose consumption (g day^{-1}), when added to Tween 80 culture medium. The same authors, in an experiment using Tween 80 as the only carbon source, did not verified fungal growth suggesting the absence of enzymes in this species capable of degrading Tween 80. The authors suggest that Tween 80 surfactant acts as a facilitator of nutrient absorption medium, mainly due to the increase in the rate of glucose consumption, thus providing a higher concentration of biomass. In this study, an increase in the biomass concentration in the culture with addition of Tween 80, but lower glucose consumption, was observed. Calculating the values of glucose conversion factor in biomass for the cultures with and without addition of Tween 80 gives values equal to 1.6 and 0.63, respectively, suggesting a better utilization of the substrate by the fungus in the presence of Tween 80. Few literature reports on the mechanism of action and the influence of Tween 80 on the production of biomass by lignocellulolytic fungi.

As for laccase activity, comparing Figs. 1 and 2, it was observed that the presence of Tween 80 in the culture medium positively affected laccase production, which was $3,016 \text{ U L}^{-1}$ in six days of culture. After this period, laccase activity decreased, with the productivity in the time of six days being $502.7 \text{ U L}^{-1} \text{ day}^{-1}$ ($21 \text{ U L}^{-1} \text{ h}^{-1}$). This yield value was 33.5 times higher than that obtained in the culture

without addition of Tween 80. ELSAYED et al. (2012) and EL-BATAL et al. (2015) also observed increased laccase production by *P. ostreatus* when added to Tween 80 culture medium. According to POZDNYAKOVA (2004), the release of enzymes, such as laccase, into the culture medium can be enhanced/stimulated by the use of detergents, non-ionic and non-toxic surfactants for biological membranes, such as Tween 80, in the composition of culture medium. According to UREK; PAZARLIOGLU (2007), these compounds would have the capacity to alter the structure of the cell membrane and, thus, facilitate the passage of the enzymes to the culture medium.

The value of laccase productivity obtained in this work in the presence of Tween 80 ($502 \text{ U L}^{-1} \text{ day}^{-1}$, *i.e.*, $21 \text{ U L}^{-1} \text{ h}^{-1}$) was higher than the one obtained by TINOCO-VALENCIA et al. (2014) ($14 \text{ U L}^{-1} \text{ h}^{-1}$), as well as higher than that obtained by AGGELIS et al. (2003) ($0.38 \text{ U L}^{-1} \text{ h}^{-1}$). These authors evaluated the potential of *P. ostreatus* in producing laccase using waste from the olive oil processing industry as a substrate.

PARENTI et al. (2013) used immersion water of wheat straw for the laccases production by *P. ostreatus*. The authors found compounds such as gallic acid (3, 4, 5-trihydroxybenzoic acid), $168.76 \mu\text{M}$, feluric acid (3- (4-hydroxy-3-methoxyphenyl) prop-2E-enoic acid), $11.90 \mu\text{M}$ and vanillin (3-methoxy-4-hydroxybenzaldehyde), $20.24 \mu\text{M}$, in immersion water. According to PISCITELLI et al. (2011), phenolic and aromatic compounds with a structure similar to that of lignin or lignin derivatives are usually added to the culture

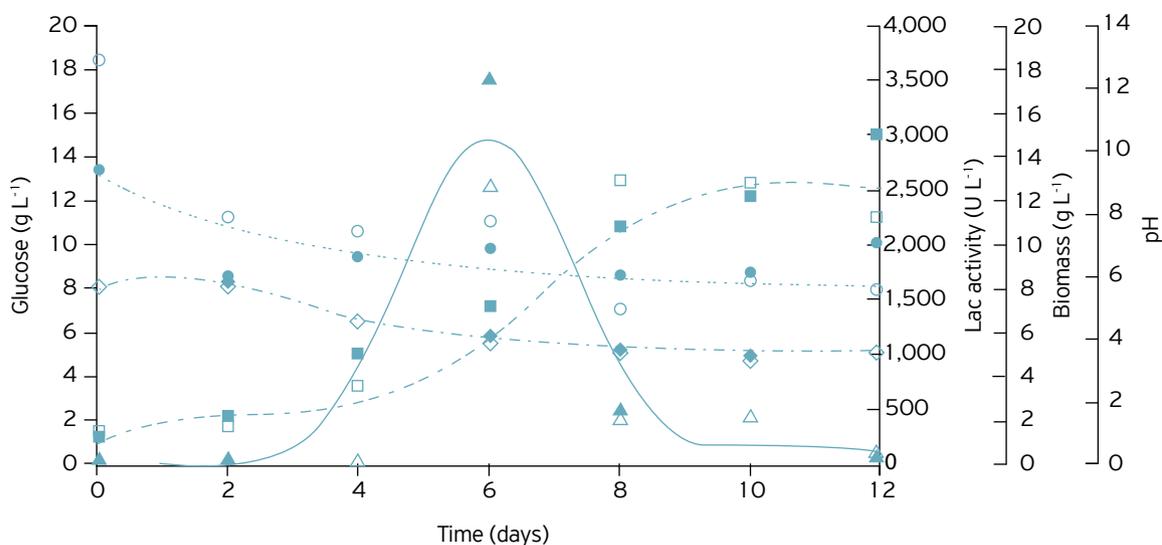


Figure 2. Profiles of biomass growth (dashed line, -■-, -□-), glucose consumption (dotted line, -●-, -○-), laccase activity (continuous line, -▲-, -△-) and pH (dash dot line, -◆-, -◇-) with time (days), in the presence of 7.5% Tween 80. The open and closed symbols represent data from duplicates.

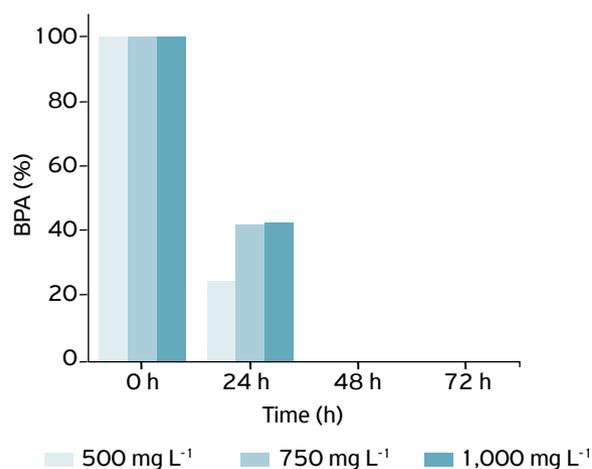
media in order to increase laccase production. According to RANCAÑO et al. (2003), this increase in laccase activity occurs because there is a response developed by the fungus, increasing the number of copies of mRNA, with increased enzyme units, as an attempt to oxidize these compounds, reducing their toxicity and, concurrently, obtaining sugars smaller and more easily assimilated. PARENTI et al. (2013) obtained laccase productivity equal to $25 \text{ U L}^{-1} \text{ day}^{-1}$, 20 times lower than that obtained in this study. The authors justify the low productivity due to the low concentrations of phenolic compounds (laccase inducers) in the final formulation of the culture medium. Based on that, it may be suggested that laccase inducers, whether or not the same as those identified by PARENTI et al. (2013), are present in banana straw immersion water.

The calculation of specific rates, according to LE DUY; ZARJIC (1973), and kinetic analysis of the process, according to BAILEY; OLLIS (1986), was performed for the culture with addition of Tween. Taking into account Gaden's classification, a partial association between laccase synthesis and fungus growth was observed, with $\mu_P = 1,788.4 \mu$. Pursuant to this equation, and since the coefficient x of the Leudeking and Piret equation is greater than 1.0, it is a kinetic that favors laccase production with association between laccase production and cell growth between the second and fifth days of culture, *i.e.*, laccase production is associated with the phase of growth acceleration (stage 2 of the growth curve, *i.e.*, onset of reproduction, in which there is a gradual increase in the rate of reproduction and growth). In associations of this type, and in the case of expanding the scale of laccase production, the use of batch process would be advisable (BORZANI, 2001).

The crude enzyme broth obtained using Tween 80 on the medium composition was also selected for the bisphenol-A removal assays. Figure 3 shows the residual percentages of bisphenol-A obtained after 0, 24, 48 and 72 hours of incubation of the crude enzyme broth with bisphenol-A solution (1:1, v/v). As can be observed in Figure 3, after 24 hours of incubation, 60% of bisphenol-A in 750 and 1,000 mg L^{-1} was removed, and approximately 75% was removed in 500 mg L^{-1} . In a 48-hour period of incubation, the percent removal was 100% for all concentrations evaluated. Since the laccase activity in the reaction medium at the beginning of the experiment was 166.11 U L^{-1} (dilution factor 2), the specific removal rate for each concentration evaluated could be calculated, 500, 750 and 1,000 mg L^{-1} , for the time of 48 h, equal to 0.063, 0.094 and 0.125 $\text{mg U}^{-1} \text{ h}^{-1}$, respectively. LIBARDI-JR et al. (2012) evaluated the removal capacity of bisphenol-A (57 mg L^{-1}) by the crude enzyme broth of *P. ostreatus* (laccase activity of 0.5 U mL^{-1}) and verified 100% removal in 24 hours (specific removal rate equal to $0.0045 \text{ mg U}^{-1} \text{ h}^{-1}$). MACELLARO et al. (2014) evaluated the ability to remove bisphenol-A (50 mg L^{-1}) by

purified and immobilized *P. ostreatus* laccases and noticed 60% removal percentage in 2 hours of incubation (specific removal rate equal to $0.017 \text{ mg U}^{-1} \text{ h}^{-1}$, lower than that obtained in this work). CHANG; CHANG (2016) studied the ability to remove bisphenol-A (20 mg L^{-1}) by the crude enzyme broth of *Pleurotus eryngii* and obtained about 97% removal after 24 hours of incubation (specific removal rate equal to $1.5 \times 10^{-5} \text{ mg U}^{-1} \text{ h}^{-1}$).

The removal of bisphenol-A occurs concomitantly with the decrease of the toxicity of the culture medium. CAJTHAML et al. (2013), in studies related to the removal of bisphenol-A by various lignocellulolytic fungi, observed higher efficiency on removal bisphenol-A by the species *P. ostreatus*, and estrogenic activity near zero at the end of the experiment (14 days). FREITAS et al. (2017) studied both the toxicity of the final solution and the products originated from the degradation of bisphenol-A by laccases of two species of *Pleurotus* (*P. ostreatus* and *P. pulmonarius*). The authors identified seven aliphatic and six aromatic compounds in the reaction medium, five of which were identified in the reactive medium containing laccases of both species. The three metabolites ethyl 3-oxobutanoato, benzaldehyde and nonadecane were identified in the culture media of *P. ostreatus*. 2,6-dimethyldecane, 9-pentadecen-1-ol, p-hydroxybenzoic acid, 2-(2-hydroxypropyl)-benzene, 1,4-diol and 2-(6-(tert-butyl)-1, 1-dimethyl-2,3-dihydro-1H-inden-4-yl) acetic acid were identified in the culture media of *P. pulmonarius*. The authors suggest these differences are due to the intrinsic characteristics of each enzyme, such as redox potential, among others. Regarding toxicity, the authors observed at the end of the cultivation, when the presence of bisphenol-A was practically zero in trials with the *P. ostreatus* species, 95%



BPA: Bisphenol-A.

Figure 3. Relative percentage of BPA during treatment by *P. sajor-caju* crude enzyme broth.

decrease in toxicity (Microtox toxicological test). The same was not verified for *P. pulmonarius*, suggesting that one or more metabolites present in the medium are, individually or not, more toxic than bisphenol-A.

CONCLUSIONS

The banana straw immersion water presented pH close to neutrality, ideal for laccase production, glucose and galactose percentages equal to 31.6 and 31.5%, respectively, ash percentage 28.6% and C:N ratio 13,8. The addition of Tween 80 to the culture medium resulted in an increase in laccase production by *P. sajor-caju*, more specifically, an increase of 50 (3,016.47 U L⁻¹) and 33.5 (502.7 U L⁻¹ day⁻¹) times in the values of maximum activity and productivity, respectively, in relation to the same culture performed without addition of Tween 80. Regarding the ability to remove bisphenol-A,

the crude enzyme broth was able to remove 100% of the compound (regardless of the concentration assessed) within 48 hours of incubation. Thus, the use of banana straw immersion water and Tween 80 in the composition of culture media for the laccase production by *P. sajor-caju* species is suggested, with the crude enzyme broth obtained in this work being efficient in the removal of bisphenol-A in synthetic solutions.

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