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Production of fungal enzymes in Macaúba coconut and enzymatic degradation of textile dye

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ABSTRACT

The reduction of environmental pollutants has been important for the sustainability (environmental, economic, and social) of the manufacturing companies. The agro-industry annually produces tons of vegetable residues that is discarded in the environment. Textile industries have a high rate of dye discharges in water resources. Fungal enzymes produced in plant residues can degrade recalcitrant organic compounds. Thus, the aim of the study was the production, purification, characterization, and use of dye discoloration of enzymes produced by *Pleurotus* ostreatus (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05) in Macaúba coconut (*Acrocomia aculeate)* residue. The initial time of colonization of the substrate was of 18 (*P. eryngii)* and 22 days (*P. ostreratus)*. After this period, 50 g of substrate were collected every five days to evaluate the enzymatic activity before and after the purification in sephadex G-100 column. All fungal strains had activity of laccase, lignin peroxidase (LiP), manganese peroxidase (MnP), xylanase, cellulase, and lipase in crude protein extract. The enzymes kept the activities from 4 to 60 °C for about 16 h and these activities were higher in acidic than alkaline pH. Furthermore, the enzymes of *P. eryngii* degraded about 100% of Carmine indigo dye after 24 h of incubation. Therefore, the Macaúba coconut residue is a good substrate for the enzyme production and these enzymes have high degradation capacity of the indigo dye.

1. Introduction

The white-rot fungi produce oxidative (e.g. laccase, manganese peroxidase, and lignin peroxidase) and hydrolytic (xylanases and cellulases) enzymes in agricultural residues (Ozcirak and Urek, 2017). These enzymes are used in several industrial processes, and their production has increased in the last decades with an income of US$ 8 billion (Jaramillo et al., 2015).

The agro-industry annually produces tons of vegetable residues that is discarded in the environment (Sadh et al., 2018; Silva et al., 2019; Xie et al., 2016). The Macaúba palm (*Acrocomia aculeate*) residues are produced in large quantities in Brazil. In oil extraction, about 14.5 tons/-year of coconut residue are produced. These residues could be used in the production of fungal enzymes and edible mushrooms, since they are rich in cellulose, hemicellulose, lignin, and lipids (Sobreira, 2011).

Pleurotus spp. is producer of these enzymes that degrade components of plant biomass, including cellulose, hemicellulose, and lignin (Xie et al., 2016; Manawadi et al., 2019). The fungal genus has mycelial growth in wood, by-products of wood (e.g. sawdust, paper, and pulp sludge), cereal straws, corn cobs, sugarcane bagasse, coffee residues (e. g. coffee, hooves, stalks, and leaves), cotton bark, soybean pulp, sorghum, and other lignocellulosic materials (Cardoso et al., 2017; Kalmiş and Sayit, 2004; Silva et al., 2019; Oliveira et al., 2018). Furthermore, it is producer of edible mushrooms with high nutritional value, therapeutic properties, and several biotechnological applications (Knop et al., 2016).

Textile industries have a high rate of dye discharges in water resources (Mishra and Maiti, 2018, 2019; Manawadi et al., 2019). However, the textile dyes degradation has been observed for microbial enzymes (Manawadi et al., 2019; Mehandia et al., 2020; Mishra and Maiti, 2019, Mishra et al., 2019). Lignocellulolytic enzymes (laccase, manganese peroxidase, lignin peroxidase, xylanases, and cellulases) and

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lipases produced in plant residues can degrade recalcitrant organic compounds, textile dyes, and others (Manawadi et al., 2019; Mehandia et al., 2020; Jankowska et al., 2020; Silva et al., 2019; Oliveira et al., 2018).

Thus, the aims of this study were to evaluate the production of lignocellulolytic enzymes and lipases by *Pleurotus* spp. in Macaúba coconut residue, to characterize these enzymes, and to determine the potential of these enzymes in discoloration of a textile dye.

2. Material and methods

The study was carried out at the Laboratory of Mycorrhizal Associations/Department of Microbiology/BIOAGRO/Universidade Federal de Viçosa (UFV).

2.1. Inoculum

The fungi used belong to the fungal collection from the UFV. The *Pleurotus ostreatus* strain PLO 02, *P. ostreatus* strain PLO 06 (da Luz et al., 2013) and *Pleurotus eryngii* strain PLE 05 were used. These fungi had mycelial growth in Petri dishes containing 20 mL of culture medium, potato-dextrose-agar (39 g L^{-1}), and lignin (1 g L^{-1}) for 15 days at 25 ± 1 °C. The spawn was produced in sorghum grains. Four disks of agar with the mycelia were added in an Erlenmeyer flasks (150 mL) containing 50 g sorghum grains previously cooked and autoclaved for 20 min at 121 °C. After 15 days at 25 °C, the spawn was used as inoculum in solid-state fermentation (SSF) for enzyme production.

2.2. Solid-state fermentation

The solid-state fermentation (SSF) was performed in Erlenmeyer flasks (250 mL) containing 280 g of Macaúba coconut residue and 65 mL of water. These substrates were autoclaved for 1 h at 121 °C. Ten grams of spawn were added in each flask. After 23 days of incubation at 25 °C, three flasks were removed each 5 days for analysis. The total fermentation time was of 63 days.

2.3. Obtaining of crude protein extract

Crude protein extract (CPE) was performed with 50 mL of sodium citrate buffer (50 mmol L^{-1}, pH 4.8) and 25 g of substrate/mycelia. This mixture was homogenized in a porcelain mortar for 5 min and centrifuged at 250 rpm for 30 min. The supernatant was filtered on filter paper (Whatman nº 1). The permeate (CPE) was used for the analysis of enzymatic activity, soluble protein determination, and enzyme purification.

2.4. Determination of enzymatic activities and soluble proteins content

The lipase activity was determined using 5 mL of soybean oil emulsion, 2 mL of phosphate buffer (10 mmol L^{-1}, pH 7.0), and 1 mL of CPE (Burkert et al., 2004). This reaction was incubated at 37 °C for 30 min. Then, 10 mL of the acetone-ethanol (1: 1 v/v) was added in each tube. The free fatty acids were titrated with sodium hydroxide (0.05 mmol L^{-1}).

The lignin peroxidase activity was measured in absorbance at 310 nm at 30 °C (Tien and Kirk, 1984). The reaction contained 1.0 mL of sodium tartarate buffer (100 mmol L^{-1}, pH 3.5), 1 mL of veratryl alcohol (4 mmol L^{-1}, ε = 9300 $M^{-1}cm^{-1}$), 250 μL of hydrogen peroxide (0.2 mol L^{-1}), and 100 μL of CPE.

The reaction for laccase activity was performed with 2300 μL of sodium acetate buffer (0.1 mol L^{-1}, pH 5.0), 100 μL of 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS, 1 mmol L^{-1}, ε = 36,000 M^{-1} cm^{-1}), and 600 μL of CPE (Buswell et al., 1996). This enzymatic reaction was incubated in a water bath at 37 °C for 2.0 min. After, the absorbance of the reaction was determined at 420 nm.

The MnP activity was determined using 500 μL of CPE, 100 μL phenol red (1 g L^{-1}, ε = 3162 M^{-1} cm^{-1}), 100 μL sodium lactate (250 mmol L^{-1}, pH 4.5), 200 μL bovine serum albumin (1% w/v), 50 μL of hydrogen peroxide, and 50 μL of manganese sulfate. This reaction was incubated in the water bath at 37 °C for 15 min. Thereafter, 40 μL of sodium hydroxide (2 mmol L^{-1}) and 2 mL of water were added. The absorbance was determined at 610 nm (Kuwahara et al., 1984).

The activities of cellulase and xylanase were performed by reducing sugars amount at 540 nm (Eveleigh et al., 2009; Bailey et al., 1992). The reaction of cellulase activity was performed using 250 μL of carboxymethylcellulose (0.1% w/v), 500 μL of sodium citrate buffer (50 mM, pH 4.8), 250 μL of CPE or partially purified protein extract (see section 2.5). For the reaction of xylanase activity, 500 μL of sodium acetate buffer, 250 μL of CPE, 250 μL of xylan (0.1% w/v) were used. These enzymatic reactions were maintained for 20 min at 50 °C in a water bath. The reducing sugars content was determined by the addition of 1 mL of 3, 5-dinitrosalicylic acid (DNS).

The soluble protein content in CPE was performed by the methodology described by Bradford at 595 nm (Bradford, 1976). The standard curve was made using serum albumin (1% w/v) with a concentration ranging from 0.05 to 9 μg mL^{-1}. For each reaction, 1.0 mL of Bradford's reagent, 1 mL of CPE, and 6 mL of water were used. This reaction was incubated for 10 min at 25 °C.

2.5. Purification of crude protein extract

The CPE was fractioned through precipitation with ammonium sulfate at 40 and 80% at 4 °C for 12 h. The suspensions (40 and 80% of saturation) were centrifuged at 4000 rpm for 15 min. The precipitates were resuspended in sodium acetate buffer (50 mmol L^{-1}, pH 5.0) followed a dialysis.

The dialysis was performed in the nitrocellulose membrane at 4 °C, for 12 h and with sodium acetate buffer (50 mmol L^{-1}, pH 5.0). After this step, the solution was applied to the Sephadex G-100 Column (80 cm × 2.0 cm), pre-equilibrated with the same buffer. The fractions were eluted with sodium acetate buffer (50 mmol L^{-1}, pH = 4.5) at 2 mL min^{-1}. Twenty milliliters of the partially purified protein extract (PPPE) were obtained. The PPPE was used for determination of the kinetic characterization of the enzymes and indigo dye degradation.

2.6. Kinetic characterization of protein extract

The temperatures of 4, 15, 30, 45, and 60 °C and pH values of 3.0, 7.0 and 10 were analyzed.

The thermal stability was performed during 30, 60, 120, 240, 480, 960 min.

The effect of the substrate concentrations for lignocellulolytic enzymes (1/8, 1/4, 1/2, 2, 4, 8, 16, and 30-fold) and lipase (1/8, 1/4, 1/2, 2, 3, 4, and 5-fold) were also determined in PPPE.

The thermal stability and effect of the substrate were carried out in optimum temperature and pH, which were determined advantages in this section.

2.7. Indigo dye degradation

The discoloration of the Carmine indigo dye (5,5′-indigodisulfonic acid sodium salt) by PPPE was evaluated after 5 min and 24 h of incubation at 35 °C (de Souza, 2012). The PPPE was a pool of the activities evaluated in section 2.4. The volume of reaction was of 1 mL with 200, 400, 600, and 800 μL of the dye (0.2 g mL^{-1}). The dye and PPPE were performed em sodium acetate buffer (50 mmol L^{-1}, pH 5.0).

The absorbance was determined at 680 nm.

2.8. Statistical analysis

The experiment in a completely randomized design was carried out

using five replicates per fungal strain. The data were submitted to an analysis of variance (ANOVA) and Tukey's test at 5% of probability. All the analysis was performed using Sigma Plot 12 software (free version).

3. Results

3.1. Enzymatic activities of the crude protein extract

All fungal strains had the activity of laccase, LiP, MnP, xylanase, cellulase, and lipase in CPE (Fig. 1).

The laccase activity was higher in the PLO 02 than other fungal strains (Fig. 1A). However, the LiP and MnP activities were highest in PLO6 (Fig. 1B and C). These lignases had maximum activity between 30 and 35 days of incubation (Fig. 1A, B, and 1C).

The maximum activity of hydrolases also was observed between 30 and 35 days of incubation (Fig. 1D and E). The xylanase activity was smaller in *P. eryngii* than *P. ostreatus* (Fig. 1D).

Lipase production was higher in PLO 06 than other fungal strains (Fig. 1F).

3.2. Temperature effect on enzymatic activities

For PLO06 and PLE05, the laccase activity was stable at all temperatures analyzed while; PLO 02 had an increase of this enzyme activity at 50 °C (Fig. 2A). The optimum temperature of LiP activity varied among the fungal strains (Fig. 2).

The activity of MnP, xylanase, and cellulase was stable at all temperatures (Fig. 2B, C, 2D, and 2E).

Lipase activity changed with increasing temperature (Fig. 2F).

3.3. Effect of pH on enzymatic activities

The optimum pH of laccase activity was similar among the fungal of this study (Fig. 3A). However, the optimum pH of LiP activity was different among them (Fig. 3A and B). Only PLO 02 had an increase in MnP activity at pH 7.0 (Fig. 3C). The xylanases and cellulases activities were also stable in the values pH analyzed (Fig. 3D and E). The maximum activity of lipases was observed in pH 3.0 for PLO 02 and pH 7.0 for PLO 06 and PLE 05 (Fig. 3F).

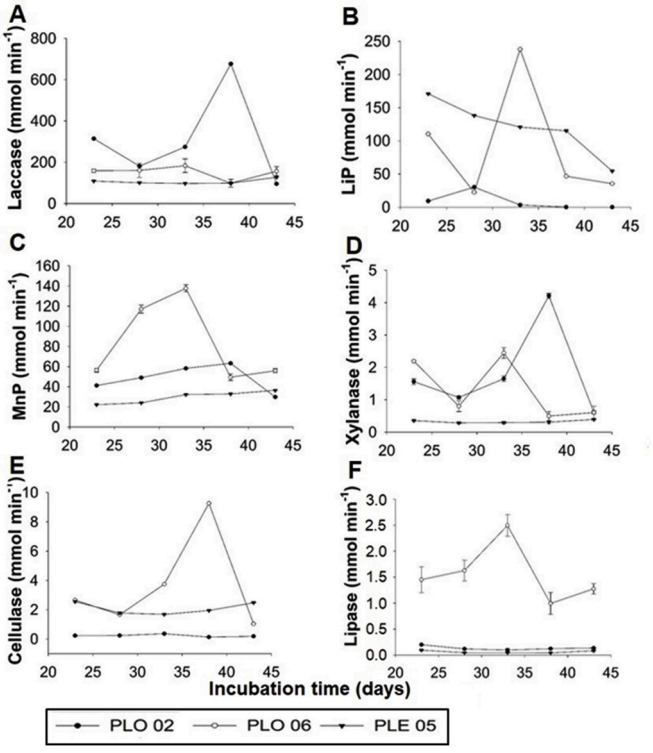

Fig. 1. Activities of laccase (A), lignin peroxidase (B), manganese peroxidase (C), xylanase (D), cellulase (E), and lipase (F) from *Pleurotus ostreatus* (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05).

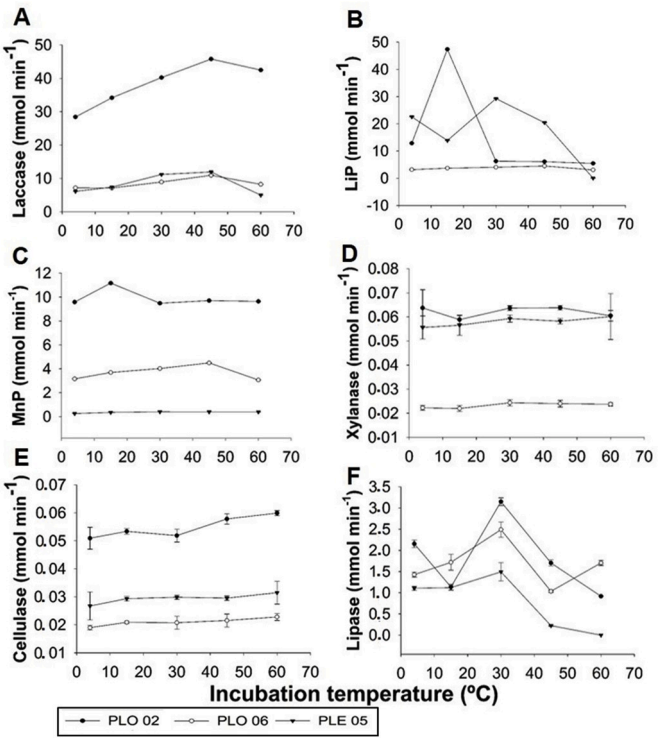

Fig. 2. Effect of temperature of incubation on the activities of laccase (A), LiP (B), MnP (C), xylanase (D), cellulase (E), and Lipase (F) produced by *Pleurotus ostreatus* (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05) when they were grown on Macaúba coconut residue.

3.4. Thermal stability

Only laccase activity was not stable at incubation times (Fig. 4).

We observed that the increase in substrate concentration caused saturation in enzymatic activity. Furthermore, the conversion rate of the substrate into the product was not proportional to this increase (Fig. 5 and Table 1).

3.5. Kinetic parameters

For laccase, the lowest K_M was observed in the PLE 05 (Fig. 5A and Table 1). The K_M for LIP, MnP, xylanases, and cellulases were similar among fungal strains (Fig. 5B, C, and 5D, Table 1).

The K_M and V_{max} are apparent values (Fig. 5 and Table 1).

3.6. Indigo dye degradation

The PPPE from all three strains was able to discolor the indigo dye (Fig. 6, Table 2). This degradation was directly proportional to the time of incubation and regardless of the amount of dye used in the reaction (Table 2). The PPPE of *P. ostreatus* had a discoloration rate of 50% (PLO 02) and 56% (PLO 6) after 24 h (Table 2). However, *P. eryngii* PLE 05 showed the highest potential to indigo degradation with 73% of discoloration in 5 min and about 100% after 24 h a 35 °C (Fig. 6, Table 2).

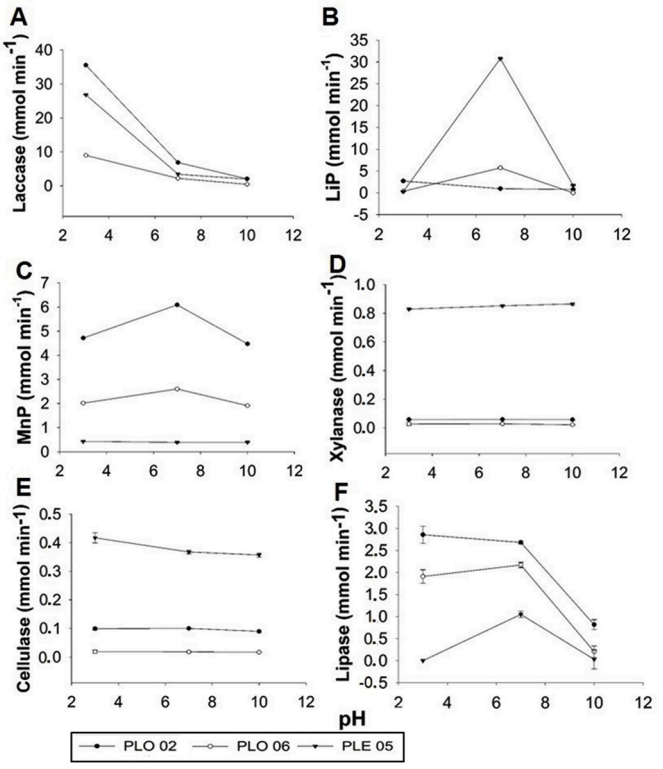

Fig. 3. Effect of pH on the activity of laccase (A), LiP (B), MnP (C), xylanase (D), cellulase (E), and Lipase (F) produced by *Pleurotus ostreatus* (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05) in crude protein extract of Macaúba coconut residue.

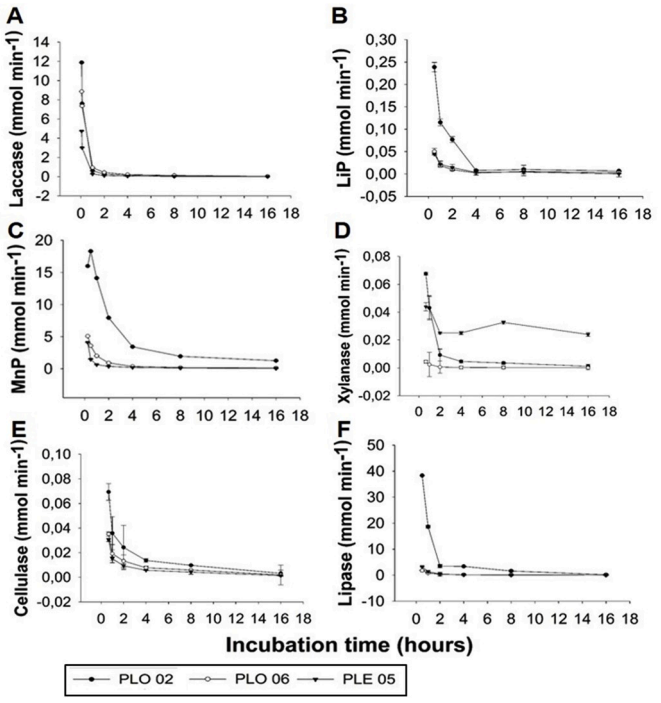

Fig. 4. Effect of incubation time on the activity of laccase (A), LiP (B), MnP (C), xylanase (D), cellulose (E), and Lipase(F) produced by *Pleurotus ostreatus* (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05) in crude protein extract of Macaúba coconut residue.

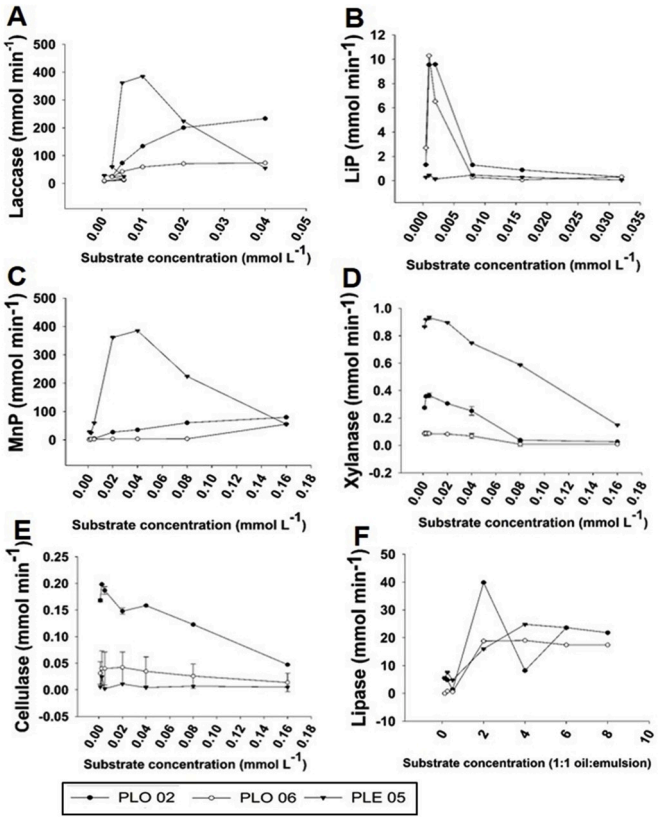

Fig. 5. Activity of lignocellulolytic enzymes and lipase produced by *Pleurotus ostreatus* (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05) in Macaúba coconut residue in relation to substrate concentration, after dialysis and molecular exclusion chromatography.

4. Discussion

The maximum peak of laccase activity of *P. ostreatus* cultivated in wheat grains was observed after 20 days, with a productivity of 70 μmol g^{-1} of substrate (Montoya and Levin, 2015). Laccase of *P. eryngii* cultivated in banana stems had maximum activity of 160.25 U mL^{-1} after 10 days (Asgher et al., 2016). MnP of *P. ostreatus* grown on wheat grains had constant production during the 40 days (Montoya and Levin, 2015). *P. ostreatus* cultivated in *Jatropha curcas*, LiP activity was of 10.60 mmol mL^{-1} after 7 days (Oliveira et al., 2018). *P. eryngii* had activity peaks for LiP and MnP of 143.15 mL^{-1} and 68.2 mL^{-1} on the fourth day of incubation in agro-industrial residues (Asgher et al., 2016).

The activities of hydrolase may vary with the fungal strains and the substrate (Figs. 1 and 2). Was observed maximum activity of cellulase (50 μmol g^{-1}) at 35 days of incubation in *P. ostreatus* grown on wheat grains (Montoya and Levin, 2015), while was showed activity of 0.00132 and 0.00133 mmol g^{-1} for cellulase and xylanase, in *P. ostreatus* when grown on sorghum straw after 20 days (Soares, 2012). This same author identified these enzymes *in P. erynggii* with activities of 0.00065 and 0.00180 mmol g^{-1} for cellulase and xylanase. These results show also the capacity of *P. ostreatus* and *P. eryngii* produced cellulolytic enzymes (Fig. 1).

All tested fungi isolates were capable of degrading lipids of the culture medium observed by the orange fluorescent halo formation that is due to the reaction of the fatty acids produced during the enzymatic hydrolysis with rhodamine B (Fig. 1). *P. eryngii* PLE 05 had lower mycelial growth than *P. ostreatus* in the first assay. Lipase production has also been reported in *Trichothecium roseum*, *Penicillium* sp., *Rhizomucor* sp., *Aspergillus* sp., and *Lentinus crinitus*, that were isolated from environments contaminated with oil (Cambri et al., 2016; Gopinath et al., 2013). Thus, *P. ostreatus* and *P. eryngii* can be used for lipase production.

Table 1
Michaelis-Menten constant (K_M) and maximum velocity (Vmax) of lignocellulolytic enzymes and lipase produced by *Pleurotus ostreatus* (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05) in Macaúba coconut residue.

Enzyme	Substrate	PLO 02		PLO 06		PLE 05	
		K_M[a]	Vmáx[b]	K_M[a]	Vmáx[b]	K_M[a]	Vmáx[b]
Laccase	**ABTS**	1.50 ± 0.01	31.40 ± 2.25	2.40 ± 0.07	48.07 ± 1.65	0.00057	57.400
LiP	Veratryl alcohol	0.30 ± 0.005	9.90 ± 1.11	0.30 ± 0.01	9.32 ± 0.17	0.00073	7.300
MnP	Phenol red	32.0 ± 0.13	17.00 ± 1.22	19.0 ± 0.22	26.24 ± 0.31	0.02200	73.00
Cellulase	Carboxymethylcellulose	1.20 ± 0.01	0.26 ± 0.01	3.40 ± 0.13	0.092 ± 0.01	0.00160	0.064
Xylanase	Xylan	6.50 ± 0.03	0.36 ± 0.04	6.00 ± 0.24	0.38 ± 0.03	0.00031	1.020
Lipase	Soybean oil	60.0 ± 0.12	7.37 ± 2.35	59.0 ± 3.24	15.20 ± 1.45	3.70000	40.800

[a] KM - μg mL^{-1}.
[b] Vmáx - mmol L^{-1} min^{-1}. ABTS - 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid. K_M and Vmax are apparent values.

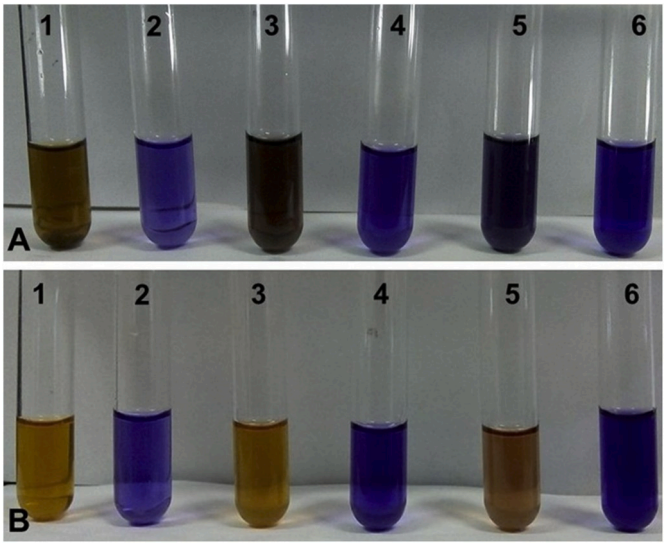

Fig. 6. Degradation of the Carmine indigo dye (0.2 g mL^{-1}) by the partially purified enzymatic extract (PPPE) of *Pleurotus eryngii* (PLE 05) after 5 min (A) and 24 h (B) of incubation. Different proportions of dye and PPPE were tested: (1) 400 µL of dye: 600 µL of PPPE; (2) 400 µL of dye: 600 µL of sodium acetate buffer (50 mmol L^{-1}, pH 5.0); (3) 600 μL of dye: 400 μL of PPPE; (4) 600 μL of dye: 400 μL of sodium acetate buffer; (5) 800 μL of dye: 200 μL of PPPE; and (6) 800 μL of dye: 200 μL of sodium acetate buffer.

Table 2
Discoloration rate of Carmine indigo dye (0.2 g mL^{-1}) by the partially purified enzymatic extract (PPPE) of *Pleurotus ostreatus* (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05) after 0.083 h (5 min) and 24 h of incubation.

Fungi	Reaction volume (μL)	Discoloration rate (%)		
	Dye	PPPE	0.083 (h)	24 (h)
PLO 02	400	600	18 ± 2.0	45 ± 2.3
	600	400	27 ± 1.3	30 ± 1.4
	800	200	42 ± 2.3	48 ± 2.6
PLO 06	400	600	19 ± 1.5	47 ± 2.4
	600	400	42 ± 1.1	45 ± 1.8
	800	200	43 ± 2.2	47 ± 2.1
PLE 05	400	600	62 ± 1.8	87 ± 2.6
	600	400	76 ± 2.9	98 ± 1.7
	800	200	74 ± 2.7	99 ± 1.2

Our lipase activity (Fig. 1) was higher than the activity of this enzyme of *P. ostreatus* grown in commercial culture medium supplemented with olive water (Piscitelli et al., 2017). These authors observed maximum lipase activity of 0.03 mmol L^{-1} at 5 days incubation.

Similar to our results (Fig. 3), 50 °C has been the optimal temperature of laccase activity produced for *P. ostreatus* (Patel et al., 2013) and *P. eryngii* (Yang et al., 2015) and MnP activity of *Ganoderma lucidum* grown in commercial medium (Muhammad et al., 2016).

The variation in the optimum temperature of LiP is reported in the literature. LiP of *P. ostreatus* cultivated in *J. curcas* residue was thermostable (Oliveira et al., 2018), but 35 °C was the optimum temperature in the growth of this fungus in commercial medium, (Sivakami et al., 2012). Furthermore, the LiP of PLE 05 showed the highest thermostability than LiP of *P. ostreatus* (Fig. 2).

A xylanase of *P. ostreatus* grown in basal medium plus xylan had its maximum activity at 50 °C (Getachew and Melaku, 2016). Similar behavior was found for a *Trichoderma inhamatum* xylanase grown in commercial medium supplemented with wheat bran (Silva et al., 2005). In *P. florida* grown in different carbon sources, maximum cellulase activity at 45 °C was found (Goyal and Soni, 2011).

The enzymes laccase, MnP, xylanase and cellulase of our fungal strains demonstrated thermostability over a wide temperature range reinforcing their potential use in different industries (Fig. 2). Furthermore, enzymes with high thermostability have advantages, such as the reduction of the enzymatic charge and longer time of enzymatic activity that caused a reduction in process costs (Viikari et al., 2007).

The lipases of *P. ostreatus* and *P. eryngii* had peak activity at 30 °C (Fig. 3F). A similar result was demonstrated in which two lipases isolated from *P. ostreatus* cultivated in commercial culture medium supplemented with olive water (Piscitelli et al., 2017). The lipase of *Schizophyllum commune* ISTL04 grown on *Leucaena leucocephala* seeds increased activity up to 60 °C (Singh et al., 2014).

The effect of three different agricultural residues (rice husk, cottonseed cake, and red gram husk) in solid-state fermentation of *Aspergillus niger* MTCC 872 was observed by Nema et al. (2019). In this study, the optimal lipase activity, using mixed substrate, was at 40 °C and the maximum activity of this enzyme was in pH 6. Furthermore, a decline in lipase activity in pH higher than 6 was observed.

Variation of optimal pH between the strains and enzymes (Fig. 3) has also been shown in laccase of *Pleurotus* spp (Patel et al., 2013; Yang et al., 2015). The laccase of *P. ostreatus* cultivated in wheat straw had a higher activity at pH 4.5 (Patel et al., 2013). This value of activity was a little higher than that of our value for PLO 02, PLO 06, and PLE 05 strains. Furthermore, the decrease in laccase activity in high value of pH can be due to the ionization of the amino acids, as for example, aspartate and glutamate (Mishra and Bisaria, 2006).

It was demonstrated the highest LiP activity of *P. ostreatus* and *G. lucidum* in pH in the range of 5–7 (Oliveira et al., 2018; Sivakami et al., 2012). However, the highest MnP activities of *P. ostreatus* were in acidic pH (Yehia, 2014). A similar result was found in the xylanase of *P. eryngii* that had optimal pH in 4 (Altaf et al., 2016).

In *P. ostreatus*, optimal activity for xylanase at pH 6 was demonstrated (Getachew and Melaku, 2016). In *Pleurotus florida* grown on different carbon sources, maximum cellulase activity was observed at pHs in the range of 4–5.5 (Goyal and Soni, 2011).

The lipase of *P. ostreatus* and *P. eryngii* had a reduction in activity in alkaline pH (Fig. 3). A similar result was observed for two lipases of *P. ostreatus* (Piscitelli et al., 2017) and *S. commune* ISTL04 (Singh et al.,

2014).

Our results of thermal stability were similar to the values of laccase *of P. ostreatus* (Patel et al., 2013). However, the LiP of *P. ostreatus* cultivated in commercial medium had an increase in activity up to 144 h (Sivakami et al., 2012). A MnP from *L. edodes* was activity after 60 days of incubation at 4 °C (Grabski et al., 1996). The xylanase of *P. eryngii* maintained activity for 288 h of incubation (Altaf et al., 2016). A cellulase of *L. edodes* had peak activity in the first hours of incubation, decreasing after 24 h (Pereira et al., 2003). These and our results demonstrate the ability of this enzyme under different thermal conditions (Fig. 4).

Our value of K_M was lower than the values observed in laccase of *P. ostreatus* produced on wheat straw (Jamil et al., 2018). According to the authors, this enzyme had K_M of 0.021. Furthermore, values of K_M and Vmax similar to our were observed in laccase of *P. ostreatus* grown on wheat straw that had K_M of 0.033 mM and Vmax of 189 U mL^{-1} (Sadia, 2011).

Our low values of K_M for xylanases are in agreement with those values obtained for microbial enzymes in which have the K_M between 25 and 1700 μ mL^{-1} (Getachew and Melaku, 2016). According to these authors, a xylanase of *P. ostreatus* produced in culture medium with xylan has K_M and Vmax of 0.19 mM and 0.011 mM min^{-1}. Furthermore, the low value of K_M was also obtained in a xylanase of *P. eryngii* grown in starch (Sugumaran et al., 2012) and *P. ostreatus* grown in sucrose that had K_M and Vmax of 0.063 mM and 0.0019 mM min^{-1} (Okereke et al., 2017).

The lipase of *P. ostreatus* PLO 02 had a higher affinity for the substrate than the lipase produced by PLO 06 and PLE 05 (Fig. 5). Furthermore, values of K_M similar to the PLO 02 lipase were observed (Piscitelli et al., 2017). According to these authors the K_M of a lipases produced by *P. ostreatus* varied of 0.035–0.35 mM min^{-1} in function of enzymatic substrate. The lipase is important to use in the industrial of food, cleaning, and cosmetics (Martins et al., 2012). Furthermore, there are few studies on the production of the lipase by white-rot fungi.

The enzymatic extract of *P. ostreatus* and *P. sajor* had also a discoloration rate of 53%–73%, respectively (Silva, 2014). Furthermore, the crude enzymatic extract of *P. ostreatus* grown on sunflower seed husks had also a degradation rate of the Carmine indigo dye between 70% and 100% (Papinutti and Forchiassin, 2010). According to the authors, this degradation rate is dependent on the dye concentration. We also obtained a discoloration rate of 100% in the enzymatic extract of *P. eryngii* PLE 05 (Fig. 6, Table 2). Thus, this high degradation of indigo dye by enzymatic extract of *P. ostreatus* (PLO 02 and PLO 06) and *P. eryngii* PLE 05 observed in this study is important due to potential of used these fungi in the bioremediation of effluent textile.

5. Conclusion

Pleurotus ostreatus (PLO 02 and PLO 06) and *Pleurotus eryngii* (PLE 05) have the ability to produce the lignocellulolytic and lipolytic enzymes using Macaúba residue. The enzymatic extract of these fungi has a high potential to degrade a textile dye. Therefore, our study shows a viable process for bioconversion of Macaúba residue in fungal enzymes (e.g. laccase, lignin peroxidase, manganese peroxidase, cellulase, xylanase, and lipase) with many industrial applications. However, the incubation time for enzyme production was very long. In future studies, the increase in the amount of inoculum, the addition of enzyme inducers, and the reduction of the carbon and nitrogen ratio in the substrate will be tested to reduce this incubation time.

Declaration of competing interest

The authors declare that there is no conflict of interest in the article: Production of fungal enzymes in Macaúba coconut and enzymatic degradation of textile dye.

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