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RESEARCH PAPER

Absorption of polycyclic aromatic hydrocarbons onto depolymerized lignocellulosic wastes by *Streptomyces viridosporus* T7A

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Abstract Preparations containing mainly lignified cell walls are effective adsorbents of hydrophobic carcinogens *in vitro*. Therefore, this innovative study aimed to structurally modify lignocellulosic materials in an enzymatic and environmentally friendly manner to improve the efficiency of sequestering three polycyclic aromatic hydrocarbons (PAHs) by adsorption. Submerged fermentations were performed to produce lignin peroxidase (LiP) in medium supplemented with three different lignocellulosic wastes (0.5% (w/v); açai seed, sugarcane bagasse, and seed coat of Brazil nut), followed by posterior hydrolysis of these three residues and subsequent adsorption to generate hydrolysate wastes. Among the three wastes, the açai seed was the most favourable carbon source for LiP production because a high enzyme activity peak was quickly achieved. Sugarcane bagasse residue was most readily hydrolysed (82.44%), and it had the highest increase in adsorption of the three PAHs tested dissolved in olive oil, from 15.67% of benzo[a]pyrene adsorbed before treatment to 39.45% after treatment. The depolymerisation of wastes may have increased binding sites for olive oil favouring the adsorption of PAHs on hydrolysed residues.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent an important class of carcinogens that are present as contaminants in various agro-food sources (Martí-Cid, Llobet, Castell, & Domingo, 2008). According to the European Food

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Safety Authority (EFSA, 2008), the summed content of chrysene, benzo[a]pyrene (BaP), benz[a]anthracene (BaA) and benzo[b]fluoranthene (BbF) is a suitable indicator of the presence and toxicity of PAHs in food. Different varieties of dietary fibres (DF) have been investigated for the adsorption of carcinogenic compounds. Insoluble DF have been most consistently associated with protection against cancer (Boki, Kadota, Takahashi, & Kitakouji, 2007; Turati, Rossi, Pelucchi, Levi, & La Vecchia, 2015). Lignin has demonstrated the ability to adsorb PAHs (Ferguson & Harris, 1998; Funk, Braune, Grabber, Steinhart, & Bunzel, 2007). Carcinogens may be removed from the body after adsorption onto DF (Ferguson, Roberton, Watson, Triggs, & Harris, 1995).

DF are found in a wide variety of foods, such as fruits, and is particularly abundant in some cereals (Turati et al., 2015); it consist of plant cell walls and are largely composed of lignocellulose. The main components of lignocellulose are cellulose, hemicellulose and lignin (Abdelaziz et al., 2016). Lignin is an aromatic polymer with a large and complex structure that contains many chemical groups, e.g., aromatic rings, phenolic groups, methoxyl groups and aliphatic alcohols. Different modification methods have been proposed to increase the sorption activity of lignin and to offer additional opportunities for its application as an adsorbent (Suhas & Carrott, 2007). Enzymatic or biological treatment is cost-effective and a clean alternative to chemical procedures. The biological route involves a depolymerization, which is the conversion of the polymer into lower molecular weight lignins (mono and oligomers). A central process in the strategy for lignin valorization as adsorbent is the breakdown of the lignin into several smaller fragments that have adsorption capacities higher than the lignin polymer (Abdelaziz et al., 2016).

The enzymatic structural modification of lignocellulosic materials, based on making the functional groups of lignin more available, is an innovative strategy that can render this material more efficient at adsorbing PAHs. Thus, new opportunities could arise for the application of the depolymerized lignocellulosic waste as a food supplement in preventing the development of cancer. *Streptomyces viridosporus* T7A produces a extracellular enzymes lignin peroxidase (LiP) (Gottschalk, Bon, & Nobrega, 2008) that extensively depolymerize lignin (Zeng, Singh, Laskar, & Chen, 2013). The peroxidases produced by Streptomyces strains are induced by lignin (Ramachandra, Crawford, & Pometto, 1987). The LiP produced by *S. viridosporus* T7A in lignocelluloses substrate have been identified and characterized since 1988 (Ramachandra, Crawford, & Hertel, 1988; Gonzalo, Colpa, Habib, & Fraaije, 2016).

The objective of this study was to increase the adsorption capacity of PAHs onto wastes after the enzymatic depolymerization of lignin. For this purpose, the production of LiP by *S. viridosporus* T7A was studied in medium supplemented with three different amazon lignocellulosic wastes, açai (*Euterpe oleracea*) seed (AS), sugarcane (*Saccharum* spp.) bagasse (SB), and seed coat of Brazil nut (BN) (*Bertholletia excelsa*), to reduce the cost of enzyme production. After the subsequent hydrolysis of these three wastes by LiP, the adsorption of three common PAHs onto the hydrolysed materials was tested for the first time.

Materials and methods

Lignocellulosic wastes

The selection of the agro-industrial wastes AS, SB and BN was based on their lignin content; these wastes were collected in Belém (Amazonia, Brazil). The wastes were dried, ground in a Wiley mill (100/200 mesh), and then sequentially extracted by the neutral detergent fibre (NDF) method, with the addition of heat-stable alpha-amylase (Sigma–Aldrich, St. Louis, USA). The residues were then air dried. Plant wastes were characterized based on their DF content (cellulose, hemicellulose and lignin) prior to enzymatic hydrolysis. Acid detergent fibre (ADF), acid detergent lignin (ADL) and NDF were determined by the Van Soest and Wine (1963) method. Hemicellulose was estimated as the difference between NDF and ADF; cellulose, the difference between ADF and ADL; and lignin, the difference between ADL and ash content.

Microorganism and inoculum culture

S. viridosporus T7A (ATCC 39115) was purchased from Colección Española de Cultivos Tipo (CECT) and imported by the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI)-UNICAMP. The sporulating cultures were obtained by growing the microorganism at 37 °C for 6–8 days on yeast extract–malt extract–dextrose agar. Spores of the stock culture of *S. viridosporus* T7A were activated in basal medium composed of 6.5 g/L yeast extract (Difco Laboratories, Detroit, USA), mineral salts and trace metal stock solution (Zerbini, Oliveira, & Bon, 1999). The preculture was prepared in 500 mL shaker flasks containing 100 mL of basal growth medium that was inoculated with 2.0 mL of a spore suspension at a concentration of 3.0×10^8 spores/mL. After 48 h of activation at 37 °C and 125 rpm, this preculture was inoculated in the fermentation medium.

Fermentations

Laboratory-scale

To determine which substrate was the best for inducing enzyme activity, batch fermentations were performed in duplicate in 500 mL shaker flasks with 100 mL of basal growth medium supplemented with the following substrates: (i) milled lignocelluloses extracted by the NDF method (100/200 mesh) at a concentration of 0.5% (w/v) each; and (ii) 0.5% (w/v) larchwood xylan (LX) (Sigma–Aldrich, St. Louis, USA), which served as a reference substrate. The flasks were inoculated with 15 mL of preculture and incubated at 37 °C and 125 rpm for 80 h. Aliquots were taken at different time intervals and centrifuged at 4000 rpm for 10 min. The cell mass was used to evaluate cell growth, and pH and LiP activity measurements were obtained from the supernatant. Cell growth was estimated based on total cellular protein from a calibration curve constructed using bovine serum albumin and was expressed as grams of cellular protein per litre of culture (Zeng, Zhao, et al., 2013).

Bioreactor

LiP was produced in a LiFlus GX bioreactor (Biotron, Seoul, Korea) containing 1.5 L of basal medium supplemented with 0.5% (w/v) lignocellulose AS and inoculated with 15% (v/v) of preculture at an initial pH of 7.0. The fermentation was conducted at 37 °C for 48 h under agitation (400 rpm) with an airflow rate of 1.0 L air/min. Then, the fermented solution was filtered through a 0.22 μm filter, and this crude enzyme extract was stored at 4 °C.

Lignin peroxidase activity

The enzymatic assay for determining LiP activity involves the oxidation of the substrate 3,4-dihydroxyphenylalanine (L-DOPA; Sigma–Aldrich, St. Louis, USA); this assay was performed according to the optimized method (Ramachandra et al., 1987). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.0), 7.5 mM L-DOPA, 100 μL of crude enzyme extract and 4 mM hydrogen peroxide in a final volume of 1 mL. The increasing absorbance was monitored at 470 nm and 37 °C for 6 min in a Microplate Spectrophotometer (BioTek™, Winooski, USA). The blank control lacked enzyme. One unit (U) of activity represented the amount of enzyme required to increase the absorbance by one unit in one minute.

Hydrolysis of lignocellulosic wastes

Fluorescence assay

Lignin breakdown was monitored by a spectrophotometric assay (Multi-Mode Microplate Reader, Synergy 2 BioTek™, Winooski, USA). The assay involves the covalent attachment of fluorescein to lignocelluloses, which elicits a time-dependent change in fluorescence after the breakdown of lignin. The fluorescent lignocelluloses were prepared and the fluorescence assay was performed as described previously (Ahmad et al., 2010), with some modifications. Briefly, a stock solution of fluorescent lignocelluloses was diluted in 4.5 mL of 0.1 M potassium phosphate buffer, pH 7.0. Measurements (λex 485 nm, λem 528 nm) were taken each minute for 60 min. Each assay was performed in triplicate, and controls were included wherein the lignocellulose solution was replaced with buffer. The fluorescence data from the controls (lacking crude enzyme extract) were subtracted from those for the experimental samples.

Evaluation of hydrolysis by HPLC

The enzymatic hydrolysis assay was performed with lignocellulose of AS, SB, and BN (200 mg); hydrogen peroxide at a final concentration of 4 mM; 32 mL of 0.1 M potassium phosphate buffer pH 7.0; and 15% (v/v) crude enzyme extract in a final volume of 40 mL. After hydrolysis for 30 min at 37 °C and magnetic stirring, the reaction mixture was centrifuged at 8000 rpm for 5 min at 4 °C. Lignin extraction from 10 mg of lignocellulose was performed thrice with ethyl acetate containing 0.1% (v/v) formic acid. The extracts were evaporated at 45 °C under vacuum before resuspension in a small volume (200 μL) of acetonitrile with 0.1% (v/v) formic acid. The degradation of the aromatic rings of lignin by lignin peroxidase was confirmed by HPLC analysis at 280 nm (Chandra et al., 2007). The samples were analysed using a Waters 2695 Separation Module (Waters, Milford, USA) equipped with an autoinjector, a 2996 photodiode array detector and Empower software. Samples (20 μL) were injected and then eluted with an isocratic mobile phase of acetonitrile/water (70:30 ratio) at a rate of 0.8 mL/min. An X-Bridge C18 (5 μm, 4.6 × 250 mm) column (Waters) and a 4.6 × 20 mm guard column were used for separation at 30 °C.

Chemical hydrolysis assay

Chemical hydrolysis with 5% (w/v) NaOH was performed at 85 °C for 60 min with a 10% (w/v) ratio of lignocellulose/solution (Gupta & Lee, 2010) for comparison with LiP enzymatic hydrolysis. The hydrolysis data were tested for significant differences by analysis of variance (ANOVA) and Tukey's test using Statistica for Windows (StatSoft). All conclusions were based on a minimum level of significance of 5% ($p < 0.05$).

Polycyclic aromatic hydrocarbon adsorption assays

BaP, BaA and BbF were incubated with hydrolysed and non-hydrolysed wastes in a system that simulated the conditions of human digestion, including the formation of a microemulsion with bile salts. An emulsifier solution was prepared by adding sodium dodecyl sulphate (10 mM) and Tween 20 (1%; wt/v) to deionized water and vortexing the resulting mixture until complete dissolution was achieved. PAH solutions (BaP, BaA and BbF) were prepared in olive oil immediately before placing 0.15 mL aliquots into eppendorf microtubes and adding 0.60 mL of the emulsifier solution; the mixture was then vortexed for 30 s to form the microemulsion. Then, 10 mg of hydrolysed lignocellulose (AS, SB, or BN) was weighed, and 0.75 mL of phosphate-buffered saline (PBS) (10 mM, pH 6.0, containing 130 mM sodium chloride) was added to each microtube, for a total volume of 1.5 mL. The PAHs were used as 10% (v/v) solutions in olive oil. The start carcinogen concentration was 15 μg/mL. The amount of PAHs added in the experiment was based on the dietary intake of an adult (12 μg/day), as reported (Martí-Cid et al., 2008).

The tubes were mixed using a shaker and incubated at 36.5 °C and 120 rpm for 4 h before centrifugation at 6000 rpm for 15 min. PAHs adsorbed onto lignocellulosic wastes were recovered by three extractions with 1 mL of hexane and 30 min of sonication for each extraction. The three extracts were combined, evaporated at 45 °C under vacuum, and resuspended by sonication in 1.5 mL of methanol for 30 min. Controls with non-hydrolysed lignocellulose and without lignocellulose were also evaluated.

The HPLC assay for PAH elution and quantification was performed using the same system as previously described. Twenty microlitres of each methanolic extract was injected and then eluted with an isocratic mobile phase of acetonitrile/water (85:15, v/v) at a flow rate of 1.5 mL/min. Samples and mobile phases were filtered through 0.22 μm Millipore filters, type GV (Millipore, Massachusetts, USA),

prior to HPLC injection. To quantify PAHs, calibration curves of the compounds were generated at five concentrations (0.05–20 μg/mL), and each one was injected in triplicate. The wavelengths were 286, 260 and 295 nm for BaA, BbF, and BaP, respectively. The identification of the peaks was confirmed by comparing the individual retention times to the respective standards. The compound concentration was expressed in μg/mL.

Results and discussion

Characterization of lignocellulosic wastes

The three lignocellulosic wastes used in this study were characterized to evaluate the content of lignin, cellulose and hemicellulose (Table 1). All residues showed high levels of NDF (>67%). BN had a higher ($p < 0.05$) lignin content (38.27%) than AS (11.22%) and SB (11.60%). AS had the highest ($p < 0.05$) cellulose content (74.43%), followed by SB (52.07%) and BN (25.91%). The hemicellulose content was similar in the three residues, but the levels were two-fold higher in BN ($p < 0.05$) than in the other residues (3.44–4.34%). The cellulose/lignin ratio of BN (0.68) was much lower than that of SB (4.49) and AS (6.63). This ratio can be an important parameter of susceptibility to enzyme production from waste.

The measures of specific surface area, volume and distribution of pores of SB, AS and BN wastes only submitted to drying and grounding are very near to zero (Alamar, 2012). Adsorbents pre-treated through carbonization followed by physical or chemical activation to activating carbon have a very porous structure with a large internal surface area ranging from 500 to 2000 m^2/g and, therefore, good adsorption capacities towards various substances (Suhas & Carrott, 2007). However, the lignocellulose degradation by LiP enzyme does not change significantly the surface area and porosity. Interestingly, the hydrolysis by *S. viridosporus* T7A results in the oxidative depolymerisation of lignin, a number of single-ring aromatic intermediates are released, increasing or enriching its content of phenolic hydroxyls, benzylic protons, and, on a less extent, carboxyl groups. (Zeng, Singh, et al., 2013). Therefore, the functional groups of lignin become more accessible to interactions with the adsorbate.

Fermentation in the submerged state

Ramachandra et al. (1987) reported that *S. viridosporus* T7A peroxidases are induced by the presence of lignin. Therefore, to evaluate the effects of the three lignocellulosic wastes at an initial concentration of 0.5% (w/v) on the production of LiP, submerged fermentations with *S. viridosporus* T7A were performed. Furthermore, larchwood xylan (LX, 0.5% w/v) served as a reference substrate (Ramachandra et al., 1988). Cell growth (Fig. 1A), LiP activity (Fig. 1B), and pH (Fig. 1C) were monitored in the submerged fermentations with the four tested carbon sources. All experimental data are presented as the average of duplicates.

The initial cell growth was higher in LX medium (0.43 g/L; 8 h), indicating that this medium was more readily metabolized than the other carbon sources. The general cellular growth profiles were similar in all media, with an exponential phase, a peak cell concentration after 24 h, and subsequent lysis. The maximum accumulation of biomass occurred in LX (0.56 g/L) and AS (0.55 g/L) media. The BN medium provided the lowest cell growth profile (0.32 g/L; 24 h), corresponding to the lowest LiP activity (98 U/L; 24 h).

In all media, the initial LiP enzyme activity (Fig. 1B) at 4 h was between 20 and 40 U/L, whereas the maximum activity was observed after the exponential phase of cell growth. The highest values were observed after 48 h of fermentation in culture medium supplemented with LX (472 U/L) or AS (285 U/L) and after 56 h in SB medium (204 U/L). Interestingly, BN medium was associated with a lower and more constant enzyme activity: 155 U/L (32 h), 134 U/L (56 h), and 157 U/L (72 h). These kinetics of enzyme production, with a decline only after 80 h, represent an advantage at a larger scale, although BN medium showed less absolute enzymatic activity, most likely due to the lower cell concentration.

Ramachandra et al. (1988) reported that after growth of *S. viridosporus* T7A for 72 h, the LiP specific activity was 0.30, 0.24, and 0.14 U/mg protein in medium supplemented with 0.5% LX, corn stover and oat spelt, respectively. When xylan from oat spelt was used, no increase in specific activity was observed, indicating that the real inducer of LiP is most likely some aromatic impurity (Ramachandra et al., 1988). Among the Amazonian lignocellulosic wastes tested, AS medium provided the best enzyme activity (285 U/L; 48 h), which was higher than that obtained by Zerbini et al. (1999) with *S. viridosporus* T7A (177 U/L; 48 h) but was lower than that reported by Macedo, Gottschalk, and Bon (1999) (350 U/L; 48 h), both of whom used 6.5 g/L glucose as the carbon source. Ramachandra et al. (1987) obtained a maximum LiP activity of 240 U/L in medium with 0.05% (w/v) corn stover lignocellulose and 72 U/L in medium without lignocellulose after 28 h of submerged fermentation with *S. viridosporus* T7A. The LiP activity obtained in this study were higher than that reported by others microorganism such as *S. lavendulae* (26.8 U/L; 120 h) (Jing, 2010), *Trametes hirsute* (12.4 U/L; 10 days) (Krumova et al., 2018) and *Phanerochaete chrysosporium* (62.02 U/L; 7 days) (Zeng, Zhao, et al., 2013).

The AS was intermediate in terms of the highest LiP activity and the cellulose/lignin ratio (6.63). *S. viridosporus* T7A not only degraded lignin, but also the hemicellulose and cellulose structures. Breakdown of β (1–4) linkages and hydrogen bonds take place in cellulose complex (Zeng, Singh, et al., 2013). Therefore, some of the cellulose contained in AS may have been hydrolysed by such cellulases, and smaller sugars that were produced could have been used as a bacterial substrate to produce biomass and increase LiP activity compared to the other residues.

There was an increase in pH during all the fermentations, starting in the range of 7.05–7.09 after 4 h and reaching 8.10–8.49 after 80 h. The enzyme stability was previously studied: LiP activity decreases dramatically at pH values higher than 8.2 (Gottschalk et al., 2008). Compared to other carbon sources, the LX medium caused a smaller variation in pH (from 7.0 to 8.1). This finding seems to be associated with the high enzymatic stability observed in the LX medium; the enzyme production remained higher than that

Table 1 Chemical composition of the lignocellulosic wastes.

Sample	NDF (%)*	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Ratio cellulose/lignin
Açai seed (AS)	89.09 ± 0.98	11.22 ± 0.32	74.43 ± 8.19	3.44 ± 0.09	6.63 ± 0.06
Sugarcane bagasse (SB)	67.88 ± 1.67	11.60 ± 0.28	52.07 ± 1.60	4.34 ± 0.05	4.49 ± 0.07
Seed coat of Brazil nut (BN)	72.17 ± 1.69	38.27 ± 1.52	25.91 ± 0.84	7.99 ± 1.15	0.68 ± 0.04

* Neutral detergent fibre.

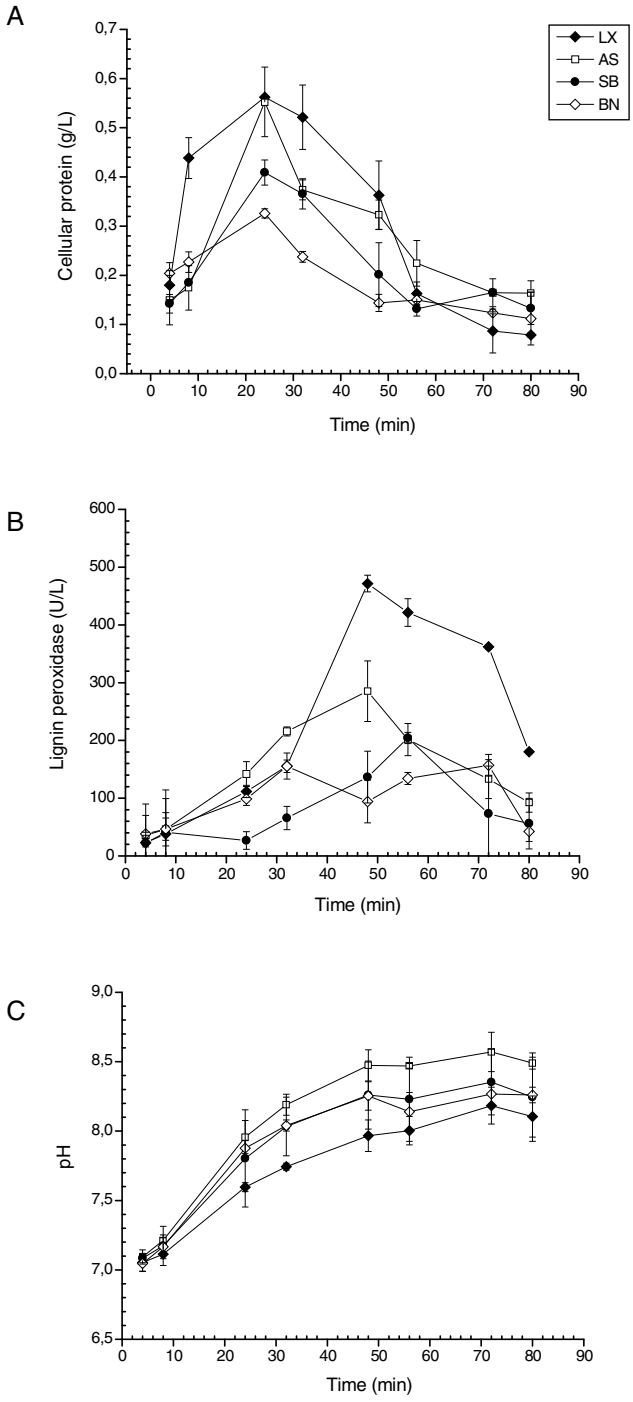

Figure 1 Profiles of *S. viridosporus* T7A grown in an agitated submerged culture at 37 °C and 125 rpm supplemented with 0.5% of waste (w/v). (A) Cellular protein (g/L). (B) LiP activity (U/L). (C) pH variation. (♦) LX: larchwood xylan; (□) AS: açai seed; (●) SB: sugarcane bagasse; and (◊) BN: seed coat of Brazil nut. Error bars indicate the standard deviation ($n = 2$).

in the other media, despite the decline after the peak at 24 h. The AS medium was associated with the largest pH range (from 7.0 to 8.6), which explains the strong decrease in enzyme activity in this medium.

The maximum LiP activity (U/L), maximum biomass accumulation (g/L), productivity (U/L h) and $Y_{LiP/X}$ (U/g) for each tested medium are compared in Table 2. According to the data, AS lignocellulosic waste provides the best nutritional conditions for enzyme production, in addition to greater productivity.

Among the tested lignocellulosic wastes, AS proved to be the most favourable carbon source for LiP production, achieving a high enzyme activity in a short time (48 h). Interestingly, this waste is also the most abundant and the cheapest. The LiP for the hydrolysis of lignocellulosic waste was produced in a bioreactor in basal medium supplemented with 0.5% (w/v) AS. After 48 h of fermentation, the LiP activity in the crude extract was 160 U/L.

Hydrolysis of lignocellulosic wastes

Fluorescence assay

The fluorescence data for the three residues are shown in Fig. 2. The lignin samples were derivatized with excess fluorescein isothiocyanate in alkaline aqueous solution (Ahmad et al., 2010). This test showed a time-dependent increase in fluorescence for all wastes after subtracting the fluorescent lignin controls. The fluorescence increased for up to 30 min, followed by stabilization. Therefore, the hydrolysis time for all wastes was defined as 30 min. Only SB presented a short decrease of 60 fluorescence units (FU) at approximately 16 min, followed by a subsequent increase. The fluorescence was markedly different between the three wastes; the increase in fluorescence after 30 min was 200, 684 and 2104 FU for SB, AS and BN, respectively. Ahmad et al. (2010) used the same assay conditions and a high molecular weight lignin to obtain an increase of approximately 1000 FU after 120 min.

Hydrolysis assay and HPLC monitoring

Table 3 shows the results of the enzymatic and chemical hydrolysis using LiP and 5% NaOH, respectively. The reduction in the peak at 280 nm has been considered lignin biodegradation by enzymatic action (Chandra et al., 2007). Interestingly, there was a significant difference ($p < 0.05$) between the hydrolysis methods for only SB residue; the chemical method resulted in 13.7% more hydrolysis than the enzymatic method. Furthermore, the enzymatic hydrolysis of the SB residue was the highest (82.44%) among the three residues. For the AS and BN coat residues, both methods of hydrolysis achieved similar results, confirming the efficiency of LiP. Yang, Zhou, Lu, Yuan, and Zhao (2012) studied the lignin biodegradation by *Streptomyces* spp. after 12 days of incubation and observed 37.6% of degradation. Fig. 3 shows the lignocellulose chromatograms for the negative controls (non-hydrolysed) and the chemically and enzymatically hydrolysed samples. HPLC analysis of degraded samples after only 30 min of contact with the crude enzyme extract showed a reduction in the peak compared to the negative controls. Additional peaks appeared in the SB and BN samples hydrolysed with LiP (Fig. 3B and C, respectively) compared to the chemically hydrolysed samples.

Several phenolic compounds with simple aromatic rings are released during the degradation of lignin by *Streptomyces* (Abdelaziz et al., 2016). The LiP preparation have ability to oxidize 1,2-diaryl propane and arylglycerol-β-aryl ether containing C_α-carbonyl and C_α-hydroxyl groups. In the presence of hydrogen peroxide, the enzyme preparation catalysed C_α–C_β bond cleavage in the side chains of the diaryl ethers. Based on the model compounds degradation, this bacterial LiP was able to oxidise C_α- as well as to cleave C_β–C_β lignin and lignin substructure model compounds (Gonzalo et al., 2016). Thus, this support the lignin depolymerization and consequently, the adsorption capacity increase onto biodegraded lignin.

The biodegradation process of biomass by *S. viridosporus* T7A is well established. The chemical composition analysis of biodegraded lignocellulose by Fourier transform infrared, pyrolysis gas chromatography/mass spectrometry,

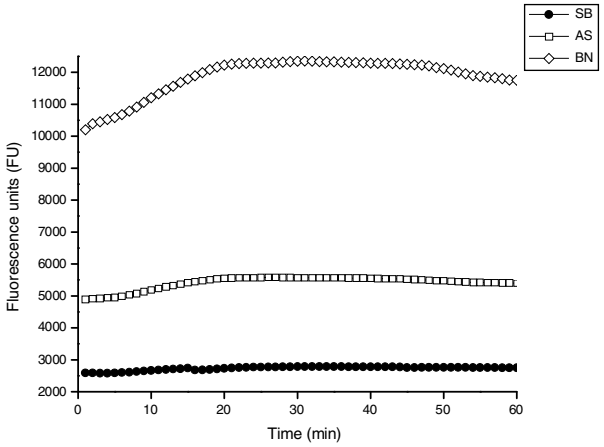

Figure 2 Fluorescence assay data over 60 min for lignocelluloses. (□) AS: açai seed; (●) SB: sugarcane bagasse; and (◊) BN: seed coat of Brazil nut.

Table 2 Parameters of lignin peroxidase production by *S. viridosporus* T7A during submerged fermentation using different lignocellulosic wastes.

Medium	Maximum LiP activity (U/L)	Maximum biomass (g/L)	Productivity (U/[L h])	Maximum $Y_{Lip/X}$ (U/g)
Larchwood xylan (LX)	471.62	0.56	9.83	1310.05
Açai seed (AS)	285.15	0.55	5.94	891.09
Sugarcane bagasse (SB)	204.27	0.41	3.65	1571.08
Seed coat of Brazil nut (BN)	155.40	0.33	4.86	655.69

Table 3 Enzymatic and chemical hydrolysis of three Amazonian lignocellulose wastes by *S. viridosporus* T7A LiP at 37 °C for 30 min or 5% NaOH at 85 °C for 60 min, respectively.

Sample	Enzymatic hydrolysis (%)*	Chemical hydrolysis (%)*
Açai seed (AS)	53.56 ± 2.74	54.03 ± 1.38
Sugarcane bagasse (SB)	82.44 ± 3.53	96.14 ± 0.17
Seed coat of Brazil nut (BN)	53.53 ± 3.97	49.76 ± 2.43

* Mean ± standard deviation, $n = 3$. Significant differences ($p < 0.05$) between enzymatic and chemical hydrolysis were only found in the case of SB.

elemental analyses, functional group analyses, and ^{13}C cross polarization magic angle spinning solid state nuclear magnetic resonance, suggest that degradation involves oxidation of side chains, demethylation of aromatic rings, ring cleavage reactions and cleavage of β-ether linkages within the polymer after partial degradation by *S. viridosporus*. Significant modification of carbonyl and methoxyl groups in the complex lignin structure was also evident (Zeng, Singh, et al., 2013).

Adsorption of polycyclic aromatic hydrocarbons

The method of preparing/activating adsorbents and biosorbents is one of the factors that influences the efficiency of adsorption processes (Suhas & Carrott, 2007). In this work, the structural modification of lignin from three lignocellulosic wastes through LiP produced by *S. viridosporus* T7A was used as a pre-treatment to increase the adsorption capacity of PAHs. The mean adsorption capacity of each adsorbate studied (BaA, BbF, and BaP) on the respective hydrolysed and non-hydrolysed adsorbents is shown in Fig. 4. The objective of this study was to increase the adsorption capacity of wastes after enzymatic lignin hydrolysis; all the hydrolysed wastes increased their adsorption of the three adsorbates compared to the non-hydrolysed wastes.

The adsorption capacities of PAHs on wastes were generally low compared to those in the literature, which range from 8.97% to 24.25% for non-hydrolysed wastes and from 14.12% to 39.45% for hydrolysed wastes. A study reported by Ferguson et al. (1995) using an aqueous system where BaP was dissolved in methanol showed that commercial cork was able to adsorb 90% of BaP. However, unlike those reported in the literature, our adsorptions were performed in aqueous solution with PAHs dissolved in olive oil to simulate the consumption of a normal meal and the conditions of gastrointestinal emulsion (Hollebeeck, Borlon, Schneider, Larondelle, & Rogez, 2013). PAHs are generally present as solute in various types of dietary lipids. Overall, 40% of PAHs remain in the lipid phase (data not shown), indicating that the hydrophobic nature of PAHs dictates a preference for remaining in the lipid phase to migrate to the adsorbent.

Liao, Mab, Chen, and Yang (2015) *studied* binding of BaP from aqueous solutions on midchain fatty acids (C_6–C_{11}) coated magnetic nanoparticles; the adsorption efficiency improved between 60 and 90% with the increase of the chain length of the coatings which suggested that a proper chain length may accommodate BaP and/or provide enough Van der Waals interactions.

The most hydrolysed residue (SB, 86%) increased the percentage of adsorption over 100% for the three PAHs (117.99, 132.38, and 151.76% for BaA, BbF, and BaP, respectively) compared to the non-hydrolysed residue; therefore, after the enzymatic pre-treatment of lignin, SB was the best residue for adsorbing PAHs (Fig. 4). The second best hydrolysed waste for adsorbing PAHs was AS, whose adsorption percentages for BbF and BaP were statistically similar to those of SB, but that for BaA was lower ($p < 0.05$).

Suberized and lignified cell walls of potato peel and commercial cork may play an important role in protecting against colorectal cancer in rats (Ferguson & Harris, 1998). The adsorption of heterocyclic aromatic amines at pH 6.5 to nonlignified control cell walls averaged only 23%, while, the adsorption at pH 6.5 to cell walls containing 8.1% of G-lignin increased to 46% (Funk et al., 2007); highlighting the impact of small amounts of lignin in enhancing adsorption. In addition to lignin, studies have shown that cellulose has ability to adsorb PAHs, but the PAH sorption to cellulose appears to be considerably lower than onto lignin (Joker, 2008; Wang, Yang, Tao, & Xing, 2007). Ferguson et al. (1995) showed that 55% of adsorbed BaP was associated with α-cellulose. The AS and BN residues showed the same percentage of lignin hydrolysis (approximately 50%) but exhibited different levels of cellulose (74.43 and 25.91%, respectively). These data may explain why AS has a higher adsorption capacity that BN. The sorbed PAHs are physically immobilized in the microfibrils of cellulose fiber and that in the neutral pH more PAHs were taken up to microspacings of powdered young barley than in acidic range (Boki et al., 2007).

Since PAHs were dissolved in olive oil, it must be considered that the wastes not only promote the adsorption of PAHs but mainly oil components. These oil components are possibly dragging PAHs molecules during the adsorption process. The binding of a fatty acid to lignocelluloses can occur between the fatty acid carboxylic functional group and the alcohol groups of cellulose, the alcohol groups of lignin, and/or the phenolic group of the lignin (Said, Ludwick, & Aglan, 2009). The depolymerisation of wastes may have increased binding sites for olive oil favoring the increased of PAHs adsorbed on hydrolysed residues.

Various adsorption plant materials have been examined for their oil adsorption capacities. The sugarcane bagasse exhibits great potential for stearic acid sorption capacity (Said, Ludwick, & Aglan, 2009). The adsorption capacity for canola oil at pH 7.6 by walnut shell media was of 60%. The mechanism of oil removal in the case of walnut shell media has been observed to be sorption. Oil is found at the interstices of the finely divided granules of the walnut shell media

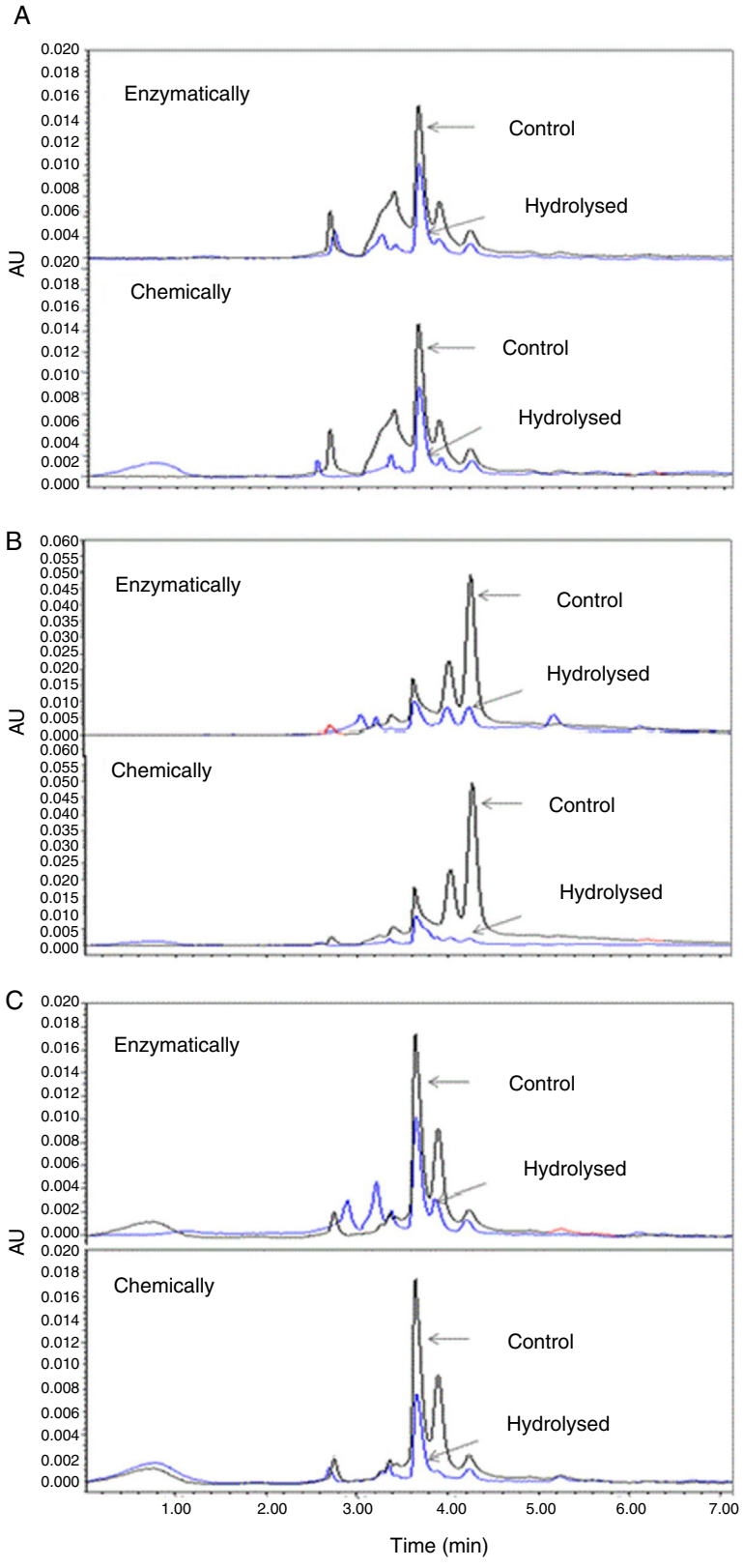

Figure 3 Comparative HPLC chromatograph of control (non-hydrolysed) and hydrolysed lignocelluloses (enzymatic: LiP; chemical: 5% NaOH). Samples: (A) AS: açai seed; (B) SB: sugarcane bagasse; and (C) BN: seed coat of Brazil nut.

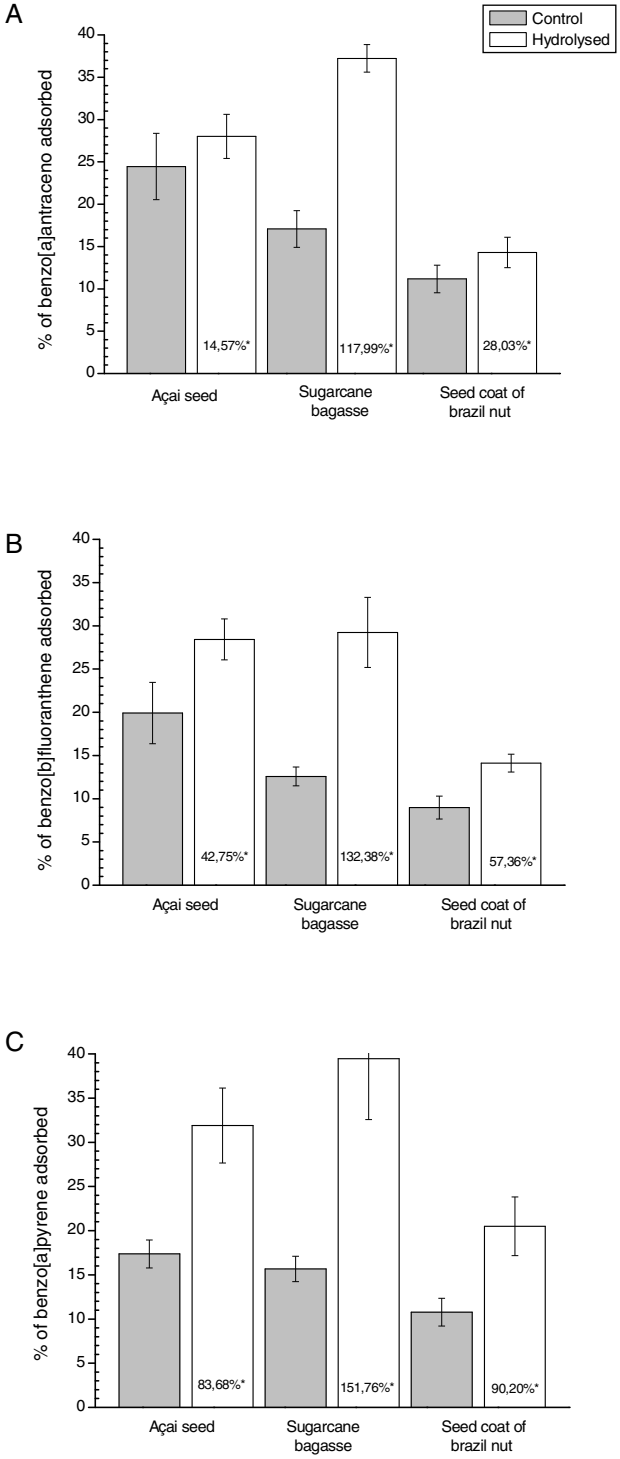

Figure 4 Adsorption (%) of PAHs on non-hydrolysed and enzymatically (LiP) hydrolysed wastes: (A) benz[a]anthracene; (B) benzo[b]fluoranthene; and (C) benzo[a]pyrene. *Percentage increase in the adsorption of hydrolysed waste compared to non-hydrolysed control.

which is observed to be an excellent coalescing material (Srinivasan & Viraraghavan, 2010).

Conclusions

Our results support the innovative use of lignocellulosic wastes as a carbon source for large-scale LiP production because lignocellulosic wastes are an abundant and low-cost raw material compared to other commonly used carbon sources. The ability of the enzymatic hydrolysis of lignin by LiP to increase the adsorption capacity of PAHs was demonstrated. Sugarcane bagasse, the most hydrolysed residue, increased the percentage adsorption of the three PAHs by over 100% for the hydrolysed compared to the non-hydrolysed forms and therefore was the best residue for PAH adsorption after the enzymatic pre-treatment of lignin. The aim of increasing the availability of the functional groups of lignin by enzymatic depolymerization to increase the adsorption capacity of the PAHs onto lignocelluloses was thus reached. The lignocellulosic wastes can potentially be used as a dietary supplement for preventing the development of cancer. The fibres of sugarcane bagasse and açai seeds are already part of the human diet. During açai pulping, part of the seed fibres are added to the juice. The ingestion of these fibers can therefore have no adverse health effects.

Conflicts of interest

The authors declare that no conflicts of interest.

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