Biotransformation of Pequi and Guavira Fruit Wastes via Solid State Bioprocess Using *Pleurotus Sajor-Caju*

Cinthia Aparecida de Andrade Silva, Maria Priscila Franco Lacerda, and Gustavo Graciano Fonseca

***Abstract*—Fungal microorganisms are widely studied in the bioconversion of substrates. Among them, *Pleurotus sajor-caju* is well recognized for its known ability to colonize and degrade wastes through solid state bioprocess (SSB). Fruit residues are inexpensive substrates that present characteristics favorable for microorganisms' colonization. The aim of this work was to investigate the mycelial growth of *P. sajor-caju* on pequi and guavira fruit wastes through SSB, in order to valorize these residues by their biotransformation. Cultivations were carried out with the substrates at pH 5 and with 60% moisture in an incubator at 30 ℃ for 25 days. Microbiological analyzes for fungi and bacteria beyond proximate composition of the substrates were evaluated every 5 days. It was observed that *P. sajor-caju* reached maximum growth at the 15th day in guavira waste and at the 25th day in pequi waste. The protein enrichments obtained were 30.31% and 37.20% for guavira and pequi wastes, respectively. It was concluded that guavira favored microbial growth and protein concentration, presenting a final product very rich in protein with potential application for animal nutrition.**

***Index Terms*—Brazilian savannah, fungi, residues, kinetic.**

I. Introduction

Pleurotus spp are popularly known as oyster mushrooms. They are primary decomposer of wood and vegetable residues. They may occur naturally in tropical and subtropical forests or cultivated [1-2]. The *Pleurotus sajor-caju* species has been widely studied for their ability to colonize and degrade a great variety of lignocellulosic residues [3], being considered a great recycler of these residues through its efficient capacity of biotransformation [4].

In the solid state bioprocess (SSB), which is usually utilized for *P. sajor-caju* production, several steps must be attempted in order to obtain a nutrient rich product. One important aspect to be considered is the choice of a suitable substrate for the proliferation of this fungus, and obtaining a mycelial growth enough to promote the protein enrichment of the substrate of even the fructification on it [5].

The use of fruits by the agroindustry generates waste that may cause environmental problems if badly discarded. These residues can be availed for SSB, adding value to the formed product. The action of fungi on solid waste can turn it into a material rich in proteins though the transformation of the medium composition [6].

Pequi (*Caryocar brasiliense*) and guavira (*Campomanesia pubescens*) are valuable fruits from the Brazilian Savannah. Pequi contains on its pulp a good amount of edible oil and is rich in vitamin A and proteins, making it an important food supplement [7]. Guavira is mostly used fresh by the food industry and as flavoring by the beverage industry due its high acidity, and ascorbic acid, minerals, dietary fiber and monoterpene hydrocarbons contents [8]. On the other hand, their wastes are marginally utilized.

The aim of this work was to evaluate the mycelial growth of *P. sajor-caju* on pequi and guavira fruit wastes through solid state bioprocess, in order to valorize these residues by their biotransformation.

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II. Materials and Methods

A. Microorganism and Maintenance

Pleurotus sajor-caju was maintained in (1) inclined potato dextrose agar (PDA) culture medium immersed in mineral oil, (2) skim milk medium, and (3) lyophilized.

B. Substrates

Pequi (*Caryocar brasiliense*) waste was obtained by mechanical press (Hauber Macanuda, Brazil) to separate the peel from other parts of the fruit. Guavira (*Campomanesia pubescens*) waste, composed by peel and seeds, was extracted using depulper (Hauber Macanuda, Brazil). 60 g of each substrate was conditioned into an Erlenmeyer flask (300 ml) and subsequently autoclaved at 121 ℃ for 15 min. Wherever necessary, these substrates were pH adjusted to 5.0 by the addition of 1M HCl and moisture to 60% by the addition of sterile distilled water.

C. Inoculum (Spawn)

For spawn preparation, wheat grains (precooked for 15 min.), gypsum and $CaCO_3$ were mixed in the proportions of 98.6%, 0.8% and 0.6%, respectively, as defined in preliminary studies, for pH maintenance. The pH was adjusted to 5.0 with addition of 1M HCl and the initial moisture content to 50%. 50g of the mixture was placed into an Erlenmeyer flask (300 ml) and subsequently autoclaved at 121 ℃ for 15 min. Inoculation of the fungus was carried out by the transfer of a 1 cm ²square area of mycelium contained in a PDA petri dish to the Erlenmeyer flask, with the aid of a spatula previously sterilized. The petri dish was previously prepared from the preserved microorganism and maintained for the maximum of 1 month at 4 ℃. The flask was incubated in bacteriological incubator (30 ℃, 168 h) to serve as inoculum on the main cultivations. 10% (w/v) of the main culture substratum weight (6 g) was distributed in 6 Erlenmeyer flasks to propagate growth, for each substrate (pequi and guavira), totaling 12 flasks.

D. Culture

The Erlenmeyer flasks were kept in a bacteriological incubator at (30 ℃, 25 days) in the absence of light for the mycelium development. Every 5 days was taken a flask of each substrate, which resulted in 6-point kinetic experiments. Time zero resulted from medium just after inoculation. Samples were homogenized and used to determine the proximal composition and the microbiological content.

E. Microbiological Analysis

For microbiological analysis, a representative product sample of 25 g was transferred to a Stomacher-bag and homogenized for 60 s in a stomacher with 225 g chilled saline peptone diluent (0.85% NaCl with 0.1% petone). Further, appropriate 10-fold dilution of the homogenate was made with saline peptone diluent. For each dilution blank, two replica were prepared. 0.1 ml from each appropriate dilution step was spread on the surface of solid media into Petri dishes. The counting plate analyses were followed by classical methodology. Fungi were determined by using potato dextrose agar in surface (25 ℃, 120 h). Mesophilic bacteria were determined by using plate counting agar (PCA) in deep (35 ℃, 48 h) and psychrotrophic bacteria using PCA in surface (7 ℃, 240 h) [9] to monitor possible contamination during experiments.

F. Proximate Composition

Moisture, crude protein, crude fat, crude fiber and crude ash contents were determined in triplicate according to the methods described by AOAC [10]. Moisture was determined by the oven drying method at 105 ℃ until constant weight (method 950.46), protein by the Kjeldhal method (method 928.08) using a 6.25 factor to convert the nitrogen content into crude protein, fat by the Soxhlet method (method 960.39), crude fiber content by the gravimetric method using a fiber determiner (method 978.10), and ash by using the muffle oven technique (method 920.153). Carbohydrates were calculated by difference according to Equation 1 (adapted from [11]).

$$\%CHO = 100 - (\%ASH + \%LIP + \%PRO + \%FIB) \quad (1)$$

where: CHO = carbohydrates; ASH = ashes; LIP = lipids; PRO = proteins; FIB = fibers. Results were expressed as % (g/100g) by the mean and standard deviation.

G. Protein Enrichment (PE)

Protein enrichment (PE) or protein variation was calculated by the percentage difference between concentration of proteins presented in cultivated medium (higher protein concentration) and treated substratum (after inoculation) according to Eq. 2 [12].

$$PE(\%) = \left(\frac{\text{final protein content (\%)}}{\text{inicial protein content (\%)}} \times 100 \right) - 100 \quad (2)$$

H. Enzyme Activity Analysis

Enzymatic analyses for xylanase, CMCase, amylase and lipase were carried out.

For the enzyme extraction, 50 ml of distilled water were added to Erlenmeyer flasks containing 5g of cultivated mediums. The microorganisms were placed on an orbital shaker (130 rpm, 35 ℃, 30 min), filtered through synthetic filters, and then centrifuged (3,000 rpm, 5 min), yielding the crude enzyme extract for the determination of enzyme activity (adapted from [13]). The supernatant crude enzyme extract was stored in polypropylene tubes with screw cap.

The activities of xylanase, amylase and CMCase were determined on 0.1 ml of the enzyme filtrate, 0.9 mL of 0.2 M acetate buffer (pH 5.0) and 1% substrate (xylan, corn starch or CMC, respectively). These activities were measured by the amount of reducing sugar present in the final enzymatic reaction, quantified by the DNS method [14]. One unit of enzyme activity was defined as the amount of enzyme able of releasing 1 μmol of the respective product per minute of reaction.

To start the reaction for lipase activity analysis, 0.1 ml of crude enzyme extract was added to 0.9 ml of substrate solution containing 3mg of *p*-nitrophenyl palmitate (*p*NPP) dissolved in 1 ml of isopropanol and 9 ml of the following solution: 2 g of Triton X-100 and 0.5 g of arabic gum in 450 ml of 0.05 M phosphate buffer pH 7.0 [15]. The mixture was incubated in a water bath (37 ℃, 20 min), then placed in an ice bath and analyzed in a spectrophotometer at 410 nm. 0.1 ml of inactivated enzyme was utilized as the control solution [16]. One unit of lipase activity was defined as the amount of enzyme able to release 1 μmol of *p*NP per minute of reaction.

III. Results and Discussion

A. Microbiological Determinations of Pleurotus Sajor-caju

The mycelial growth of *P. sajor-caju* in the substrates pequi and guavira can be observed in Fig. 1. This species is well recognized for its great capacity of degrading waste materials, which was also confirmed by the results obtained here.

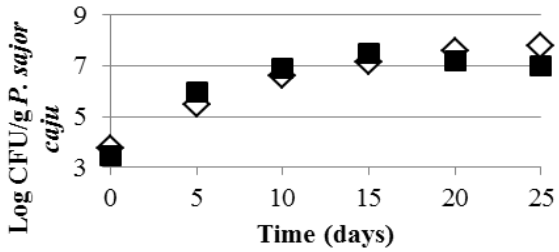

Fig. 1. Growth kinetics of *P. sajor-caju* in pequi (◊) and guavira (■) wastes substrates.

After 5 days, *P. sajor-caju* had penetrated homogeneously the two substrates. The Genus Pleurotus was already described by its rapid colonization of fruit substrates, e.g. apple [17-19]. In guavira waste substrate, *P. sajor-caju* reached its maximum growth in the 15th day. After that, it started declining. In pequi waste substrate the maximum growth was found in the 25th day. In this case, there was no growth decline, suggesting that it could still improve cell counting if the experiment had not been concluded (Fig. 1).

Bacterial growth was not detected for none of the samples, ensuring that there was no contamination during the experiment and that the thermal treatment of substrates (121 ℃ for 15 min.) was enough to guarantee only the presence of the inoculated *P. sajor-caju*.

B. Proximate Composition and Protein Enrichment

The proximate composition of pequi and guavira wastes substrates inoculated with *P. sajor-caju* over 25 days is shown in Tables I and II. The protein content has augmented in the both substrates. In pequi waste, protein content increased from 9.89% to 12.89% after 25 days, which represents an increment of 3.00% (Table I). In guavira waste, protein content increased from 8.34% to 12.72% (4.38%) after 10 days, and then decreased to 11.44% at the 25th day. Hay inoculated with *P. ostreatus* presented a protein increase of 2.1% after 35 days [20].

Table III shows the protein enrichments for guavira and pequi wastes, which improved 30.31% and 37.20%, respectively. Considering the maximum protein content obtained instead of the final protein content, the protein enrichment of guavira waste reached 52.52% after only 10 days. A maximum protein enrichment of 52.07% was observed after 10 days for *P. sajor-caju* cultivated in fresh deproteinized cheese whey, with protein increasing from 25.81% to 39.25% [21]. The reduction might be explained by a limitation in the nitrogen content. The values of protein enrichment obtained (Table 3) are in good agreement with results from others authors, with protein enrichment ranging from 15 to 65% [22-24].

TABLE I: PROXIMATE COMPOSITION OF PEQUI WASTE SUBSTRATE INOCULATED WITH P. SAJOR-CAJU OVER 25 DAYS.

Time (days)	Proteins (%)	Lipids (%)	Ash (%)	Fiber (%)	Carbohydrates (%)
0	9.89 ± 0.87	0.70 ± 0.01	1.38 ± 0.68	20.47 ± 0.60	67.56
5	9.98 ± 0.97	0.88 ± 0.39	1.43 ± 0.35	20.90 ± 0.03	66.81
10	10.73 ± 0.26	1.77 ± 0.74	1.94 ± 0.21	22.30 ± 0.64	63.26
15	10.56 ± 0.16	3.43 ± 0.58	2.10 ± 0.10	21.31 ± 0.69	62.60
20	10.77 ± 0.92	1.58 ± 0.01	2.40 ± 0.81	23.80 ± 0.03	61.45
25	12.89 ± 0.11	0.73 ± 0.04	2.19 ± 0.85	27.07 ± 0.25	57.12

Calculated on dry matter basis. Determinations were performed at least in triplicate.

TABLE II: PROXIMATE COMPOSITION OF GUAVIRA WASTE SUBSTRATE INOCULATED WITH P. SAJOR-CAJU OVER 25 DAYS.

Time (days)	Proteins (%)	Lipids (%)	Ash (%)	Fiber (%)	Carbohydrates (%)
0	8.34 ± 0.46	1.68 ± 0.80	1.35 ± 0.10	52.94 ± 0.07	35.69
5	10.70 ± 0.85	2.66 ± 0.08	1.56 ± 0.07	54.28 ± 0.04	30.80
10	12.72 ± 0.12	3.58 ± 0.12	1.34 ± 0.24	55.07 ± 0.16	27.29
15	11.51 ± 0.19	3.62 ± 0.16	2.28 ± 0.13	57.28 ± 0.38	25.31
20	11.23 ± 0.64	4.13 ± 0.28	2.58 ± 0.04	59.67 ± 0.04	22.39
25	11.44 ± 0.41	2.39 ± 0.15	1.50 ± 0.03	61.85 ± 0.06	22.82

Calculated on dry matter basis. Determinations were performed at least in triplicate.

TABLE III. PROTEIN ENRICHMENT AFTER *P. SAJOR-CAJU* CULTIVATIONS IN PEQUI AND GUAVIRA WASTES SUBSTRATES

Substrate	Protein enrichment (%)
Pequi waste	30.31
Guavira waste	37.20

Calculated from protein results of Tables I and II, using Eq. II.

In pequi waste substrate, the lipids content increased 2.73% (from 0.70% to 1.58%) until the 15th day (Table II). The lipids content increase was higher for guavira waste substrate, reaching a variation of 2.45% (from 1.68% to 4.13%) until the 20th day (Table III). After that, the lipids content decreased for both substrates. The lipid increase in media may have occurred as a consequence of the enzyme production by hyphaes during the mycelial growth. These lipids are destined for construction of the mushroom cell wall and for income induction. With growth, the lipids were excreted and maintained in the medium, raising its percentage [12]. As there was no fructification, lipids start to be consumed when cell growth reach or approximate to the stationary phase, as observed in Fig. 1.

In general, crude fiber and ash increased with time. *Pleurotus* ssp. mycelium can grow on lignocellulosic wastes mainly because it is able to produce cellulolytic and ligninolytic enzymes which allow them to degrade complex carbohydrates, increasing fiber content [12]. The concentration of minerals could be correlated with other mechanisms, such as nitrogen fixation [25]. Ash increments of 5.86% and 5.36% were reported for *P. ostreatus* and *P. sajor-caju*, respectively, when cultivated in rice straw based media [26].

Carbohydrates (quantified by difference) were the main compounds utilized for growth and so converted to other composites (Tables I and II). However, most of the reduction was probably due the fiber content increase.

C. Enzyme Activity

Fig. 2 and Fig. 3 show the enzyme activities for xylanase enzyme, lipase, CMCase and amylase in pequi and guavira waste substrates, respectively.

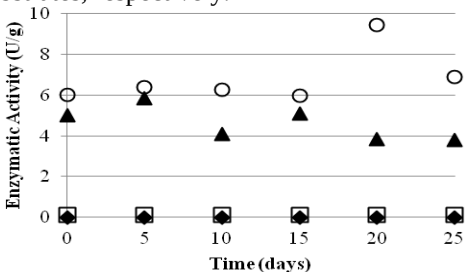

Fig. 2. Enzyme activity of amylase (::), CMCase (▲), lipase (□) e xylanase (♦) of pequi waste substrate cultivated with *P. sajor-caju* in function of the cultivation time.

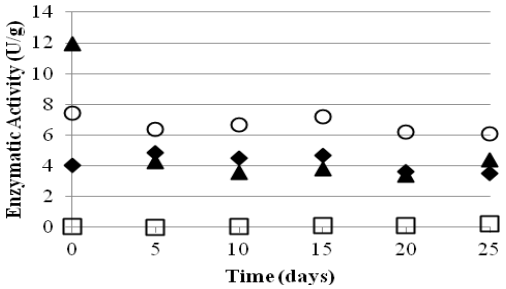

Fig. 3. Enzyme activity of amylase (::), CMCase (▲), lipase (□) e xylanase (♦) of guavira waste substrate cultivated with *P. sajor-caju* in function of the cultivation time.

Amylase activity was observed for both pequi and guavira waste substrates after cultivation. For pequi waste substrate there was a higher production (9.4 U / g) after 20 days of cultivation. For guavira waste substrate, this enzyme activity remained stable during cultivation.

There was no lipolytic activity on the analyzed substrates. The lack of lipolytic activity can be linked to the non-supplementation with any kind of fats summed to the fact that that the initial lipid content was very slow (TABLE I and II). Oil content is an important parameter for lipases synthesis because the same behaves like an inductor for the lipase production [27].

Xylanase also showed no activity on the pequi waste substrate, but showed activity in substrate composed with guavira residues. The activity was detected up to 15 days of cultivation (4.67 U / g) and then decreased after this time. *P. sajor-caju*, when grew on cane sugar bagasse produced 0.11 U / mL (1.1 U / g) of xylanase [28].

CMCase showed activity on both substrates, in the range of 3 to 4 U / g. In the pequi waste substrate the activity diminished after 10 days of cultivation. For the guavira substrate the activity decreased from the start of cultivation. These results are superior to that described for *P. sajor-caju* in sugar cane bagasse (0.8 U / g) [28].

Substrates as bagasse do not possess large amounts of macro and micronutrients, so the enzymatic productions are also not very high [29].

IV. Conclusion

Two agroindustrial fruit (pequi and guavira) wastes were successfully utilized as substrate for *P. sajor-caju* growth during solid state bioprocess. Both residues presented protein enrichment after 25 days of cultivation. Guavira favored microbial growth and protein concentration. The obtained product was very rich in protein and presents potential application for animal nutrition. The production of enzymes was also possible in these substrates, being more expressive for amylase production in pequi.

References

[1] R. Maziero, "Substratos alternativos para o cultivo de pleurotus spp," Presented at the *Instituto de biociências, Universidade São Paulo*, São Paulo, pp.136, 1990.

[2] J. Z. Wu, P. C. K. Cheung, K. H. Wong, and N. L. Huang, "Studies on submerged fermentation of pleurotus tuber-regium (Fr.) singer - Part 2: effect of carbon-to-nitrogen ratio of the culture medium on the content and composition of the mycelial dietary fiber," *Food Chemistry*, vol. 85, pp. 101-105, 2004.

[3] E. Espósito and J. L. Azevedo, "Fungos comestíveis," *Fungos Uma Introdução à Biologia, Bioquímica e Biotecnologia*, 1st ed, A. F. Eira: Educs, vol. 12, pp. 510, 2004.

[4] R. H. Zhang, X. Li, and J. G. Fadel, "Oyster mushroom cultivation with rice and wheat straw," *Bioresource Technology*, vol. 82, no. 3, pp. 277-284, May 2002.

[5] L. P. Donini, E. Bernardi, E. Minotto, and J. S. D. Nascimento, "Desenvolvimento in vitro de pleurotus spp. sob a influência de diferentes substratos e dextrose," *Arquivos do Instituto Biológico*, vol. 72, no. 3, pp. 331-338, 2005.

[6] C. M. Silveira and E. B. Furlong, "Caracterização de compostos nitrogenados presentes em farelos fermentados em estado sólido," *Ciência e Tecnolologia de Alimentos*, vol. 27, no. 4, pp. 805-811, 2007.

[7] S. D. Silva, J. D. A. Brait, F. P. Faria, S. M. Silva, S. L. Oliveira, P. F. Braga, and F. M. S. M. D. Silva, "Chemical characteristics of pequi fruits (caryocar brasiliense camb.) Native of three municipalities in the State of Goiás – Brazil," *Ciência e Tecnologia de Alimentos*, vol. 29, no. 4, pp. 771-777, 2009.

[8] M. I. Vallilo, L. C. A. Lamardo, M. L. Gaberlotti, E. D. Oliveira, and P. R. H. Moreno, "Composição química dos frutos de campomanesia adamantium (Cambessédes)," *O. Berg. Ciência e Tecnologia de Alimentos*, vol. 26, no. 4, 2006.

[9] N. Silva, V. C. A. Junqueira, and N. F. A Silveira, "Manual de Métodos de Análise Microbiológica de Alimentos, Brasil," *SP: Livraria Varela*, 1997.

[10] *AOAC - Association of Official Analytical Chemists, Official methods of analysis*, 16 ºed, Washington: AOAC, 1995.

[11] B. S. Rodrigues, "Resíduos da agroindústria como fonte de fibras para a elaboração de pães integrais," *MSc. Dissertation*, University of São Paulo, Brazil, 2010.

[12] G. G. Fonseca, E. A. Gandra, L. F. Sclowitz, A. P. C. Antunes, and J. Costa, "Protein enrichment and digestibility of soft rush (Juncus effusus) and rice residues using edible mushrooms Pleurotus ostreatus and Pleurotus sajor-caju," *World Journal of Microbiology and Biotechnology*, vol. 25, pp. 449-456, 2009.

[13] R. R. S. R. Leite, D. A. Bocchini, E. S. Martins, D. D. Silva, E. Gomes, and R. D. Silva, "Production of cellulolytic and hemicellulolytic enzimes from Aureobasidium pulluans on solid state fermentation," *Applied Biochemistry and Biotechnology*, vol. 137, pp. 281-288, 2007.

[14] G. L. Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugars," *Analytical Chemistry*, vol. 31, pp. 426-428, 1959.

[15] W. O. B. Silva, S. Mitidieri, A. Schrank, and M. H. Vainstein, "Production and extraction of an extracellular lipase from the entomopathogenic fungus metarhizium anisopliae," *Process Biochemistry*, vol. 40, pp. 321-326, 2005.

[16] Z. Liu, C. Zhenming, L. Wang, and L. Jing, "Production, purification and characterization of an extracellular lipase from aureobasidium pullulans HN2.3 with potential application for the hydrolysis of edible oils," *Biochemical Engineering Journal*, vol. 40, pp. 445-451, 2008.

[17] R. C. Upadhyay and H. S. Sohi, "Apple pomace - a good substrate for the cultivation of edible mushrooms," *Current Science*, vol. 59, pp. 1189-1190, 1988.

[18] J. J. Worrall and C. S.Yang, "Shiitake and oyster mushroom production on apple pomace and sawdust," *Hortscience*, vol. 27, pp.1131-1133, 1992.

[19] P. Bustamante, J. Ramos, V. Zuniga, H. S. Sabharwal, and R. A. Young, "Biomechanical pulping of bagasse with the white rot fungi ceriporiopsis subvermispora and pleurotus ostreatus," *Tappi Journal*, vol.82, no. 6, pp. 123-128, 1999.

[20] P. Schmidt, F. S. Wechsler, J. S. D. Nascimento, and F. M. Vargas Junior, "Tratamento do feno de braquiária pelo fungo pleurotus ostreatus," *Revista Brasileira de Zootecnia*, vol. 32, no. 6, pp. 1866-1871, 2003.

[21] R. Mukhopadhyay, B. P. Chatterjee, and A. K. Guha, "Biochemical changes during fermentation of edible mushroom pleurotus sajor-caju in whey," *Process Biochemistry*, vol. 38, pp. 723-725, 2002.

[22] M. Adamović, G. Grubić, I. Milenković, R. Jovanović, R. Protić, L. Sretenović, and L. Stoićević, "The biodegradation of wheat straw by pleurotus ostreatus mushrooms and its use in cattle feeding," *Animal Feeding Science Technology*, vol. 71, pp. 357-362, 1998.

[23] O. A. Abu, O. O. Tewe, D. M. Losel, and A. A. Onifade, "Changes in lipid, fatty acids and protein composition of sweet potato (ipomoea batatas) after solid-state fungal fermentation," *Bioresource Technology*, vol. 72, pp.189-192, 2000.

[24] D. Wang, A. Sakoda, and M. Suzuki, "Biological efficiency and nutritional value of pleurotus ostreatus cultivated on spent beer grain," *Bioresource Technology*, vol. 78, pp. 293-300, 2001.

[25] E. M. Moda, J. Horii, and M. H. F. Spoto, "Edible mushroom pleurotus sajor-caju production on washed and supplemented sugarcane bagasse," *Scientia Agricola (Piracicaba, Braz.)*, vol. 62, pp. 127-132, 2005.

[26] M. Bonatti, P. Karnopp, H. M. Soares, and S. A. Furlan. "Evaluation of pleurotus ostreatus and pleurotus sajor-caju nutritional characteristics when cultivated in different lignocellulosic wastes," *Food Chemistry*, vol. 88, pp.425-428, 2004.

[27] P. V. Rao, K. Jayaraman, and C. M. Lakshmanan, "Production of lipase by Candida rugosa in solid state fermentation. 1: determination of significant process variables," *Process Biochemistry*, vol. 28, pp. 385-389, 1993.

[28] C. R. Menezes, I. S. Silva, and L. R. Durrant, "Bagaço de cana: fonte para produção de enzimas ligninocelulolíticas," *Estudos Tecnológicos*, vol. 5, pp. 68-78, 2009.
[29] E. S. Dias, E. M. S. Koshikumo, R. F. Schwan, and R. Silva, "Cultivo do cogumelo pleurotus sajor-caju em diferentes resíduos agrícolas," *Ciência e Agrotecnologia*, vol. 27, pp. 1363-1369, 2003.

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