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Bioconversion of hydrolyzed cashew peduncle bagasse for ethanol and xylitol production

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ABSTRACT

The agro-industrial waste deposited in the environment causes problems in nature that can be solved with the use and generation of bioproducts. Thus, the objective was to study the lignocellulosic fraction of cashew (*Anacardium occidentale* L.) peduncle bagasse and fermentation on large scale (8-16 times) using the strain *Candida guilliermondii* CCT-3544 as production agent. According to the obtained results, it can be noted that the dry cashew peduncle bagasse has 21.45% of cellulose, 10.96% of hemicellulose and 35.39% of lignin. During fermentation, *C. guilliermondii* 3544-CAT was able to grow on medium containing hydrolysate, with maximum cell growth concentration of 3.5 g L⁻¹. The behavior of the sugars in the fermentation process was similar in the different variables, with maximum production of ethanol and xylitol at 48 h of fermentation.

Palavras-chave:

Anacardium occidentale L.
Candida guilliermondii
hemicelulose
bioprodução

Bioconversão do hidrolisado do bagaço do pedúnculo do caju para produção de etanol e xilitol

RESUMO

Os resíduos agroindustriais depositados no meio ambiente favorecem problemas na natureza, passíveis de serem solucionados com o aproveitamento e geração de bioprodutos. Assim, objetivou-se estudar a fração lignocelulósica do bagaço do pedúnculo do caju (*Anacardium occidentale* L.) e a fermentação em escala ampliada (8 a 16 vezes) utilizando a estirpe *Candida guilliermondii* CCT-3544 como agente de produção. De acordo com os resultados obtidos pode-se ressaltar que o bagaço do pedúnculo do caju seco possui 21,45% de celulose, 10,96% de hemicelulose e 35,39% de lignina. Durante a fermentação, a *C. guilliermondii* CCT-3544 foi capaz de crescer no meio com hidrolisado sendo sua concentração máxima de crescimento celular (3,5 g L⁻¹). O comportamento dos açúcares no processo fermentativo foi similar nas diferentes variáveis com produção máxima de etanol e xilitol em 48 h de fermentação.



Introduction

The Brazilian agricultural economy remains as one of the most important in the world, with performance superior to 186.1 million tons in the 2012/2013 season (Econ, 2013). Concomitantly with these levels, tons of agro-industrial wastes are generated, which are eliminated in the environment, producing excessive accumulation of organic matter in nature.

In this context, many of these wastes from the agro-industry can be used in biotechnological processes as raw material to obtain products with commercial value. Thus, various studies have demonstrated the potential of use of fermentable sugars, such as glucose and xylose, in the biosynthesis of products like ethanol and xylitol.

In Brazil, there are innumerable agro-industrial wastes favorable to the development of bioproducts, such as cashew (*Anacardium occidentale* L.), which is a tropical plant that has a pseudofruit with great potential for biotechnological development, since it is little used by the industry, with estimate of loss of more than 80% of the peduncle (Prommajak et al., 2014).

The utilization of cashew is recommended, due to its nutritional composition containing phenolic compounds, flavonoids, tannins, sugars like glucose and xylose and ascorbic acid (Sousa et al., 2016). Hence, the production of second-generation bioethanol has allowed the use of these biomasses in its production, which guarantees high selectivity of the product, low cost and safety (Chen et al., 2010).

Xylitol is a bioproduct that has attracted global attention for its sweetening power similar to that of sucrose, with low calories. In addition, it has anticariogenic property and is also considered by the Food and Drug Administration (FDA) as sugar substitute used in the food industry, being approved in more than forty countries (Mussato & Roberto, 2002; Prakasham et al., 2009).

Given the large availability of cashew peduncle in the Northeast region, with more than 95% of the national production, and the need to employ a clean technology and minimize wastes from the industries, this research aimed to study the production of ethanol and xylitol using the liquor from the acid treatment of cashew peduncle bagasse as substrate, and the yeast *Candida guilliermondii* CCT-3544 as transformation agent.

MATERIAL AND METHODS

The utilized raw material was cashew peduncle bagasse provided by the fruit pulp company IDEAL, João Pessoa, PB. Cashew peduncle bagasse was subjected to washings with distilled water at temperature of 50 °C, 20 min each washing, to remove dirt and sugars that remain after pseudofruit pulp processing at proportion of 1:4, until reaching the content of total soluble solids equal to zero, expressed in °Brix.

The bagasse was then taken on trays to convective dryers and subjected to temperature of 55 °C for about 2 h. The dry biomass was fragmented in knife mill to reduce fiber size to 48 mesh.

The liquor 1 used in the fermentation was obtained using a stainless-steel reactor with thermal controller (automation

system for time and internal/external temperature) and maximum capacity of 0.7 L. This reactor was loaded with 50 g of cashew peduncle bagasse for every 0.6 L of 3% sulfuric acid solution, a proportion that was defined based on previous studies. The hydrolysis time of 1 h in the reactor was recorded from the moment at which the temperature established in the experimental planning to obtain the fermentable sugars was achieved.

The liquor 2 was obtained from the dry cashew peduncle bagasse subjected to hydrolysis using 3% sulfuric acid solution for 1 h to remove the hemicellulose fraction in a rotary reactor with nominal capacity of 20 L. For that, 1.250 kg of bagasse were mixed with 15 L of 3% sulfuric acid solution. After that, the reactor was closed and the time started to be recorded when the process reached temperature of 160 °C with pressure of 8 kgf cm⁻² and the desired temperature was achieved at 105 min (1 h 45 min). Then, temperature was reduced to 90 °C and, immediately after, the liquor was unloaded, filtered and centrifuged to remove the remaining impurities, resulting in 10 L of hydrolysate.

Part of the hydrolysate was concentrated in a digital rotary evaporator connected to a vacuum pump attached to a water cooling system through the SL 152/10 Ultra Thermostatic Bath using, as experimental parameters, rotation of 0.60 rpm, temperature of 80 ± 5 °C and 250 mL in each step, with time of about 3 h from the beginning to the end of the concentration.

This step aimed to increase the proportion of sugars in the liquors, especially xylose. Concentration was performed through the reduction of 2.5 times in its initial volume.

The determinations of extractives and analyses of lignocellulosic materials (lignin, cellulose, hemicellulose and holocellulose) were performed according to the methodology described by Morais et al. (2010).

The contents of sugar in the hydrolyzed liquor (xylose, glucose and arabinose) and inhibitors of fermentation (acetic acid, 5-Hydroxymethylfurfural and furfural) were determined using a High-Frequency Liquid Chromatograph (Varian, Waters, California, USA), equipped with an isocratic solvent system, "Rheodyne" valve with 20-µL sample loop; attached to a Agilent Hi-Plex H column (7.7 x 300 mm, 8 µ), at a temperature of 65 °C, refractive index detector (Varian) and the processing Software Galaxie Chromatography Data System. The mobile phase was 0.009 mol L⁻¹ H₂SO₄, at a flow rate of 0.6 mL min⁻¹, and the run lasted for 1 h.

The yeast used in the experiment was *Candida guilliermondii* CCT 3544, obtained in the Collection of Tropical Cultures (CCT) of the André Tosello Tropical Foundation of Research and Technology - FAT - Campinas, SP, which was maintained lyophilized at temperature of 28 °C until the moment of reactivation. Cells were reactivated by adding 0.2 mL of sterile distilled water, using a Pasteur pipette, in the vial with the lyophilized yeast, in sterile environment.

After 15 min, all the content was transferred from the vial to a test tube containing 5.0 mL of the liquid culture medium yeast-malt extract (3 g L⁻¹ of yeast extract, 3 g L⁻¹ of malt extract, 5 g L⁻¹ of peptone and 10 g L⁻¹ of dextrose) and incubated at 28 °C for 10 days.

The culture grown in the juice was subcultured to Petri dishes containing yeast-malt extract agar (3 g L⁻¹ of yeast

extract, 3 g L⁻¹ of malt extract, 5 g L⁻¹ of peptone and 20 g L⁻¹ of agar) and incubated at 28 °C for 48 h. Yeast growth was studied using cells of *Candida guilliermondii* CCT 3544, which were transferred under aseptic conditions from the Petri dishes to tubes containing 5 mL of sterilized distilled water. Subsequently, 2 mL aliquots of this suspension were transferred to erlenmeyer flasks containing 100 mL of the culture medium with 30 g L⁻¹ of xylose, 2 g L⁻¹ of ammonium sulfate, 0.1 g L⁻¹ of calcium chloride and 20 g L⁻¹ of rice bran extract (obtained from heating in autoclave at 111 °C - 0.5 atm for 15 min with later centrifugation for 30 min under aseptic conditions at 2000 x g, subsequently using only the supernatant as rice bran extract solution) employed in the study of Lima et al. (2014).

This culture medium was autoclaved and its pH was adjusted to 5.5. After inoculation, the flasks were incubated at 28 °C in shaker, with rotation of 200 rpm for 24 h. After this period, the cells were separated by centrifugation at 2000 x g for 30 min and re-suspended in sterilized distilled water, to obtain a solution with cell concentration of 50 g L⁻¹. This suspension was used to calculate the volume necessary to provide the initial cell concentration of 3 g L⁻¹.

Cell biomass was monitored, in duplicate, based on the measurement of optical density at 600 nm in spectrophotometer, whose cell concentration in g L⁻¹ was calculated using a calibration curve that correlates the absorbance and concentration in dry weight of the cells. Distilled water was used as control sample. The calibration curve was determined by reading the optical density of the suspensions, in duplicate, obtained through dilution of a cell suspension with known optical density and concentration in dry weight.

The concentration in dry weight of the initial cell suspension was determined gravimetrically. Cultured juice (2 mL) were centrifuged in previously dried and weighed tubes, in five replicates. After centrifugation, the supernatant was discarded and the cell biomass was dried in an oven at 105 °C, for 24 h.

The cultures were conducted considering, as a reference, the best results obtained in the study of Lima et al. (2014), using the hydrolysate supplemented with 1 g L⁻¹ of ammonium sulfate, 0.5 g L⁻¹ of calcium chloride, 5 g L⁻¹ of rice bran, pH 4.0, with agitation of 200 rpm at temperature of 28 °C, for 120 h. The medium with hydrolyzed liquor 1 was placed in 1 L erlenmeyer flasks, with 0.4 L of medium, being sterilized, cooled and inoculated with *C. guilliermondii*, provided that the initial concentration was 3 g L⁻¹.

On the other hand, the test with Bioreactor (hydrolyzed liquor 2) used 1.6 L of liquor, pre-treated, concentrated and supplemented under the same culture conditions on stirring table, in duplicate. The experiments were carried out in TEC-

BIO bioreactor-fermenter, with capacity for 4.5 L and height of the reservoir with lid of 53 cm.

The fermentation process was evaluated considering the parameters volumetric productivity (Q_p), conversion factor (Y_{p/s}) efficiency (η) with 0.40 and 1.6 L. The parameters were calculated after 48 h of fermentation.

RESULTS AND DISCUSSION

According to the characterization of the lignocellulosic composition of the dry cashew peduncle bagasse (Table 1), it consisted of 21.45% of cellulose, 10.96% of hemicellulose, 35.39% of lignin and 9.51% of extractives.

Albuquerque et al. (2014) evaluated xylitol production from cashew peduncle bagasse using *Kluyveromyces marxianus* CCA510 and reported values of 17.73% of cellulose, 19.22% of hemicellulose, 33.41% of lignin and 6.41% of extractives, indicating a substantial amount of hemicellulose, compared with the results found in the present study.

Similar values were found by Rocha et al. (2009), who analyzed dried and hydrolyzed cashew peduncle bagasse and observed values of 19.21, 12.0 and 38.11% for cellulose, hemicellulose and lignin, respectively. In addition, Lima et al. (2012) also observed similar results for dry cashew peduncle bagasse (18.31% cellulose; 27.18% hemicellulose and 23.91% lignin).

The values presented in the characterization, despite being close to those cited in the literature, differ due to the differences of season and cultivated species.

Table 2 shows the data of the hydrolyzed liquor of cashew peduncle bagasse. According to the results, xylose is the predominant sugar in the hydrolysate in the concentrated liquor, followed by glucose and arabinose originated during the acid hydrolysis of the lignocellulosic biomass. Regarding the amount of glucose in the hydrolysate, Lima et al. (2012) found similar amount (1.78 g L⁻¹); however, the amounts of xylose and arabinose (1.43 and 7.12 g L⁻¹, respectively) were different in the present study.

During the concentration process, the values of 5-hydroxymethylfurfural (HMF) and furfural were minimized due to the drag of these compounds along with the condensate.

Table 1. Lignocellulosic composition of the dry cashew peduncle bagasse

Composition	Dry cashew bagasse (%)
Extractives	9.51 ± 0.50
Cellulose	21.45 ± 0.31
Hemicellulose	10.96 ± 0.31
Holocellulose	32.41 ± 0.33
Lignin	35.39 ± 0.97

Table 2. Characteristics of hemicellulosic and concentrated hydrolysate of cashew peduncle bagasse

Composition	Hydrolyzed liquor 1	Hydrolyzed liquor 2	Concentrated hydrolyzed liquor (g L ⁻¹)	Condensed
Glucose	2.1756	2.5497	5.1972	0.0000
Xylose	5.4311	5.1706	10.5521	0.0000
Arabinose	3.8136	3.0317	6.0515	0.0000
Acetic acid	0.2039	0.0844	0.0865	0.0000
5-hydroxymethylfurfural	0.3947	0.1526	0.1154	0.5069
Furfural	0.4545	0.2869	0.1316	0.4581

However, compounds toxic to the yeasts could be observed, such as acetic acid, 0.2039 g L⁻¹ in the hydrolyzed liquor 2, 0.0844 g L⁻¹ in the hydrolyzed liquor 1 and 0.0865 g L⁻¹ in the concentrate; furfural (0.4545; 0.2869 and 0.1316 g L⁻¹) and hydroxymethylfurfural (0.3947; 0.1526; 0.1154 g L⁻¹), which were generated from the degradations of pentoses and hexoses during the heating through chemical reactions originated in the acid hydrolysis.

Silva et al. (2007) reported that concentrations above 3 g L⁻¹ of acetic acid, 1.5 g L⁻¹ of hydroxymethylfurfural and 1 g L⁻¹ of furfural have toxic effect enough to inhibit the action of microorganisms in the fermentation process.

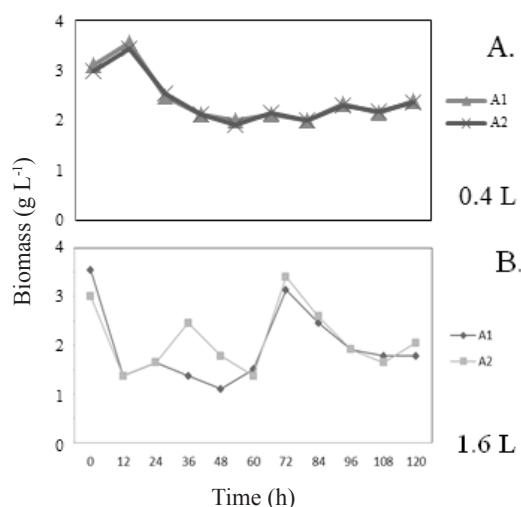
In addition, the concentrations found by Lima et al. (2012) were 0.3437 g L⁻¹ of acetic acid, which is similar to that found in the hydrolyzed liquor 2 (0.2039 g L⁻¹); for furfural (0.00002 g L⁻¹) and hydroxymethylfurfural (0.00009 g L⁻¹), the results were different from those of the present study.

Regarding the biomass data presented in Figure 1, cell growth was observed in the hydrolyzed liquor 1 up to 12 h of fermentation (3 to 3.5 g L⁻¹), maximum growth with decrease at 36 h (2 g L⁻¹), stabilizing until 120 h of operation. Bier et al. (2007) studied growth and xylose consumption using *Candida guilliermondii* in the hydrolyzed liquor of sugarcane bagasse and found maximum growth at 48 h of fermentation.

In the 1.6 L hydrolysate (Figure 1B), there was a reduction of cell concentration up to 12 h, with growth from 12 to 36 h (2.5 g L⁻¹) for A2; nevertheless, for A1 the growth only started from 48 h of fermentation, reaching its maximum at 72 h (3.5 g L⁻¹), after which there was a decrease in cell concentration.

According to Figure 2A, there was a variation in the concentrations of glucose, xylose, arabinose and xylitol production during the 120 h of fermentation. It is noticed that the sugars present in the fermented hydrolysate were consumed in the first 12 h, with slight consumption between 12 and 48 h, and remaining constant along the fermentation. The values confirmed by the xylitol production were 100% with 48 h.

Zhang et al. (2012) studied xylitol production using *Candida athensensis* SB18 and observed total xylose consumption at



A1 and A2 are replicates

Figure 1. Variation of biomass concentration during the fermentation of the hemicellulosic hydrolysate of cashew peduncle bagasse with hydrolyzed liquor 1 - 0.40 L (A) and hydrolyzed liquor 2 - 1.6 L (B)

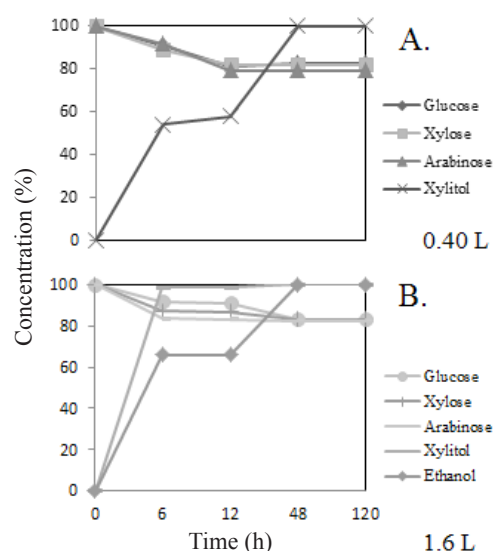


Figure 2. Variation in the concentration of glucose, xylose, arabinose, ethanol in the fermentation of the hydrolyzed liquor 1 (A) and hydrolyzed liquor 2 (B) of cashew peduncle bagasse

288 h of fermentation and maximum xylitol concentration at 264 h. The detoxified hemicellulosic hydrolyzed concentrate was supplemented with nitrogen source of the yeast extract; urea, Na₂HPO₄; KH₂PO₄. The pH of the medium was adjusted to 7.0 and controlled with 2M NaOH solution.

Figure 2B shows that the sugars were consumed in the first 6 h and remained constant until 12 h. Maximum consumption of sugars occurred at 48 h, values that were confirmed by the xylitol concentration, equal to 98.6% in the first 6 h, obtaining 100% with 48 h of fermentation. Ethanol was produced simultaneously to the obtainment of xylitol, with glucose consumption at 48 h of fermentation and maximum ethanol production in the first 48 h, remaining constant until 120 h.

Huang et al. (2011) studied the kinetic profile of *Candida tropicalis* JH030 during the fermentation of non-detoxified rice straw hydrolysate and reported the consumption of sugars (glucose, xylose and arabinose) at 80 h of fermentation, with simultaneous production of ethanol and xylitol, using 60 mL of hydrolysate with pH adjusted to 6.0, temperature of 30 °C and rotation in shaker of 100-150 rpm. The pre-inoculum was prepared with 10 g L⁻¹ of yeast extract, 20 g L⁻¹ of peptone and 20 g L⁻¹ of glucose.

Sene et al. (2000) report the preference for the consumption of glucose, compared with xylose, a trend found in this study for *Candida guilliermondii* CCT 3544.

CONCLUSIONS

1. The characterization of the lignocellulosic composition of dry cashew peduncle bagasse demonstrates the potential of this agro-industrial waste for xylitol production.

2. *C. guilliermondii* CCT-3544 obtained maximum cell growth concentration (3.5 g L⁻¹) in the medium with concentrated hydrolysate (0.40 L) in the first 12 h; for the hydrolysate fermented in the bioreactor (1.6 L), there was a variation in its cell concentration, reaching maximum of 3.5 g L⁻¹ at 72 h.

3. The behavior of the sugars in the fermentation process was similar in the different variables (0.40 L in shaker and 1.6 L in bioreactor).

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LITERATURE CITED

- Albuquerque, T. L. de; Silva Júnior, I. J. da; Macedo, G. R. de; Rocha, M. V. P. Biotechnological production of xylitol from lignocellulosic wastes: A review. *Process Biochemistry*, v.49, p.1779-1789, 2014. <https://doi.org/10.1016/j.procbio.2014.07.010>
- Bier, M. C. J.; Maranhão, L. T.; Azevedo, J. A. M.; Silva Júnior, L. S. da. Crescimento e consumo de xilose de *Candida guilliermondii* na fermentação submersa utilizando-se bagaço de cana-de-açúcar. *Evidência*, v.7, p.119-130, 2007.
- Chen, X.; Jiang, Z.-H.; Chen, S.; Qin, W. Microbial and bioconversion production of D-xylitol and its detection and application. *International Journal of Biological Sciences*, v.6, p.834-844, 2010. <https://doi.org/10.7150/ijbs.6.834>
- Econ, L. BRASIL: Ministério da Agricultura, Pecuária e Abastecimento - MAPA Secretaria de Política Agrícola - SPA, 2013. 1-7p.
- Huang, C. F.; Jiang, Y. F.; Guo, G. L.; Hwang, W. S. Development of a yeast strain for xylitol production without hydrolysate detoxification as part of the integration of co-product generation within the lignocellulosic ethanol process. *Bioresource Technology*, v.102, p.3322-3329, 2011. <https://doi.org/10.1016/j.biortech.2010.10.111>
- Lima, F. C. dos S.; Silva, F. L. H. da; Gomes, J. P.; Muniz, M. B.; Santiago, A. M.; Silva, C. G. Biotechnological production of xylitol: Evaluation of detoxification process with residual lignin using response surface methodology. *Chemical Engineering Transactions*, v.38, p.415-420, 2014. <https://doi.org/10.4236/aces.2012.24064>
- Lima, F. C. dos S.; Silva, F. L. H. da; Gomes, J. P.; Silva Neto, J. M. da. Chemical composition of the cashew apple bagasse and potential use for ethanol production. *Advances in Chemical Engineering and Science*, v.2, p.519-523, 2012.
- Morais, J. P. S.; Rosa, M. F.; Marconcini, J. M. Procedimentos para análises lignocelulósicas. Campina Grande: Embrapa Algodão, 2010. 21-42p.
- Mussato, S. I.; Roberto, I. C. Xylitol: A sweetener with benefits for human health. *Revista Brasileira de Ciências Farmacêuticas*, v.38, p.401-413, 2002.
- Prakasham, R. S.; Rao, R. S.; Hobbs, P. J. Current trends in biotechnological production of xylitol and future prospects. *Current Trends in Biotechnology and Pharmacy*, v.3, p.8-36, 2009.
- Prommajak, T.; Leksawasdi, N.; Rattanapanone, N. Biotechnological valorization of cashew apple: A review. *Chiang Mai Journal of Natural Sciences*, v.13, p.159-182, 2014. <https://doi.org/10.12982/cmujns.2014.0029>
- Rocha, V. M. P.; Rodrigues, T. H. S.; Macedo, G. R. de; Gonçalves, L. R. B. Enzymatic hydrolysis and fermentation of pretreated cashew apple bagasse with alkali and diluted sulfuric acid for bioethanol production. *Applied Biochemistry and Biotechnology*, v.155, p.104-114, 2009. <https://doi.org/10.1007/s12010-008-8432-8>
- Sene, L.; Vitolo, M.; Felipe, M. G. A.; Silva, S. S. Effect of environmental conditions on xylose reductase and xylitol dehydrogenase production in *Candida guilliermondii*. *Applied Biochemistry and Biotechnology*, v.84, p.371-80, 2000. <https://doi.org/10.1385/ABAB:84-86:1-9:371>
- Silva, D. D. V. da; Mancilha, I. M. de; Silva, S. S. da; Felipe, M. das G. de A. Improvement of biotechnological xylitol production by glucose during culture of *Candida guilliermondii* in sugarcane bagasse hydrolysate. *Brazilian Archives of Biology and Technology*, v.50, p.207-215, 2007. <https://doi.org/10.1590/S1516-89132007000200005>
- Sousa, S. L.; Morais, B. A. de; Ribeiro, L. C.; Costa, J. M. C. da. Stability of cashew apple juice in powder dehydrated in spouted bed. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.20, p.678-682, 2016. <https://doi.org/10.1590/1807-1929/agriambi.v20n7p678-682>
- Zhang, J.; Geng, A.; Yao, C.; Lu, Y.; Li, Q. Xylitol production from D-xylose and horticultural waste hemicellulosic hydrolysate by a new isolate of *Candida athensensis* SB18. *Bioresource Technology*, v.105, p.134-141, 2012.