Saccharification of Sugarcane Bagasse Using an Enzymatic Extract Produced by *Aspergillus fumigatus*

Kênia F. R. Lamounier[1], Patrisia O. Rodrigues[1], Daniel Pasquini[2]* and Milla A. Baffi[1]

[1]*Institute of Agricultural Sciences, Federal University of Uberlândia (UFU), Uberlândia, MG, 38408-100, Brazil*
[2]*Chemistry Institute, Federal University of Uberlândia (UFU), Uberlândia, MG, 38408-100, Brazil*

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ABSTRACT: This study investigates the efficiency of a crude enzymatic extract produced by *Aspergillus fumigatus* SCBM6 by solid state fermentation (SSF) in the hydrolysis of alkali pretreated sugarcane bagasse (PTB). After SSF using *in natura* sugarcane bagasse (SCB), the enzymatic extract presented 21.33 $U.g^{-1}$ of *β*-glucosidase and 544.46 $U.g^{-1}$ of xylanase. The alkaline pretreatment with sodium hydroxide (2% NaOH (w/v) removed 43% of the lignin from PTB and the cellulosic fraction increased to 75%. The hydrolysis was optimized as a function of time, temperature, and concentration of PTB. After hydrolysis, the maximum yield (30.05%) of total released reducing sugars (TRS) was obtained under the following conditions: 24 h, 55 °C, 2% of PTB and 3 $U.g^{-1}$ of *β*-glucosidase (CBU). Furthermore, an approximate TRS value (26.4%) was also obtained after saccharification carried out during 6 h, 55 °C, 4% of PTB and 1 CBU. These results indicate that hydrolysis can be performed in a short incubation period and with low enzymatic load for reasonable TRS release.

KEYWORDS: *A. fumigatus*, enzymatic hydrolysis, sugarcane bagasse, alkaline pretreatment, solid state fermentation

1 INTRODUCTION

Climatic changes and the increase of oil costs have contributed to the rising search for sustainable energy sources and alternative fuels from renewable resources [1]. In Brazil, bioethanol is one of the most promising biofuels because it has great potential to replace gas and the country is in a prominent geographic position for the production of sugarcane [2]. The sugarcane industry produces several different kinds of by-products, such as sugarcane bagasse (SCB), which can be reused for additional production of ethanol. Around 75% of this residue can be employed as an energy source or as raw material for the production of low-value products such as mulch or ceiling tiles. The remaining 25% is considered as solid waste and is dumped into landfills [3]. This residual portion of SCB which is discarded can be applied as a promising source for additional production of ethanol (second generation ethanol or 2G ethanol) [4].

Sugarcane bagasse (SCB) can be used as substrate for the growth of microorganisms and production of hydrolytic enzymes, such as cellulases and hemicellulases, which are commonly used in saccharification processes of residual biomass such as cellulases and hemicellulases [5]. SCB is a lignocellulosic waste composed of approximately 40–60% cellulose, 20–30% hemicellulose and 15–25% lignin [6], whose strong interaction among them limits their utilization and represents the main limitation for its conversion into fermentable carbohydrates for second generation ethanol production [7]. Thus, in order to recycle this lignocellulosic by-product, three steps are necessary: pretreatment (physical, physical-chemical or biological) of biomass, hydrolysis and alcoholic fermentation [8, 9].

The first stage consists of chemical pretreatments with the aim to promote the delignification, decrease the cellulose crystallinity and increase the porosity of the material. Among them, the alkaline pretreatment with sodium hydroxide (NaOH) can be very advantageous since it allows an effective removal of lignin, with low degradation of sugars and minimal production of inhibitors [10]. The second step involves hydrolysis using cocktails of cellulases and hemicellulases (with mixed hydrolytic activities such as *β*-glucosidases, xylanases and *β*-xylosidases) produced mainly by filamentous fungi to hydrolyze polymers in free monosaccharides. This procedure has several advantages: absence of secondary reactions, low formation

*Corresponding author: daniel.pasquini@ufu.br

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of fermentation inhibitors and elevated activity under mild conditions of temperature and pH [11].

Filamentous fungi from the *Aspergillus* genus are known as one of the main producers of exo-enzymes which catalyze the hydrolysis of cellulose and hemicelluloses [12, 13]. Some strains of *A. fumigatus* have been described as good producers of enzymes from cellulolytic and hemicellulolytic complexes, such as *β*-glucosidases and xylanases, respectively [12, 14]. This fungal species has also been investigated in studies of hydrolysis of lignocellulosic materials [14]. In this context, the present study analyzed the efficiency of alkaline pretreatment of SCB with NaOH and the hydrolysis of PTB using an enzymatic extract produced by *A. fumigatus* (SCBM6 strain). Variable conditions of hydrolysis, including time, temperature, concentration of substrate and concentration of enzymatic extract, were also evaluated.

2 MATERIAL AND METHODS

2.1 Alkaline Pretreatment (APT)

Samples of sugarcane bagasse (SCB) (obtained from a mill in Vale do Tijuco with sugar and alcohol manufactured by Usina Uberaba S/A, Uberaba, MG, Brazil) were washed with distilled water for 1 h with constant stirring and dried at 50 °C for 48 h. Then, the residue was crushed in an agricultural grinder in order to obtain particles of around 5 mm of size, homogenized and stored in plastic bags free of humidity. Samples of 100 g of SCB were weighed and placed in a Soxhlet extractor with 2.5 L of NaOH (2% w/v) [14]. This mixture was refluxed under heating process for 4 h at 70 °C. The residue obtained was filtered and washed with distilled water until neutral pH and dried for 12 h at 50 °C.

2.2 Characterization of Untreated and Pretreated Sugarcane Bagasse

The chemical composition of untreated (SCB) and pretreated (PTB) sugarcane bagasse was determined in order to evaluate the effects of alkaline pretreatment on the substrate structure. Humidity was determined according to standard TAPPI T264 cm-97 and ash content was evaluated in accordance with TAPPI T211 om-02. The concentration of acid insoluble lignin was quantified by the TAPPI T222 om-02 method.

Holocellulose was obtained by the sodium chloride method based on the reaction between lignin, the chlorine dioxide (ClO_2) and hypochlorite ion (ClO^-) [15]. Cellulose was referred to the fibrous residue after two extractions of holocellulose with KOH solutions of 5 and 24% (w/v). The hemicellulose content was determined as the percentage difference between the levels of holocellulose and cellulose with respect to the initial mass of dry sample, considering the calculated yield from holocellulose analysis [15]. All the assays were performed in triplicate.

2.2.1 Solid State Fermentation (SSF)

Solid state fermentations (SSF) for enzyme production were conducted in 250 mL Erlenmeyer flasks containing 2.5 g of *in natura* sugarcane bagasse (SCB) and 2.5 g of wheat bran (WB) at the proportion of 1:1 ($g.g^{-1}$) and 5 mL of sterile nutrient solution consisting of 0.35% ammonium sulfate ($(NH_4)_2SO_4$), 0.3% potassium dihydrogen phosphate (KH_2PO_4), 0.05% magnesium sulfate heptahydrate ($Mg_2SO_4.7H_2O$) and 0.05% calcium chloride ($CaCl_2$). The flasks were sealed and autoclaved at 121 °C, 1 atm for 20 min. Each flask was cultivated with a thermotolerant *A. fumigatus* SBCM6 inoculum equivalent to 10^7 $spores.g^{-1}$ substrate cultivated 2–3 days at 45 °C. Afterwards, 15 mL of sterile nutrient solution was added to the flasks and the surface was scraped to release spores and suspended in the sterile nutrient solution. *A. fumigatus* SBCM6 was obtained from the Fungal Collection of Environmental Microbiology Laboratory (LAMIC), Federal University of Uberlândia (UFU), Brazil. This strain was previously isolated from SCB piles, identified at species level and evaluated for enzyme production, demonstrating high *β*-glucosidase and xylanase activities [12]. After counting spores, sterile nutrient solution was added to obtain a final volume of 20 ml and moisture of 80% [12]. The SSF process was carried out for seven days at 45 °C. After 96 h of cultivation, a volume of 50 mL of distilled water was added to the flasks at intervals of 24 h and manually stirred with a glass rod. Samples were then subjected to mechanical agitation in an orbital shaker at 150 g for 1 h. The material was filtered, centrifuged for 20 min at 10,000 × *g* and the supernatant (crude extract) was stored in aliquots at −20 °C. All experiments were performed in triplicate.

2.2.2 Enzyme Activity Measurement

After SSF, *β*-glucosidase (cellulase) and xylanase (hemicellulose) activities were measured. The assays of both enzymes were performed in triplicate. The quantification of *β*-glucosidase or cellobiase (CBU) was performed at 60 °C using a reaction mixture containing 50 µL of crude enzymatic extract, 250 µL of 0.1 $mol.L^{-1}$ acetate buffer, pH 5.5, and 250 µL of ρ-nitrophenyl-*β*-D-glucopyranoside 4 $m.mol.L^{-1}$ (PNPG, Sigma-Aldrich) as substrate. After 10 min, the reaction was stopped by

adding 2 mL of sodium carbonate solution (Na_2CO_3) 2 mol.L^{-1}. The concentration of released ρ-nitrophenol (ρNP) was determined by spectrophotometry at 410 nm. One unit of enzymatic activity (U) was defined as the amount of enzyme required to release 1 µmol of ρNP per minute of reaction from a ρNP standard curve [16]. Activity was expressed in units of enzymatic activity per gram of dry substrate used in SSF (U.g^{-1}).

Xylanase activity was assayed by 3.5-dinitrosalicylic acid (DNS) method in a reaction mixture containing 10 µL of enzymatic extract, 90 µL of 1% xylan (xylan from beechwood, Sigma-Aldrich) in acetate buffer 0.1 mol.L^{-1} at 60 °C for 10 min. The reaction was stopped by the addition of 100 µl DNS [17]. The final volume was boiled during 10 min and afterwards 800 µL of distilled water was added. The amount of released xylose was measured at 540 nm. One unit of enzymatic activity was defined as the amount of enzyme required to release 1 µmol of xylose per minute of reaction, using a xylose standard curve.

2.2.3 *Enzymatic Hydrolysis*

The hydrolysis of pretreated sugarcane bagasse (PTB) was carried out using the crude enzymatic extract produced by *A. fumigatus* SCBM6. The saccharification conditions: enzyme load, temperature, time and substrate concentration were evaluated. The enzyme load was based on units of *β*-glucosidase or cellobiase (CBU) in the crude enzymatic extract per gram of cellulose.

Initially, saccharifications were performed in 250 ml Erlenmeyer flasks sealed with a rubber stopper containing 2% PTB in sodium citrate buffer 0.1 mol.L^{-1}, pH 4.0 and 3U.g^{-1} of *β*-glucosidase (3 CBU) in a final volume of 20 mL. The reaction mixture was incubated at 55 °C, 200 rev.min^{-1} and samples were taken after 6, 12, 24 and 48 h of incubation in order to determine the ideal period in which to release the total reducing sugars (TRS).

After the determination of the best time of hydrolysis, the reactions were carried out at variable temperatures (50, 55 and 60 °C) at the previously selected period under the same conditions. Lastly, the concentration of the substrate was also assessed using 2, 4, 6, 8 and 10% (w/v) of PTB at the formerly designated time and temperature. In addition, an additional hydrolysis was carried out for a shorter period of time (6 h), in order to improve its application in sugar industries, under the same conditions as the third experiment. All the hydrolysates were filtered, centrifuged at 5000 × *g* for 30 min and used for quantitative analysis of total reducing sugars (TRS) by DNS method [17]. All the experiments were performed in triplicate.

2.2.4 *Quantification of Total Reducing Sugars (TRS)*

The quantification of TRS was performed by the incubation of 100 µL of each hydrolysate and 100 µL DNS for 10 min in the boiling water. Subsequently, 800 µL of distilled water was added. The amount of released TRS was determined at 540 nm. Assays were executed in triplicate.

3 RESULTS AND DISCUSSION

3.1 Chemical Characterization of Untreated and Pretreated SCB

Composition of ash, lignin, hemicelluloses and cellulose content was analyzed in order to evaluate the alterations of the SCB composition before and after alkali pretreatment with NaOH (Table 1).

Before alkali pretreatment, 26.1% of lignin was observed in untreated bagasse (SCB). In the studies of Rabelo *et al.* [18] and Nascimento *et al.* [19] close values of lignin in raw bagasse samples were obtained (25.8 and 21.7%, respectively). After pretreatment, the lignin fraction decreased from 26.1 in SCB to 14.9% in pretreated SCB and was reduced by 43.0%, indicating a significant removal of this macromolecule in PTB. As regards the hemicellulosic fraction in SCB, only 13.5% was noted, a value below the rates found by other studies, such as the one by Teixeira *et al.* [20] in which 29.7% of hemicelullose was obtained. Rabelo *et al.* [18] and Nascimento *et al.* [19] also presented higher percentages (19.7 and 29.4%, respectively). In PTB, it was also observed that a small portion of hemicellulose was removed (around 5.5%). Concerning the cellulosic fraction, 54.4% was observed in SCB. This value was slightly superior to that found in the literature. Rabelo *et al.* [18] and Nascimento *et al.* [19] found 39.6 and 38.8%, respectively. After pretreatment, the cellulose portion increased by around 21% in PTB (75%), indicating that the cellulose was preserved in PTB. Furthermore, a low hemicellulose decrease was observed.

Table 1 Chemical characterization of untreated (SCB) and alkali pretreated (PTB) sugarcane bagasse.

Components	SCB (%)	PTB (%)
Ash Content	0.6	0.2
Lignin	26.1	14.9
Cellulose	54.4	75.0
Hemicellulose	13.5	12.8
Total balance	94.6	102.9

Alkaline pretreatment of SCB with NaOH has been studied in other works. Guilherme *et al.* [21] studied the alkali pretreatment with 4% NaOH (w/v) and reported increases by 22.33% and 5.01% in the cellulosic and hemicellulosic fractions, respectively, and a reduction by 72.62% in the lignin content. Gao *et al.* [22] analyzed the alkaline pretreatment of SCB with 1.8% NaOH (w/v) and noticed increases in the content of cellulose from 41.95% to 63.19% and hemicellulose from 21.7% to 26.63%, and a decrease in the lignin composition from 23.6% to 8.95%. In accordance with these previous studies, the alkali pretreatment carried out in the present work was efficient for lignin reduction and holocellulose preservation in pretreated SCB. This effective removal of lignin can significantly contribute to the access of the hydrolytic enzymes to biomass [10].

3.1.1 Enzyme Production

In this study, *β*-glucosidase and xylanase production in SSF by *A. fumigatus* SCBM6 was investigated as a function of time, using untreated sugarcane bagasse (SCB) and wheat bran (WB) as substrates. The measurements were performed between 96 and 144 h since the greatest production of enzymes was observed in a previous study using the same fungal strain and conditions [11]. For *β*-glucosidase (CBU), the maximum activity was obtained at 144 h (21.33 $U.g^{-1}$ of *β*-glucosidase), while for xylanase, the maximum yield occurred at 96 h (544.46 $U.g^{-1}$ of xylanase) (Figures 1a,b). Other cellulases and hemicellulases were not detected at relevant concentrations.

Low-grade production of *β*-glucosidase was described in previous studies using sugarcane bagasse and wheat bran. Inferior *β*-glucosidase biosynthesis was obtained by Moretti *et al.* [23] in SSF of *A. fumigatus* M7.1 using the same substrates (around 5 $U.g^{-1}$ of substrate). For xylanase, a close value was observed (573 $U.g^{-1}$ of substrate) by Su *et al.* [11] using *A. fumigatus* SBC4 as inoculum and SCB and WB as substrates in SSF. Based on these comparisons, it can be concluded that *A. fumigatus* SCBM6 was a good producer of these enzymes, demonstrating superior production in comparison to other studies. Some strains of *Aspergillus sp.* have also been described in other studies as good producers of extracellular enzymes by SSF, mainly xylanases [12,13]. In addition, previous works described some *A. fumigatus* strains useful in processes for production of cellulolytic and hemi-cellulolytic enzymes [14, 23].

3.1.2 Optimization of Enzymatic Hydrolysis

Hydrolysis assays were conducted using crude enzymatic extract produced by *A. fumigatus* SCBM6. Time, temperature, substrate concentration and *β*-glucosidase (CBU) load were evaluated in order to determine the best conditions to obtain the highest yields of total reducing sugars (TRS). After each different saccharification experiment, the yields of released TRS were evaluated. In the first stage, the time of hydrolysis was evaluated. The highest production of TRS (209.04 TRS $mg.g^{-1}$ of residue or 4.11 $g.L^{-1}$) was obtained after hydrolysis carried out during 24 h at 55 °C with 2% PTB and 3 $U.g^{-1}$ of *β*-glucosidase (CBU), with a yield of 30.05% of TRS per g cellulose (Table 2).

Table 2 Production of total reducing sugars (TRS) after hydrolysis varying the time (temperature fixed at 55 °C, 2% of PTB and 3 $U.g^{-1}$ of *β*-glucosidase).

Time (h)	$g.L^{-1}$	$mg.g^{-1}$ [a]	Yield [b] (%)
6	3.70	185.19	23.51
12	2.72	135.90	16.59
24	4.11	209.04	30.05
48	1.62	81.04	10.34

[a]mg of TRS.g^{-1} of bagasse
[b]g of TRS.g^{-1} of cellulose × 100

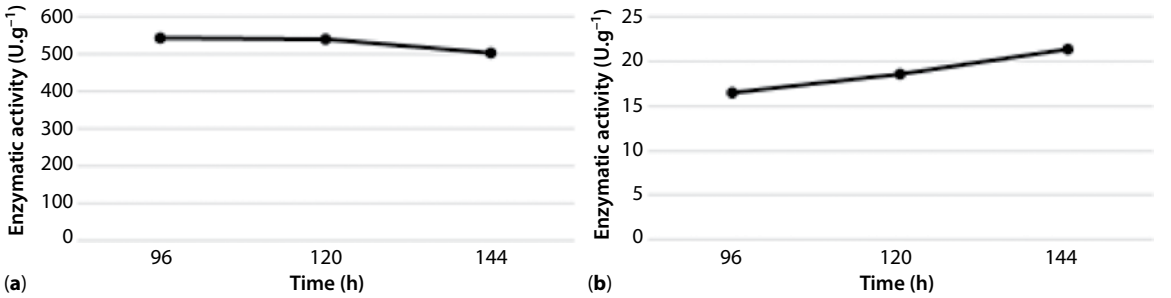

Figure 1 Production of enzymes by SSF using *A. fumigatus* SCBM6 as inoculum and sugarcane bagasse and wheat bran as substrates: (**a**) xylanase and (**b**) *β*-glucosidase.

Other authors also obtained maximum yields of TRS after hydrolysis performed during 24 h. Moretti *et al.* [24] analyzed the hydrolysis of SCB pretreated with glycerol and microwave using a mixed extract containing endoglucanase, *β*-glucosidase and xylanase (47.0, 1.0 and 220.0 $U.g^{-1}$ dry bagasse, respectively) from a *Myceliophthora thermophila* M.7.7 cultivation and also obtained approximately 50 mg $TRS.g^{-1}$ bagasse in 24 h. Comparing the present results with those obtained by Moretti *et al.* [24], the amount of TRS (209.04 TRS $mg.g^{-1}$ of residue) was superior to that detected by these authors, indicating the effectiveness of *A. fumigatus* SCBM6 enzymatic extract in hydrolysis of lignocellulosic biomass. These data suggested that the hydrolysis performed during 24 hours and using a low quantity of enzyme extract (only 3 $U.g^{-1}$ of *β*-glucosidase) was very successful for the liberation of worthy amounts of TRS. Thus, the time of 24 h was fixed for the next hydrolysis experiments.

Temperature was the second evaluated condition for saccharification. The highest production of TRS was obtained in hydrolysis carried out at 55 °C (209.04 mg $TRS.g^{-1}$ bagasse) (Table 3). This result is in accordance with other studies which indicated temperatures among 40 to 60 °C favoring the liberation of sugars after saccharification and reducing the probability of inhibitor production and enzyme denaturation [25,26]. Compared to the literature, lower yields of TRS were described in previous studies of hydrolysis of sugarcane bagasse performed at the same temperature, such as Moretti *et al.* [24], in which approximately 50 mg $TRS.g^{-1}$ bagasse was obtained in hydrolysis using a *M. thermophila* M.7.7 enzymatic extract.

Substrate concentration was the third investigated condition. In this assay, the highest yield of TRS was obtained in the hydrolysis using 2% PTB (209.04 mg $TRS.g^{-1}$ bagasse), with a yield of 30.05% per g of cellulose present in the substrate after 24 h at 55 °C (Table 4).

Our data was superior in comparison to previous studies in the literature. Barrera-Martínez *et al.* [27] obtained 27.17% TRS after 2 h of saccharification at 50 °C using 10% ozonate sugarcane bagasse and a commercial enzyme cocktail provided by ENMEX (containing 416.25 FPU g^{-1} of biomass).

Lastly, in order to increase the application of the present enzymatic extract in residual biomass saccharification in the alcohol industry, hydrolysis procedures were evaluated under the previous selected conditions (55 °C and with 4% PTB), but in a shorter period of time (6 h) and with lower concentration of *β*-glucosidase (1 $U.g^{-1}$ of substrate) than the previous experiments. In this experiment, it was observed that after a period of 6 h, the TRS yield was 26.40% (3.64 $g.L^{-1}$ and 182.19 $mg.g^{-1}$ bagasse). This result was close to that achieved in the period of 24 h (27.57%) under the same conditions (Table 4). Thus, these data suggest that the ideal selected hydrolysis condition was observed in the assay performed during 6 h and using only 1 $U.g^{-1}$ of *β*-glucosidase (CBU) (26.40%). Pereira *et al.* [26] obtained 36% of glucose (quantified by HPLC) after only 8 h of saccharification, which can be considered a satisfactory glucose release in a shorter hydrolysis time, also indicating that it is not necessary to extend the experiment to a longer period to obtain higher yields of glucose. Based on these data, it was suggested that the enzymatic hydrolysis performed in a shorter incubation period and with low enzymatic concentrations is feasible and more economically worthwhile for industrial applications.

4 CONCLUSIONS

After solid state fermentation using *A. fumigatus* SCM6 as inoculum, significant amounts of *β*-glucosidase and xylanase were obtained. The best release of total reducing sugars was obtained after hydrolysis carried out during a period of 24 h at 55 °C, using 3 $U.g^{-1}$ of *β*-glucosidase and with 2% of pretreated sugarcane bagasse (30.05%). However, it was found that hydrolysis performed during 6 h with 4% of PTB and with 1 $U.g^{-1}$ of *β*-glucosidase showed very close yields of TRS (26.40%), suggesting that hydrolysis performed

Table 3 Production of total reducing sugars (TRS) after hydrolysis varying the temperature (time fixed at 24 h, 2% of PTB and with 3 $U.g^{-1}$ of *β*-glucosidase).

Temperature (°C)	$g.L^{-1}$	$mg.g^{-1}$ [a]	Yield[b] (%)
50	1.77	88.33	11.40
55	4.11	209.04	30.05
60	3.79	189.47	27.46

[a]mg of $TRS.g^{-1}$ of bagasse
[b]g of $TRS.g^{-1}$ of cellulose × 100

Table 4 Production of total reducing sugars (TRS) after hydrolysis varying the substrate concentration (time fixed for 24 h at 55 °C and with 3 $U.g^{-1}$ of *β*-glucosidase).

[Substrate] (%)	$g.L^{-1}$	$mg.g^{-1}$ [a]	Yield [b] (%)
2	4.11	209.04	30.05
4	7.61	190.24	27.57
6	7.80	129.97	18.84
8	8.16	102.05	14.79
10	5.66	56.61	8.20

[a]mg of $TRS.g^{-1}$ of bagasse
[b]g of $TRS.g^{-1}$ of cellulose × 100

in a relatively shorter time and with lower enzymatic load can be viable for an appreciable release of TRS and prospective use in industrial applications.

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REFERENCES

1. B. Hah-Nhägerdal, M. Galbe, G. Gorwa-Grauslund, G. Liden, and G. Zacchi, Bio-ethanol: The fuel of tomorrow from the residues of today. *Trends Biotechnol.* **24**, 549–556 (2006).
2. P. Reyes, R.T. Mendonça, M.G. Aguayo, J. Rodríguez, B. Veja, and P. Fardim, Extraction and characterization of hemicelluloses from *Pinus radiata* and its feasibility for bioethanol production. *Rev. Árvore* **37**, 175–180 (2013).
3. C.W. Chang and C. Webb, Production of a generic microbial feedstock for lignocellulose biorefineries through sequential bioprocessing. *Bioresource Technol.* **227**, 35–43 (2017).
4. C.D.O.G. Silva, E.N. Aquino, C.A.O. Ricart, G.E.O. Midorikawa, R.N.G. Miller, and E.X. Ferreira Filho, GH11 xylanase from *Emericella nidulans* with low sensitivity to inhibition by ethanol and lignocellulose-derived phenolic compounds. *FEMS Microbiol. Lett.* **362,** 1–8 (2015).
5. A.K. Chandel, B.C. Gonçalves, J.L. Strap, and S.S. Da Silva, Biodelignification of lignocellulose substrates: An intrinsic and sustainable pretreatment strategy for clean energy production. *Crit. Rev. Biotechnol.* **35**(3), 1–13 (2013).
6. J. Vanneste, T. Ennaert, A. Vanhulsel, and B. Sels, Unconventional pretreatment of lignocellulose with low-temperature plasma. *ChemSusChem* **10**, 14–31 (2017).
7. M. Maza, H.F. Pajot, M.J. Amoroso, and M.G. Yasem, Post-harvest sugarcane residue degradation by autochthonous fungi. *Int. Biodeterior. Biodegration* **87**, 18–25 (2014).
8. H. Rabemanolontsoa and S. Saka, Various pretreatments of lignocellulosics. *Bioresour. Technol.* **199**, 83–91 (2015).
9. S. Pandey, Cellulases in conversion of lignocellulosic waste into second-generation biofuel. *Int. J. Adv. Res.* **3**, 392–399 (2015).
10. J.S. Kim, Y.Y. Lee, and T.H. Kim, A review on alkaline pretreatment technology bioconvertion of lignocellulosic biomass. *Bioresour. Technol.* **199**, 42–48 (2015)**.**
11. C.H. Su, M.H. Chung, H.J. Hsieh, Y.K. Chang, J.C. Ding, and H.M. Wu, Enzymatic hydrolysis of lignocellulosic biomass in ionic liquid media for fermentable sugar production. *J. Taiwan Inst. Chem. Eng.* **43**, 573–577 (2012).
12. B.S.L. Dos Santos, A.F.S. Gomes, E.G. Franciscon, J.M. Oliveira, and M.A. Baffi, Thermotolerant and mesophylic fungi from sugarcane bagasse and their prospection for biomass-degrading enzyme production. *Braz. J. Microbiol.* **46**, 903–910 (2015).
13. A.F.S. Gomes, B.S.L. Santos, E.G. Franciscon, and M.A. Baffi, Substract and temperature effect on xylanase production by *Aspergillus fumigatus* using low cost agricultural wastes. *Biosci. J.* **32**, 915–921 (2016).
14. P.O. Rodrigues, B.V. Dos Santos, L. Costa, M.A. Henrique, D. Pasquini, and M.A. Baffi, Xylanase and *β*-glucosidase production by *Aspergillus fumigatus* using commercial and lignocellulosic substrates submitted to chemical pre-treatments. *Ind. Crops Prod.* **95**, 453–459 (2016).
15. B.L. Browning, *Methods of Wood Chemistry*, vol. 2, pp. 561–587, Wiley & Sons, Interscience Publishers, New York (1967).
16. M.A. Baffi, T. Tobal, J. Henrique, G. Lago, R.S. Leite, M. Boscolo, E. Gomes, and R.A. Da Silva, Novel *β*-glucosidase from *Sporidiobolus pararoseus*: Characterization and application in winemaking. *J. Food Sci.* **76**, 997–1002 (2011).
17. G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31**, 426–428 (1959).
18. S.C. Rabelo, R. Maciel Filho, and A.C.A Costa, Comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production. *Appl. Biochem. Biotechnol.* **148**, 45–58 (2008).
19. V.M. Nascimento, A. Manrich, P.W. Tardioli, R. de Campos Giordano, G.J. de Moraes Rocha, and R. de Lima Camargo Giordano, Alkaline pretreatment for practicable production of ethanol and xylooligosaccharides. *Bioethanol* **2**, 112–125 (2016).
20. L.C. Teixeira, J.C. Linden, and H.A. Schroeder, Simultaneous saccharification and cofermentation of peracetic acid-pretreated biomass. *Appl. Biochem. Biotechnol.* **84–86**, 111–127 (2000).
21. A.A. Guilherme, P.V.F. Dantas, E.S. Santos, F.A.N. Fernandes, and G.R. Macedo, Evaluation of composition, characterization and enzymatic hydrolysis of pretreated sugar cane bagasse. *Braz. J. Chem. Eng.* **32**, 23–33 (2015).
22. Y. Gao, J. Xu, Z. Yuan, Y. Zhang, Y. Liu, and C. Liang, Optimization of fed-batch enzymatic hydrolysis from alkali-pretreated sugarcane bagasse for high-concentration sugar production. *Biores. Technol.* **167**, 41–45 (2014).
23. M.M.S. Moretti, D.A. Bocchini-Martins, R. Da Silva, A. Rorigues, L.D. Sette, and E. Gomes, Selection of thermophilic and thermotolerant fungi for the production of celulases and xylanases under solid-state fermentation. *Braz. J. Microbiol.* **43**, 1062–1071 (2012).
24. M.M.S. Moretti, D.A. Bocchini-Martins, C.C.C Nunes, M.A. Villena, O.M. Perrone, R. Silva, M. Boscolo, and E. Gomes, Pretreatment of sugarcane bagasse with microwaves irradiation and its effects on the structure and on enzymatic hydrolysis. *Appl. Energy* **122**, 189–195 (2014).
25. J. de Cassia Pereira, R. Travaini, N.P. Marques, S. Bolado-Rodríguez, and D.A.B. Martins, Saccharification of

ozonated sugarcane bagasse using enzymes from *Myceliophthora thermophila* JCP 1-4 for sugars release and etanol production. *Bioresour. Technol*. **204**, 122–129 (2016).
26. J. de Cassia Pereira, N. Paganini Marques, A. Rodrigues, T. Brito de Oliveira, M. Boscolo, R. da Silva, E. Gomes, and D.A. Bocchini Martins, Thermophilic fungi as new sources for production of cellulases and xylanases with potential use in sugarcane bagasse saccharification. *J. Appl. Microbiol*. **118**, 928–939 (2015).
27. I. Barrera-Martínez, N. Guzmán, E. Peña, T. Vásquez, R. Cerón-Camacho, J. Folch, J.A.H. Salazar, and J. Aburto, Ozonolysis of alkaline lignin and sugarcane bagasse: Structural changes and their effect on saccharification. *Biomass Bioenerg*. **94**, 167–172 (2016).

