Applied Biochemistry and Biotechnology (2019) 189:37–48
https://doi.org/10.1007/s12010-019-02991-6

Use of an (Hemi) Cellulolytic Enzymatic Extract Produced by *Aspergilli* Species Consortium in the Saccharification of Biomass Sorghum

Beatriz Vieira dos Santos[1] · Patrísia Oliveira Rodrigues[1] · Carlos Juliano Brant Albuquerque[2] · Daniel Pasquini[3] · Milla Alves Baffi[1]

Received: 18 December 2018 / Accepted: 1 March 2019 /
Published online: 13 March 2019

Abstract

This study evaluated the production of lignocellulose-degrading enzymes by solid-state fermentation (SSF) using a microbial consortium of *Aspergillus fumigatus* SCBM6 and *A. niger* SCBM1 (AFN extract). The fungal strains were cultivated in sugarcane bagasse (SCB) and wheat bran (WB) as lignocellulosic substrates for 7 days at 30 °C. After SSF, the highest peaks of enzyme production were 150 and 80 U g^{-1} for β-xylosidase and β-glucosidase at 48 h, 375 U g^{-1} for xylanase at 96 h, and 80 U g^{-1} for endoglucanase and 4 U g^{-1} for cellulase activity on filter paper (FPase) at 144 h. The efficiency of the produced AFN extract was investigated in the enzymatic hydrolysis of crude biomass sorghum (BS) and after the removal of extractives (ES). After saccharification, the glucose and xylose concentrations were 10× superior in ES than in BS hydrolysate (2.5 g L^{-1} after 12 h). The presence of inhibitors of alcoholic fermentation, such as formic acid, was also reduced in ES hydrolysates, indicating that the removal of extractives positively contributed to the effectiveness of enzymatic hydrolysis of biomass sorghum using AFN extract.

Keywords Cellulases · Hemicellulases · *Aspergillus* · Biomass sorghum · Enzymatic hydrolysis

Introduction

The increasing energy dependence, the reduction of oil reserves, and environmental problems have stimulated the search for new sources of fuels [1]. In this scenario, the use of

✉ Milla Alves Baffi
millabaffi@yahoo.com.br; milla.baffi@ufu.br

[1] Agricultural Sciences Institute (ICIAG-UFU), Uberlândia Federal University, Uberlândia, Minas Gerais 38405-320, Brazil

[2] Agricultural Sciences Institute (ICA-UFMG), Minas Gerais Federal University, Montes Claros, Minas Gerais 39404-547, Brazil

[3] Chemical Institute (IQ-UFU), Uberlândia Federal University, Uberlândia, Minas Gerais 38400-902, Brazil

lignocellulosic biomass as raw material for biofuel production can be a viable alternative since they are renewable and widely available [2]. Among them, the second generation ethanol (2G ethanol) has potential to substitute the gasoline and can be synthesized after the bioconversion of lignocellulosic biomass in fermentable sugars [3]. Thus, agroindustrial wastes such as sugarcane bagasse (SCB), sweet sorghum bagasse, and maize straw have been highlighted for this purpose [4]. Plant cultures of rapid growth and high productivity, such as *Sorghum bicolor* (L.), can also be suitable as sugar sources (starch, sucrose, and lignocellulose), with different kinds of sorghum (grain, fodder, hay, or biomass) and strategies to convert them in ethanol [5, 6].

In Brazil, researches related to the production of vegetal varieties with elevated biomass composition have been performed. For example, The Sorghum Genetic Improvement Program from Brazilian Agricultural Research Agency (Embrapa), in collaboration with the Agriculture Research Company of Minas Gerais (EPAMIG), recently developed a sorghum variety with increased biomass content, entitled *biomass sorghum*—BS [7]. Biomass sorghum usually contains around 32–50% of cellulose, 16–28% of hemicellulose, and 6–11% of lignin, resembling to other conventionally cultures investigated for 2G ethanol production [8]. In addition, BS has vigorous and rapid development, being able to grow up to 5 m of height and produce more than 50 t ha^{-1} of dry matter per half-yearly average cycle [8]. The use of BS for the production of biofuels is a relatively new field of research with high potential for application in the generation of ethanol due to its high biomass productivity and elevated composition in polysaccharides [9].

For its bioconversion in 2G-ethanol, BS might be previously submitted to enzymatic hydrolysis employing cellulases and hemicellulases in order to liberate fermentable sugars. However, commercial enzyme cocktails have been currently employed in hydrolysis procedures, increasing the cost of the process. Alternatively, these enzymes can be produced by microorganisms by solid-state fermentation (SSF), employing low cost lignocellulosic substrates [10]. Filamentous fungi are the most adapted ones to grow up in these substrates and have been studied for enzyme production by SSF [11]. In recent studies, some strains of *Aspergillus fumigatus* and *A. niger* were described as excellent producers of some enzymes from the hemi- and cellulolytic complex such as xylanase, β-xylosidase, and β-glucosidase after cultivation in SCB and wheat bran (WB) [12]. These enzymes produced by SSF have been applied in enzymatic hydrolysis of SCB and other lignocellulosic biomass in order to obtain efficient and economically viable bioconversions [13].

Due to the recalcitrant lignocellulose structure, a step of pretreatment of the biomass can be necessary before the enzymatic hydrolysis, with the aim to reduce the lignin content, increase the porosity of the fibers and the access to cellulose [14]. After pretreatment, the polymers are hydrolyzed in monomers of hexose (C6) and pentose (C5), and then, these last ones are submitted to alcoholic fermentation [15]. Physical, chemical, and biological pretreatments can be employed to reduce the resistance of the material and facilitate the transformation of carbohydrates in fermentable sugars [16]. However, inhibitor compounds of the saccharification and fermentative process may be produced during pretreatments [17]. In addition, pretreatment stages can contribute to the increase of cost of process. Therefore, despite of its benefits, a step of pretreatment sometimes can be onerous for biomass bioconversion.

Lignocellulosic biomasses are also composed by extractives containing fat acids, phenolic substances, wax, and other organic compounds that can be extracted with water or organic solvents [17]. The presence of extractives in the material can hinder the hydrolysis, since these compounds can reduce the access to the polysaccharide fraction. High concentrations of

extractives can also influence the lignin quantification, harming the accurate characterization of the material. Thus, it is advisable that such compounds might be removed from the material [18]. In this context, the present work investigated the use of an enzymatic extract produced by SSF from a consortium between *A. fumigatus* SCBM6 and *A. niger* SCBM1 (AFN extract) in the hydrolysis of biomass sorghum before (BS) and after the removal of extractives (ES). The effect of elimination of extractives on the fermentable sugars and inhibitor yields after hydrolysis was evaluated.

Materials and Methods

Materials

Biomass sorghum sample, LE 299 variety, genetically improved to increase the biomass content was kindly donated by EPAMIG. The crop of LE 299 variety was carried out in May 2016 at the experimental station Capim Branco (Uberlândia, Minas Gerais, Brazil). After harvest, BS was washed in running water, oven dried at 50 °C, triturated in a blender for reduction of particle size, and stored at room temperature for further studies.

Chemical Characterization of Biomass Sorghum

The quantification and removal of the extractives of raw biomass sorghum was carried out using a special cartridge for extraction of the Soxhlet type, previously weighed and tared. The extraction was conducted for 48 h in a 500-mL volumetric flask, wherein 125 mL of cyclohexane and 125 mL of ethanol (99%) were added. Then, the cartridge and biomass sorghum sample were washed with distilled water (100 mL). After washing, the cartridge was dried at 60 °C for 72 h. After this step, the cartridges and extracted biomass sorghum were weighed. For the calculation of mass loss, ES sample was removed and submitted to moisture analysis. The extractive content was determined by gravimetry [19]. The ash content was determined according to the TAPPI T211 OM-93 standard. Around 1000 g of biomass sorghum was added in a crucible and the whole was heated at 800 °C in muffle for 2 h. The sample was then cooled in a desiccator up to reach room temperature and weighed. Ash content was determined by considering the percentage of mass of the residue (sample after calcination) with regard to the initial mass of dry sample.

The insoluble lignin content was determined according to the TAPPI Standard T222 om-02, with minor modifications. Around 0.7133 g (dry basis) of BS and ES was weighed and transferred to a 50-mL tube containing 10.7 mL of 72% sulfuric acid solution (H_2SO_4) (d = 1.6338 g mL^{-1}). The insoluble lignin content was determined gravimetrically. To quantify the soluble lignin content, the filtrate obtained during the previous step was measured as absorbance at 215 nm and 280 nm [19]. Cellulose and hemicelluloses contents were determined by chromatographic analysis of solution components resulting from the determination of insoluble lignin by detecting the following compounds: cellobiose, glucose, xylose, arabinose, formic acid, acetic acid, 2-furfuraldehyde (FF), and 5-hydroxymethyl-2-furfuraldehyde (HMF). The analyses of the sugars were carried out in a high-performance liquid chromatography (HPLC) model SHIMADZU. The mixture was composed of 0.005 mol L^{-1} H_2SO_4 and the eluent flow was 0.6 mL min^{-1}. The column temperature was maintained at 45 °C in a SHIMADZU CTO-10A furnace [19].

Solid-State Fermentation

The enzyme production was performed by SSF employing a consortium of mesophilic fungi *A. niger* SCBM1 and *A. fumigatus* SCBM6 as inocula, previously isolated from SCB piles and identified by gene sequencing [20]. These strains were obtained from the Laboratory of Environmental Microbiology, Federal University of Uberlândia (LAMIC-UFU, Minas Gerais, Brazil), and maintained in Petri dishes containing sterile Sabouraud agar medium at 30 °C.

For SSF, 2.5 g of *in natura* SCB and 2.5 g of crude WB were used as substrates, plus 5 mL of nutrient solution, composed by 0.5% of ammonium sulfate, 0.3% of potassium monobasic, 0.05% of magnesium sulfate heptahydrate, and 0.05% of calcium chloride per liter of distilled water. The mixture was autoclaved for 20 min, and then, similar three mycelial disks of around 0.5 cm in diameter/disk from each fungal strain precultured in Sabouraud agar plates were added in nutrient solution (final volume of 20 mL) according to Manfrin et al. (2018).

The SSF was executed for 7 days at 30 °C. After each 24 h of cultivation, a volume of 50 mL of distilled water was added to the flasks for extraction of the enzymes, followed by mechanical stirring on an orbital shaker at 200 rev min^{-1} for 1 h. The material was filtered, centrifuged for 30 min at 5000 rev min^{-1} and the supernatant containing the crude enzymatic extract was collected and aliquoted at − 20 °C. All experiments were performed in triplicate.

Enzymatic Assays

After SSF, the activities of cellulases (β-glucosidase and carboxymethylcellulose/endoglucanase) and hemicellulases (xylanase and β-xylosidase) were quantified. The activities were expressed in units of enzymatic activity per gram of dry substrate (U g^{-1}). All assays were performed in triplicate. The quantification of β-glucosidase was performed at 60 °C in a reaction mixture containing 50-μL crude enzyme extract, 250-μL 0.1 mol L^{-1} sodium acetate buffer, pH 5.5, and 250 μL ρ-nitrophenyl-β-D-glucopyranoside 4 m mol L^{-1} (PNPG, Sigma-Aldrich) as substrate. After 10 min, the reaction was stopped by adding of 2 mL of 2 mol L^{-1} sodium carbonate solution. The concentration of the released ρ-nitrophenol (pNP) was determined by spectrophotometry at 410 nm. One unit of β-glucosidase activity (U) was defined as the amount of enzyme required to release 1 μmol of ρNP per min of reaction from a standard curve of pNP [20].

Endoglucanase activity (CMCase) was determined by the 3.5-dinitrosalicylic acid (DNS) method in a reaction mixture containing 30 μL of crude enzyme extract and 270 μL of 1% carboxymethylcellulose (CMC, Sigma-Aldrich), respectively, prepared in 0.1 mol L^{-1} sodium citrate buffer, pH 4.5 [21]. The reactions were conducted at 40 °C for 10 min and stopped by the addition of 300 μL of DNS. Afterwards, the samples were cooled in an ice bath and the final volume (3 mL) was completed with 2400 μL of distilled water. One unit of CMCase activity was defined as the amount of enzyme required to release 1 μmol of glucose per min of reaction, obtained from a standard glucose curve [13].

The total cellulase activity per filter paper units (FPU) was determined in a reaction mixture containing 1 mL of 0.05 mol L^{-1} citrate buffer, pH 4.8, 0.5 mL of enzyme extract, and a filter paper tape of 1 cm wide by 6 cm long, weighing approximately 50 mg during 60 min. Afterwards, the reaction was stopped by the addition of 3 mL of DNS and the final volume was homogenized and brought to the boiling bath for 5 min. Then, the samples were taken to the ice bath and 20 mL of distilled water was added and homogenized. After 20 min, the filter paper was macerated with a glass stick. The amount of glucose released was measured at 540 nm [22].

The β-xylosidase dosage was determined by the same procedure for β-glucosidase, however, using p-nitrophenyl-β-D-xylopyranoside (PNPX, Sigma-Aldrich) as substrate. One unit of β-xylosidase activity (U) was defined as the amount of enzyme required to release 1 μmol of ρNP per min under the assay conditions from a standard curve [20]. Xylanase activity was determined by the same method employed to quantify endoglucanase, but with 1% xylan (Sigma-Aldrich) as substrates [22]. The amount of xylose released was measured by spectrophotometry at 540 nm. One unit of xylanase activity was defined as the amount of enzyme required to release 1 μmol of xylose per min of reaction, obtained from a standard xylose curve [13].

Enzymatic Hydrolysis of Biomass Sorghum

Crude and free of extractives biomass sorghum were submitted to enzymatic hydrolysis in 50-mL Erlenmeyer flasks containing 3% *w*/*w* dry solid, sealed with rubber stopper, in 0.1 mol L^{-1} sodium citrate buffer, pH 4.5, with 10 FPU g^{-1} cellulose (dry basis) of AFN enzymatic extract in a final volume of 20 mL. The mixture was incubated at 50 °C at 150 rev min^{-1} during 12 and 24 h in triplicate. All hydrolysates were filtered, centrifuged at 5000 rev min^{-1} for 10 min [36]. The concentrations of sugars and inhibitors released in the hydrolysates were quantified by HPLC in a SHIMADZU chromatograph. The mixture was composed of 0.005 mol L^{-1} H_2SO_4 and the eluent flow was of 0.6 mL min^{-1}. The column temperature was maintained at 45 °C in a SHIMADZU CTO-10A furnace [19].

Results and Discussion

Chemical Characterization of Biomass Sorghum

Samples of raw and free of extractive biomass sorghum were characterized for ash, extractive, lignin, hemicellulose, and cellulose contents. In BS, 13.11% of extractives, 30.72% of cellulose, 28.49% of hemicelluloses, and 24.71% of total lignin were observed (Table 1). In ES, there was a slight reduction in the lignin content (about 7%). It was also observed that the polysaccharide fraction was preserved in ES, with a small increase due to the redistribution of the masses (Table 1).

The chemical characterizations were compared with those in other studies. Castro et al. [23] evaluated the chemical composition of three cultivars of sorghum and observed close values for lignin content (between 19.6 and 22.2%) to that one in the present study (Table 1). However, different values for cellulose (between 42.4 and 44.5%) and hemicelluloses (between 18.7 and

Table 1 Characterization of raw (BS) and extracted (ES) biomass sorghum

Composition (%)	BS	ES
Ash	3.40 ± 0.11	4.35 ± 0.10
Extractives	13.11 ± 0.08	0.00 ± 0.12
Cellulose	30.72 ± 0.16	32.11 ± 0.19
Hemicellulose	28.49 ± 0.20	34.66 ± 0.17
Insoluble lignin	21.01 ± 0.18	19.67 ± 0.20
Soluble lignin	3.70 ± 0.22	2.95 ± 0.21
Mass balance	100.43 ± 0.10	93.74 ± 0.16

19.5%) were found by these authors. Koradiya et al. [24] also reported different percentages of polysaccharides in sorghum: 43.8% cellulose and 24.55% hemicelluloses. Corredor et al. [25] characterized four types of forage sorghum for the production of fermentable sugars. Among them, all four varieties of sorghum presented cellulose content between 24 and 38%, values compatible with the percentages found in the present study both for BS as ES.

Enzyme Assays

In the present study, significant activities were obtained for the investigated enzymes after SSF with *A. niger* SCBM1 and *A. fumigatus* SCBM6 consortium. Regarding hemicellulases, the maximum peak of xylanase production (375 U g^{-1}) was observed at 96 h of fermentation (Fig. 1). In the period between 72 and 120 h, about 95% of the activity was maintained, and from 120 h, there was a marked decrease in the production of this enzyme (Fig. 1). For β-xylosidase, the production remained high and constant throughout the fermentation (150 U g^{-1}), with maximum peak in 48 h. *Aspergillus* strains have been described as good xylanase producers in previous studies [20, 22, 26]. Lamounier et al. [27] obtained similar values of maximum xylanase production (544.46 U g^{-1}) in 96 h of fermentation, with SCB and WB as substrates at the same proportions and with isolated *Aspergillus* strains. Moretti et al. [26] observed maximum production of xylanase by *A. fumigatus* M.7.1 of 1040.0 U g^{-1} in 144 h of culture at the same conditions. Rodrigues et al. [13] obtained a peak of 78.00 U g^{-1} of β-xylosidase by *A. niger* SCBM1 in 144 h of SSF also with SCB and WB in the same proportions. However, the isolated *A. fumigatus* SCBM6 strain did not produce this enzyme. Dos Santos et al. [20] also analyzed the production of β-xylosidase and xylanase by *A. niger* SCBM3, using the same substrates and obtained lower values than those found in the present study (73 and 39 U g^{-1} after 144 and 48 h of SSF, respectively). These authors also reported the production of β-xylosidase (9 U g^{-1}) and xylanase (19 U g^{-1}) by *A. sydowii* SCBM7 with production peaks between 144 and 96 h, respectively. Thus, our data demonstrated that the growth of *A. niger* SCBM1 and *A. fumigatus* SCBM6 in a consortium was favorable to the induction of hemicellulases, mainly β-xylosidase with a very high production at the beginning of the fermentation (Fig. 1).

For cellulase production, the maximum peak of β-glucosidase was observed in 24 h of fermentation (80 U g^{-1}), with a high decrease after 48 h. After that period, the activity increased between 72 and 96 h (Fig. 2). Afterwards, the activity substantially decreased. This result may indicate the presence of two β-glucosidases in the extract; probably one produced by *A. niger* SCBM1 and the other by *A. fumigatus* SCBM6. For CMCase, the biosynthesis peak (80 U g^{-1}) was observed in 144 h of fermentation (Fig. 2).

Previous studies have also demonstrated the capacity of *Aspergillus* strains as good cellulase producers. Santos et al. [17] selected three species as good β-glucosidase producers: *A. sydowii* SBCM7 and *A. niger* SBCM3 with peaks of production in 240 h of culture (60 and 54 U g^{-1}, respectively) and *A. fumigatus* SBC4 after 96 h (54 U g^{-1}). However, the values of β-glucosidase activity obtained by these authors were inferior to that found in the present work (Fig. 2). Rodrigues et al. [13] evaluated the production of β-glucosidase by the same isolated strains under the same conditions and observed a maximum yield of 78.47 U g^{-1} after 120 h by *A. fumigatus* SCBM6, whereas *A. niger* SCBM1 showed a peak of 43.02 U g^{-1} in 144 h. Comparing the production obtained in the present work with these previous studies, we observed that the growth of fungi in a consortium was an important factor to activate the β-glucosidase production, since higher activity (80 U g^{-1}) was observed at the beginning of the fermentation with the fungal consortium (Fig. 2). Other authors also evaluated β-glucosidase

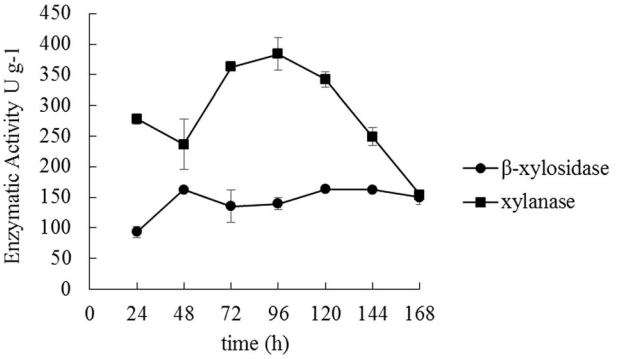

Fig. 1 Production of hemicellulases by SSF by fungal consortium (*A. niger* SCBM1 e *A. fumigatus* SCBM6). Enzymatic activity is expressed as U g^{-1}. Values represent mean values of experiments in triplicate ($p < 0.05$)

production by *Aspergillus* strains separately and obtained lower values of β-glucosidase than in the present study. Moretti et al. [26] observed maximum production of β-glucosidase of 5.0 U g^{-1} by *A. fumigatus* M.7.1 in 96 h of culture, also with the same substrates. Bansal et al. [28] evaluated the production of β-glucosidase by *A. niger* NS-2 using only SCB as substrate and obtained only 3 U g^{-1} in 96 h of fermentation. Thus, comparing our results with literature, the obtained data suggested that the cultivation of both species in a consortium favored the β-glucosidase production.

The CMCase biosynthesis was compared to other studies. Basso et al. [29] studied CMCase production by SSF with SCB as substrate (28 °C/15 days) using two strains of *Trichoderma reesei* and obtained lower values than those found in the present work. The strain *T. reesei* RUT C30 produced 3.5 U g^{-1} and QM9414 strain produced 5.7 U g^{-1}. Oliveira et al. [30] used SCB, green coconut husk, and cashew bagasse as substrates for the production of cellulases by *Aspergillus fumigatus* 5.8S and observed that the highest values for CMCase were 4.20 U g^{-1} for SCB, 4.07 U g^{-1} for coconut shell, and 0.64 U g^{-1} for cashew bagasse; values also inferior than those obtained by the *Aspergillus* consortium of the present study.

With respect to FPase, the production remained constant with maximum peak (4 FPU g^{-1}) in 144 h of fermentation (Fig. 3). Previous studies reported similar results. Saladini et al. [3] cultivated *A. fumigatus* SK1 strain in oil palm and found maximum activity of 3.36 FPU g^{-1} in 264 h of SSF, a comparable value to that obtained in this work. Soni et al. [31] studied the

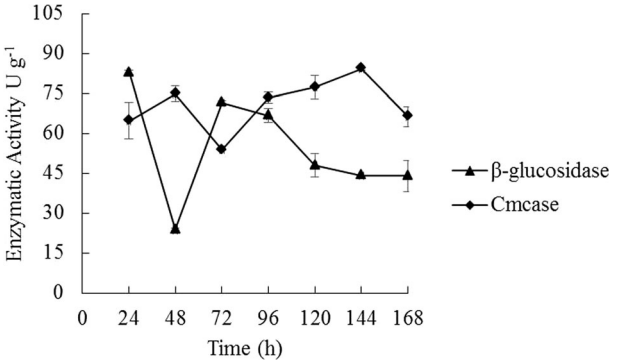

Fig. 2 Production of cellulases by SSF by fungal consortium (*A. niger* SCBM1 e *A. fumigatus* SCBM6). Enzymatic activity is expressed as U g^{-1}. Values represent mean values of experiments in triplicate ($p < 0.05$)

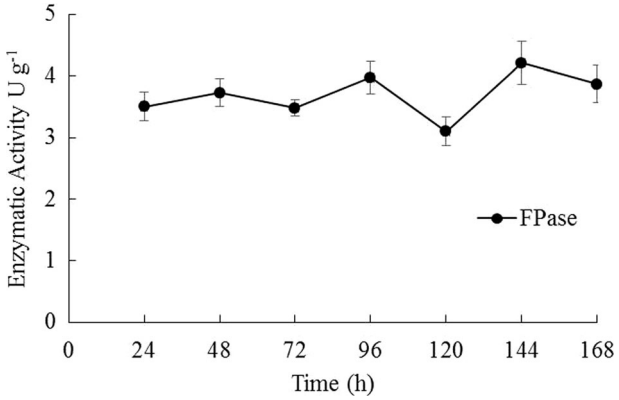

Fig. 3 Production of FPase by SSF by fungal consortium (*A. niger* SCBM1 e *A. fumigatus* SCBM6). Enzymatic activity is expressed as U g^{-1}. Values represent mean values of experiments in triplicate ($p < 0.05$)

production of cellulases by *A. fumigatus* AMA by SSF employing SCB as substrate and observed 1.43 FPU g^{-1}; value lower than that obtained in the present study. Falkoski et al. [32] cultivated *Chrysoporthe cubensis* in WB and obtained 2.5 FPU g^{-1} in 96 h of SSF; activity also inferior to that obtained in the present study. Dias et al. [22] evaluated the FPase production by *A. niger* SCBM1 using biomass sorghum as substrate and also found inferior FPase peak (2.11 FPU g^{-1} in 144 h of fermentation). Thus, it was concluded that the growth of the fungal strains *A. niger* SCBM1 and *A. fumigatus* SCBM6 by SSF in SCB and WB as substrates was favorable for the production of hemicellulolytic and cellulolytic enzymes, with yields higher than those found in prior studies [20, 33].

Enzymatic Hydrolysis

In this work, enzymatic hydrolysis of the biomass sorghum was carried out before and after the removal of the extractives employing AFN extract produced in the previous step by consortium among *A. niger* SCBM1 and *A. fumigatus* SCBM6. The results demonstrated that the release of monosaccharides (glucose and xylose) was superior in ES than in BS (Table 2). In ES hydrolysate, an increase of around 10× in glucose production was obtained in comparison to BS hydrolysate (2.5 g L^{-1} after 12 h of hydrolysis). It was also observed that the presence of inhibitor compounds of hydrolysis was also inferior in ES than in BS hydrolysates (mainly

Table 2 Production of sugars and inhibitors after enzymatic hydrolysis of raw (BS) and extracted (ES) biomass sorghum. Values expressed in g L^{-1}

Compounds	BS		ES	
	12 h	24 h	12 h	24 h
Cellobiose	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00
Glucose	0.186 ± 0.09	0.000 ± 0.00	2.538 ± 0.10	0.685 ± 0.11
Xylose	0.494 ± 0.08	0.000 ± 0.00	0.832 ± 0.11	0.000 ± 0.19
Arabinose	0.285 ± 0.23	0.000 ± 0.27	0.761 ± 0.19	0.454 ± 0.21
Formic acid	0.468 ± 0.09	0.332 ± 0.05	0.000 ± 0.00	0.000 ± 0.00
HMF	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00
FF	0.000 ± 0.00	0.010 ± 0.06	0.004 ± 0.08	0.000 ± 0.00

formic acid), indicating that these compounds might have been removed together to extractives (Table 2). These results indicated that the step of removal of extractives may have positively influenced the effectiveness of the saccharification.

Enzymatic hydrolysis is an advantageous technique, since the reactions are highly specific, minimizing the generation of undesirable by-products [34]. In addition, it provides good yields in glucose with low by-product formation, favoring the use of the hydrolysate in bioconversion processes [35]. Here, the results indicated that the extractives may influence the saccharification efficiency, so that in ES hydrolysates, increase in xylose and glucose production was observed (Table 2). This increase was more expressive (about 10×) in glucose production. Since it is the first report of the use of biomass sorghum in enzymatic hydrolysis experiments, our data were compared to hydrolysis studies performed with other lignocellulosic biomass sources. The liberation of glucose observed here was superior to that found by Pereira et al. [36] in the hydrolysis of pre-treated ozonolysis sugarcane bagasse using a commercial enzymatic combination (Celluclast 1.5 L and Novozym 188) in the proportion of 10 FPU and 30 CBU per g cellulose and also using an extract of *Myceliophthora thermophila* JCP1-4 in the same proportions (0.95 and 1.40 g L^{-1}, respectively). Nascimento et al. [37] performed enzymatic hydrolysis with 10% NaOH pretreated SCB with 20 FPU g^{-1} of Accelerase 1500 enzyme and obtained 38.8 g L^{-1} of glucose in the hydrolysate, value much higher than that found in the present work. However, these authors employed a commercial enzyme cocktail and the double of enzymatic load in hydrolysis, which may increase the cost of the process. Moreover, these authors employed SCB previously submitted to alkaline pre-treatment, step which can favor the enzymatic release of reducing sugars but can also increase the cost of the method and, consequently, of the final product.

Thus, comparing our results with those in previous studies, we concluded that the amount of glucose released after the hydrolysis of biomass sorghum free of extractives was satisfied, since the material was only submitted to the withdrawal of extractives, without the necessity of a pre-treatment stage. Furthermore, the use of an enzymatic consortium produced through the co-cultivation of *A. niger* SCBM1 and *A. fumigatus* SCBM6 by SSF with agroindustrial residues could also positively contribute to the economic viability of the process.

It was also observed that the removal of extractives may have also positively contributed to the decrease of inhibitor compounds of hydrolysis, such as formic acid (Table 2). Previous studies have shown that compounds originally present in the extractive fraction, such as short chain carboxylic acids, can act as inhibitors of hydrolysis and fermentation processes [38]. Thus, the increase in glucose and xylose production after hydrolysis of ES may also be related to the removal of these compounds. Other compounds such as furanic derivatives (HMF and FF) are among the most important inhibitory compounds of hydrolysis [39]. However, in our study, these compounds were obtained in very low amounts, mainly in free of extractive biomass sorghum hydrolysates. Neves et al. [40] compared the composition of raw and ethanol extracted SCB and observed that HMF and FF decreased considerably in the sample of extracted SCB. In conclusion, these results indicated that only the stage of removal of extractives was sufficient to favor the decrease in the concentration of these compounds.

Conclusions

In the present study, significant amounts of CMCase, β-glycosidase, and β-xylosidase were obtained in AFN extract after the cultivation of *A. niger* SCBM1 and *A. fumigatus* SCBM6 strains in consortium by SSF. After saccharification with AFN extract, expressive increases in

glucose, xylose, and arabinose release were obtained in hydrolysates of free of extractive sorghum biomass in comparison to crude biomass sorghum hydrolysates. The highest glucose yield and lowest inhibitor formation were obtained after 12 h of hydrolysis.

Acknowledgments The authors thank to Dr. Bruno Eduardo Lobo Baeta for the assistance in chemical characterization of sorghum biomass.

Funding Information This study received financial support from Minas Gerais State Foundation for Research Development-FAPEMIG (PRONEM-FAPEMIG-APQ-01360-14).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Karagöz, P., Rocha, I. V., Özkan, M., & Angelidaki, I. (2012). Alkaline peroxide pretreatment of rapeseed straw for enhancing bioethanol production by same vessel Saccharification and co-fermentation. *Bioresource Technology, 104*, 349–357.
2. Saladini, F., Patrizi, N., Pulselli, F. M., & Marchettini, N. (2016). Guidelines for emergy evaluation of fi rst , second and third generation biofuels. *Renewable and Sustainable Energy Reviews, 66*, 221–227.
3. Toquero, C., & Bolado, S. (2014). Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing. *Bioresource Technology, 157*, 68–76.
4. Thangavelu, S. K., Ahmed, A. S., & Ani, F. N. (2016). Review on bio ethanol as alternative fuel for spark ignition engines. *Renewable and Sustainable Energy Reviews, 56*, 820–835.
5. Carrillo, M. A., Staggenborg, S. A., & Pineda, J. A. (2014). Washing sorghum biomass with water to improve its quality for combustion. *Fuel., 116*, 427–431.
6. Dahlberg, J. (2019). The role of sorghum in renewables and biofuels. In Humana Press (Ed.), *Sorghum Methodos Protoc*, 1st edn (pp. 269–277).
7. Parrella, R. A. C. (2011). Desempenho agronômico de híbrido de sorgo biomassa. Sete Lagoas: Embrapa Milho e sorgo. *Boletim de Pesquisa e Desenvolvimento*. 41, 19p.
8. May, A., Parrela, R. A. C., Damasceno, C. M. B., & Simeone, M. L. F. (2014). Sorgo como matéria-prima para produção de bioenergia: etanol e cogeração. *Informe Agropecuário., 35*(278), 73–81.
9. Demirbaş, A. (2003). Relationships between lignin contents and fixed carbon contents of biomass samples. *Energy Conversion and Management, 44*(9), 1481–1486.
10. Chandel, A. K., Singh, O. V., Narasu, M. L., & Rao, L. V. (2011). Bioconversion of Saccharum spontaneum (wild sugarcane) hemicellulosic hydrolysate into ethanol by mono and co-cultures of *Pichia stipitis* NCIM3498 and thermotolerant Saccharomyces cerevisiae-VS3. *New Biotechnology, 28*(6), 593–599.
11. Alfenore, S., & Molina-Jouvea, C. (2016). Current status and future prospects of conversion of lignocellulosic resources to biofuels using yeasts and bacteria. *Process Biochemistry, 51*(11), 1747–1756.
12. Gomes, A. F. S., dos Santos, B. S. L., Franciscon, E. G., & Baffi, M. A. (2016). Substrate and temperature effect on xylanase production by aspergillus fumigatus using low cost agricultural wastes. *Bioscience Journal*, 915–921.
13. Rodrigues, P. d. O., Pereira, J. d. C., dos Santos, D. Q., Gurgel, L. V. A., Pasquini, D., & Baffi, M. A. (2017). Synergistic action of an Aspergillus (hemi-) cellulolytic consortium on sugarcane bagasse saccharification. *Industrial Crops and Products, 109*, 173–181.
14. Rocha, G. J. d. M., Nascimento, V. M., Gonçalves, A. R., Silva, V. F. N., & Martín, C. (2015). Influence of mixed sugarcane bagasse samples evaluated by elemental and physical-chemical composition. *Industrial Crops and Products, 64*, 52–58.
15. Tomás-Pejó, E., Alvira, P., Ballesteros, M., & Negro, M. J. (2011). Pretreatment technologies for lignocellulose-to-bioethanol conversion. *Biofuels*, 149–176.
16. Kim, J. S., Lee, Y. Y., & Kim, T. H. (2016). A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource Technology, 199*, 42–48.
17. Santos, F. A., Queiróz, J. H., Colodette, J. L., Fernandes, S. A., Guimarães, V. M., & Rezende, S. T. (2012). Potencial da palha de cana-de-açúcar para produção de etanol. *Química Nova., 35*(5), 1004–1010.

18. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2012). *Determination of structural carbohydrates and lignin in biomass: Laboratory analytical Producere (LAP), NREL/TP-510-42618*. Golden: National Renewable Energy Laboratory (NREL).
19. Baêta, B. E. L., Lima, D. R. S., Ardame, O. F. H., Gurgel, L. V. A., & de Aquino, S. F. (2016). Optimization of sugarcane bagasse autohydrolysis for methane production from hemicellulose hydrolyzates in a biorefinary concept. *Bioresource Technology, 200*, 137–146.
20. Dos Santos, B. S. L., Gomes, A. F. S., Franciscon, E. G., De Oliveira, J. M., & Baffi, M. A. (2015). Thermotolerant and mesophylic fungi from sugarcane bagasse and their prospection for biomass-degrading enzyme production. *Brazilian Journal of Microbiology, 46*(3), 903–910.
21. Baffi, M. A., Tobal, T., Henrique, J., Lago, G., Leite, R. S. R., Boscolo, M., Gomes, E., & Da-Silva, R. (2011). A novel β-glucosidase from *Sporidiobolus pararoseus*: Characterization and application in winemaking. *Journal of Food Science, 76*, 997–1002.
22. Dias, L. M., dos Santos, B. V., Albuquerque, C. J. B., Baeta, B. E. L., Pasquini, D., & Baffi, M. A. (2017). Biomass sorghum as a novel substrate in solid-state fermentation for the production of hemicellulases and cellulases by Aspergillus Niger and A. Fumigatus. *Journal of Applied Microbiology, 124*, 708–718.
23. Castro, E., Nieves, I. U., Rondón, V., Sagues, W. J., Fernández-Sandoval, M. T., Yomano, L. P., York, S. W., Erickson, J., & Vermerris, W. (2017). Potential for ethanol production from different sorghum cultivars. *Industrial Crops and Products, 109*, 367–373.
24. Koradiya, M., Duggirala, S., Tipre, D., & Dave, S. (2016). Pretreatment optimization of Sorghum pioneer biomass for bioethanol production and its scale-up. *Bioresource Technology, 199*, 142–147.
25. Corredor, D. Y., Salazar, J. M., Hohn, K. L., Bean, S., Bean, B., & Wang, D. (2009). Evaluation and characterization of forage sorghum as feedstock for fermentable sugar production. *Applied Biochemistry and Biotechnology, 158*(1), 164–179.
26. Moretti, M. M. S., Bocchini-Martins, D. A., Da Silva, R., Rodrigues, A., Sette, L. D., & Gomes, E. (2012). Selection of thermophilic and thermotolerant fungi for the production of cellulases and xylanases under solid-state fermentation. *Brazilian Journal of Microbiology, 43*(3), 1062–1071.
27. Lamounier, K. F. P., Rodrigues, P. O., Pasquini, D., & Baffi, M. A. (2018). Saccharification of sugarcane bagasse using an enzymatic extract produced by *Aspergillus fumigatus*. *Journal of Renewable Materials, 6*(2), 169–175.
28. Bansal, N., Tewari, R., Soni, R., & Soni, S. K. (2012). Production of cellulases from Aspergillus niger NS-2 in solid state fermentation on agricultural and kitchen waste residues. *Waste Management, 32*(7), 1341–1346.
29. Basso, T. P., Gallo, C. R., & Basso, L. C. (2010). Atividade celulolítica de fungos isolados de bagaço de cana de açúcar e madeira em decomposição. *Pesquisa Agropecuária Brasileira, 45*(11), 1282–1289.
30. Oliveira Júnior, S. D., Padilha, C. E. A., De Macedo, G. R., Asevedo, E. A., Carvalho, V. P., & Araujo, F. R. (2018). Utilization of agroindustrial residues for producing celulases by Aspergillus fumigatus on semi-solid fermentation. *Journal of Environmental Chemical Engineering, 6*(1), 937–944.
31. Soni, R., Nazir, A., & Chadha, B. S. (2010). Optimization of cellulase production by a versatile Aspergillus fumigatus fresenius strain (AMA) capable of efficient deinking and enzymatic hydrolysis of Solka floc and bagasse. *Industrial Crops and Products, 31*(2), 277–283.
32. Falkoski, D. L., Guimarães, V. M., de Almeida, M. N., Alfenas, A. C., Colodette, J. L., & de Rezende, S. T. (2013). Chrysoporthe cubensis: A new source of cellulases and hemicellulases to application in biomass saccharification processes. *Bioresource Technology, 130*, 296–305.
33. Ang, S. K., Shaza, E. M., Adibah, Y., Suraini, A. A., & Madihah, M. S. (2013). Production of cellulases and xylanase by *Aspergillus fumigatus* SK1 using untreated oil palm trunk through solid state fermentation. *Process Biochemistry, 48*(9), 1293–1303.
34. Klinke, H. B., Olsson, L., Thomsen, A. B., & Ahring, B. K. (2003). Potential inhibitors from wet oxidation of wheat straw and their effect on ethanol production of Saccharomyces cerevisiae: Wet oxidation and fermentation by yeast. *Biotechnology and Bioengineering, 81*(6), 738–747.
35. Martin, C., Almazan, O., Marcet, M., & Jonsson, L. J. (2007). A study of three strategies for improving the fermentability of sugarcane bagasse hydrolysates for fuel ethanol production. *International Sugar Journal, 109*, 33–33.
36. Pereira, J. C., Travaini, R., Marques, N. P., Bolado-Rodríguez, S., & Martins, D. A. B. (2016). Saccharification of ozonated sugarcane bagasse using enzymes from *Myceliophthora thermophila* JCP 1-4 for sugars release and ethanol production. *Bioresource Technology, 204*, 122–129.
37. Nascimento, V. M., Manrich, A., Tardioli, P. W., de Campos Giordano, R., de Moraes Rocha, G. J., & Giordano, R. d. L. C. (2016). Alkaline pretreatment for practicable production of ethanol and xylooligosaccharides. *Bioethanol, 2*, 112–125.
38. Travaini, R., Otero, M. D. M., Coca, M., Da-Silva, R., & Bolado, S. (2013). Sugarcane bagasse ozonolysis pretreatment: Effect on enzymatic digestibility and inhibitory compound formation. *Bioresource Technology, 133*, 332–339.

39. Moreira, L. R. S., Milanezi, N. V. G., & Filho, E. X. F. (2011). Enzymology of plant Cell Wall breakdown: An update. In M. S. E. Buckeridge & G. H. Goldman (Eds.), *Routes to Cellulosic Ethanol* (Vol. 6, pp. 73–96). New York: Springer.
40. Neves, P. V., Pitarelo, A. P., & Ramos, L. P. (2016). Production of cellulosic ethanol from sugarcane bagasse by steam explosion: Effect of extractives content, acid catalysis and different fermentation technologies. *Bioresource Technology, 208*, 184–194.