Bioconversion of cassava bagasse and sugarcane bagasse using cheap home-made enzymatic cocktails

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Research Article

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Abstract

The agricultural industries generate lignocellulosic wastes that can be modified by fungi to generate high value-added products. The aim of this work was to analyze the efficiency of the bioconversion of sugarcane bagasse and cassava bagasse using two cheap home-made enzymatic cocktails from *Aspergillus niger* LBM 134 (produced also from agroindustrial wastes) and compare the hydrolysis yield with that obtained from the bioconversion using commercial enzymes. Sugarcane bagasse and cassava bagasse were pretreated with a soft alkaline solution before the hydrolysis carried out with home-made enzymatic cocktails of *A. niger* LBM 134 and with commercial enzymes to compare their performances. Mono and polysaccharides were analyzed before and after the bioconversion of both bagasses as well as their microscopic structure. The maximal yield was the 80% of total glucans saccharified from cassava bagasse. The bioconversion of both bagasses were better when we used the home-made enzymatic cocktails than commercial enzymes. We obtained high added-value products from agroindustrial wastes, home-made enzymatic cocktails and hydrolysates rich in fermentable sugars. The importance of this work lays in the higher performance of the cheap home-made enzymatic cocktails over the hydrolytic performance of commercial

29 enzymes due to the cost of producing the home-made enzymatic cocktails were more
30 than 500 times lower than commercial enzymes.

31 **Keywords:** agroindustrial wastes, *Aspergillus niger*, pretreatment, enzymatic
32 hydrolysis, bioethanol simulation, cost-effective process

33 1. Introduction

34 Biomass is the core of the bioeconomy concept where the efficient and sustainable
35 use of this renewable resource constitutes the basis of bioeconomy development [1]. In
36 this context, biorefineries are a key pillar in the development of a future bioeconomy-
37 based society based on the development of biorefineries to produce biofuels and
38 bioproducts from renewable biomass sources and efficient bioprocesses to achieve
39 sustainable production [1]. Renewable feedstocks can be obtained from primary
40 biomass sources or wastes derived from household, industrial and agricultural activities.
41 Using wastes from agricultural activities adds value to the whole chain and those from
42 worldwide crops are an interesting resource.

43 Cassava (*Manihot esculenta Cranz*) and sugarcane (*Saccharum* sp.) are two of the
44 major tropical and subtropical agricultural crops [2]. The root of cassava is processed
45 to isolate the starch or to sell cassava as a pre-cooked meal [3]. The industry of cassava

46 generates CB as one of the solid by-products; this waste is a problem due to its high
47 percentage of water, which makes more expensive drying and transporting operations
48 [3]. Sugarcane is used for sugar and bioethanol 1G production and SCB is one of the
49 by-products of this industry. Both CB and SCB are generated in large quantities by their
50 respective industries [4]. The improper disposal of these material represents an
51 environmental problem increasing the pollution; however, these agroindustrial wastes
52 can be used for obtaining added-value products while reducing the environmental
53 pollution [5]. The starch, cellulose and hemicelluloses in CB and SCB can be converted
54 into monomeric sugars that can fermented into bioethanol [6, 7].

55 The conversion of hemicellulosic biomass to bioethanol involves a pretreatment to
56 open up the biomass structure following by an acid or enzymatic hydrolysis of the
57 complex carbohydrates into simple sugars and their fermentation into ethanol that must
58 be purified for its use as a fuel [5]. The enzymatic hydrolysis has advantages over the
59 acidic hydrolysis, the enzymatic hydrolysis requires less energy and milder
60 environmental conditions and does not require harsh conditions or high temperature
61 and pressure [5, 8]. Moreover, the use of enzymes, i.e., cellulases and hemicellulases is
62 the most promising method for hydrolysis of polysaccharides to monomer sugars due

63 to hemicellulases facilitate cellulose hydrolysis by exposing the cellulose fibers, thus
64 making them more accessible and promoting the commercial production of
65 lignocellulosic ethanol [9]. However, the cost of enzymes production is one of the most
66 important factors that improve the total costs in the bioethanol production [10, 11].
67 Therefore, research have focused on reducing the costs of enzymes by improving the
68 activity of enzymes or by proposing new low-cost enzymatic cocktails that can perform
69 the conversion of polysaccharides to fermentable monosaccharides [12].

70 The current challenge on SCB and CB hydrolysis consist in using enzymatic
71 cocktails instead of pure commercial enzymes due to many enzymatic classes are
72 required to convert agroindustrial wastes such as SCB and CB into fermentable sugars
73 [12, 13]. The home-made enzymatic cocktails of *A. niger* LBM 134 grown on SCB and
74 CB were selected to carrying out the hydrolysis of these two agroindustrial wastes. The
75 rationale for using these home-made cocktails was the saccharification potential they
76 presented because the wide spectrum of enzymes they showed [14, 15].

77 In this context, the aims of this work were to analyze the efficiency of the
78 conversion of two agroindustrial wastes, SCB and CB using two home-made enzymatic
79 cocktails of *A. niger* LBM 134 grown on the respective agroindustrial wastes and to

compare these conversions with that carried out with commercial enzymes.

2. Materials and methods

2.1 Fungal material

The fungus *A. niger* LBM 134 was isolated from rotten wood of Misiones rainforest and deposited in the collection of the Molecular Biotechnology Laboratory (LBM, from Spanish *Laboratorio de Biotecnología Molecular*), of the Biotechnology Institute Misiones "María Ebe Reca", National University of Misiones. Stock cultures were maintained in 39 g L^{-1} potato dextrose agar medium (PDA) at 28 °C under static conditions until its mycelial development and conserved at 4 °C.

2.2 Feedstock preparation and chemical composition analysis

Two different types of agroindustrial wastes were used: sugarcane bagasse (SCB) and cassava bagasse (CB), both generated by the agroforestry industries of Misiones (Argentina). SCB was sampled from a sugarcane mill at San Javier locality and CB was donated by San Alberto Cooperative in Puerto Rico, Misiones. SCB and CB were dried at 60 °C overnight, respectively, and milled to produce material retained through a 40-mesh screen.

The chemical composition of raw material was determined according to the

97 laboratory analytical procedure (LAP) and biomass analysis of the National Renewable
98 Energy Laboratory (NREL, https://www.nrel.gov). Carbohydrates were determined by
99 high performance liquid chromatography (HPLC). Concentration of sugars and acetic
100 acid (mg mL^{-1}) was calculated using standard curves of pure compounds (Sigma-
101 Aldrich, USA): glucose, cellobiose, xylose, arabinose and acetic acid. All results are
102 expressed on a dry wood basis (OD).

103 *2.3 Fungal cultivation and preparation of home-made enzymatic cocktails*

104 To obtain the two home-made enzymatic cocktails, *A. niger* LBM 134 was grown
105 in two optimized media containing SCB and CB as carbon sources and incubated under
106 optimal conditions according to Díaz et al. [4]. Then, the culture broths were
107 centrifuged at 10,000 g for 20 min at 4 °C and clarified and sterilized by Chromafil
108 Xtra PET-20/25 (0.20 μm) filters (MachereyNagel; Düren, Germany) to obtain the cell-
109 free enzymatic cocktails and finally concentrated using 3 kDa Amicon Ultra centrifugal
110 filters (Merck KGaA; Darmstadt, Germany) to achieve the enzyme levels for carrying
111 out the hydrolysis assays.

112 *2.4 Effect of the bioprocess conditions on enzymatic stability activities*

113 The effect of the optimal temperature (30 °C) and pH (5.0) of the hydrolysis process

114 was evaluated on the stability of endoxylanase (EX), β-xylosidase (BXL), filter paper
115 activity (FPase) and β-glucosidase (BGL) activities in both home-made enzymatic
116 cocktails. For that, the enzymatic cocktails were incubated at 30 °C and pH 5.0 at
117 different intervals (6, 12, 24, 48, 72 and 96 h). Residual activity of each enzyme was
118 determined and expressed as a percentage, taking the initial enzymatic activity as 100%.
119 The buffer solution used was 0.05 M sodium acetate buffer for achieving pH 5.0.

120 *2.5 Determination of enzyme activities*

121 EX activity was determined according to Bailey [16] and FPase activity, according
122 to Ghose & Bisaria [17] through the quantification of released reducing sugars using
123 beechwood xylan (Sigma-Aldrich, USA) and Whatman no. 1 filter paper as substrates,
124 respectively. Reducing sugars were measured by 1,3-dinitrosalicylic acid (DNS) assay
125 [18] using xylose and glucose as standard curve for EX and FPU activities, respectively.
126 Absorbance was measured at 540 nm. EX activity was expressed as international units
127 (U), defined as the amount enzyme needed to produce 1 μmol of xylose per min at
128 50 °C while FPase activity was expressed as filter paper unit (FPU), defined as the
129 amount of enzyme releasing 1 μmol of reducing sugar from filter paper per min at 50 °C.
130 BGL activity was determined according to Ghose & Bisaria [17] using ρ-nitrophenyl-

β-D-glucobioside (PNPG) as substrate; and BXL activity was determined according to Ghose and Bisaria [17] using ρ-nitrophenyl-β-D-xylobioside (PNPX) as substrate, through the quantification of ρ-nitrophenol method. Absorbance was measured at 410 nm. BGL and BXL activities were expressed as U, defined as the amount of enzyme releasing 1 μmol of ρ-nitrophenol per min at 50 °C.

2.6 Bioconversion of SCB and CB

SCB and CB were pretreated with an alkaline solution of NaOH 0.85% (w/v) to remove lignin and avoid the holocellulose hydrolysis. For that, 10 g of bagasse was mixed with 200 mL of the alkaline solution for a consistence of 5% (w/v) at 121 °C during 30 min. Then, the bagasses were washed with water and 0.5 M sodium acetate buffer pH 5.0 at 80 rpm, 25 °C for 12 h; bagasses were dried at 45 °C during 24 h. The enzymatic hydrolysis of both agroindustrial wastes were carried out by the home-made enzymatic cocktails of *A. niger* LBM 134 and by commercial enzymes for comparing their performance. Also, two controls of these enzymatic hydrolysis were carried out: 1) incubation of bagasses without enzymes for determining the reducing sugars previous the hydrolysis; 2) incubation of the home-made enzymatic cocktails without the bagasses enzymes for determining the reducing sugars of the cocktails. The home-

148 made enzymatic cocktail for carrying out the hydrolysis of SCB was obtained from *A.*
149 *niger* LBM 134 grown on SCB and in the same way, the home-made enzymatic cocktail
150 for the CB hydrolysis was obtained from the fungus grown on CB. For that, 1 g of
151 pretreated bagasse was incubated with 25 mL of reaction solution consisting of 0.05 M
152 sodium acetate buffer pH 5.0, 30 °C and the corresponding enzymatic cocktail
153 containing (in Ug^{-1} of biomass): EX 300, FPU 10 and BGL 20. The commercial
154 enzymes used were EX of Xylanase (Sigma-Aldrich, USA) 300 U g^{-1}, FPU of
155 Celluclast (Sigma-Aldrich, USA) 10 U g^{-1} and BGL of Viscozyme (Sigma-Aldrich,
156 USA) 20 U g^{-1}. All the enzymatic hydrolysis and the control assays were carried out at
157 30 °C, pH 5.0, 200 rpm during 24 h without the addition of any antibiotic for no
158 increasing the cost of the bioprocess. After this period, the assays were vacuum filtered
159 and centrifugated at 12,000 g during 20 min. The resulting supernatants were used to
160 quantify reducing sugars with the DNS method [18] and to identify and quantify
161 monomeric sugars by HPLC analysis.
162 The values were presented as the means of the triplicates ± the standard deviation.
163 *2.7 Hydrolysis yield*
164 Saccharification percentages were calculated using reducing sugars with the

following equation [17]:

$$\%\ saccharification = \frac{reducing\ sugars\left(\frac{mg}{ml}\right)*0.9*100}{[S]\ (\frac{mg}{ml})} \quad \text{(Eq. 1)}$$

where, [S] is substrate concentration.

While saccharification percentage may be an acceptable measure of the rate of enzyme activity for calculations of enzymatic synergy, it does not indicate whether monomer sugars suitable for bioethanol production are present [19]. For that, hydrolysis percentages were determined based on the monomer sugars released after the hydrolysis of bagasses using the following equation proposed by the NREL:

$$\%\ Hydrolysis = \frac{glucose\ \ cellobiose\ or\ xylose\left(\frac{mg}{ml}\right)*100}{polysaccharides\ in\ the\ substrate*FC} \quad \text{(Eq. 2)}$$

where, FC corresponds to the conversion factor, that is 1.11 for glucose, 1.05 for cellobiose, and 1.13 for xylose.

2.8 Electron microscopic structure of SCB and CB before and after the hydrolysis

Bagasses were observed by scanning electron microscopy (SEM) to evaluate the changes in their microscopic structure during each step of the bioprocess: before and after of the alkaline pretreatment and after the hydrolysis with the home-made enzymatic cocktails and with the commercial enzymes. For that, 0.01 g of bagasses were fixed in each evaluated step with formaldehyde:alcohol:acid (FAA, 10:50:5).

182 Then, the samples were dehydrated with increasing concentrations of acetone solutions
183 and dried by the method of critical point with CO_2. Finally, the samples were metalized
184 with gold and observed with a scanner electron microscope (JEOL 5800LV).

185 *2.9 Simulation model for bioethanol production from SCB and CB*

186 Key parameters such as yield coefficients and rate constants used in the generic
187 flowsheet were assessed, based on experimental and theorical data. To establish a
188 simulation model for potential yield of bioethanol production from a combined
189 fermentation of glucose and xylose, experimental concentrations of these sugars
190 reported by Kamoldeen et al. [20] were used (Supplementary table 1).

191 The apparent reaction rate constants for each component were obtained using the
192 experimental concentration values of the components in a progressive reaction. A first
193 order reaction model for glucose and xylose decomposition and conversion rates were
194 proposed. These models were validated with experimental data from the work of
195 Kamoldeen et al. [20]. These models were used to stablish a simulation process of
196 bioethanol production.

197 *2.10 Statistical analysis*

198 The experimental and theorical results were analyzed and graphed with the software

199 GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

200 **3. Results and discussion**

201 *3.1 Characterization of SCB and CB*

202 The bioprocesses carried out in this study as a strategy to convert both SCB and CB
203 into enzymatic cocktails and fermentable sugars offered the possibility of obtaining
204 these high added-value products from agroindustrial wastes. Firstly, to know the
205 chemical composition of both SCB and CB for comparing then with monomeric sugars
206 after the enzymatic hydrolysis, the main components of the raw bagasses were
207 identified according to NREL analytical procedure (Table 1). SCB presented more
208 quantities of extractives (fat, proteins, wax), hemicelluloses and lignin than CB.
209 Conversely, CB had more glucans than SCB.

210 TABLE 1

211 *3.2 Characterization of the home-made enzymatic cocktails of A. niger LBM 134*

212 The pH and thermostability of the key enzymes involved in the hydrolysis of
213 lignocellulosic biomass was studied (FPase, BGL, EX and BXL) in the home-made
214 enzymatic cocktails of *A. niger* LBM 134 due to the pH and the temperature are two
215 main factors affecting the stability of the enzyme activity. The enzymes of both home-

made cocktails showed considerable stability, making them promising to be used in the bioconversion of SCB and CB.

The polysaccharide hydrolytic activities, FPU, BGL, EX and BXL, of the home-made enzymatic cocktails of *A. niger* LBM 134 were measured (Table 2) and the enzymatic levels demonstrated that these cocktails were suitable for carrying out the bioconversion of SCB and CB. Also, the effect of temperature (30 °C) and pH (5.0) on the stability of the enzyme activities were studied due to the importance of the enzymatic stability of in any bioprocess (Figure 1). Thermostability of enzymes was above 50% after 24 h (Figure 1a-b) and pH stability was above 50% after 24 h (Figure 1c-d). Therefore, the hydrolysis assays were carried out under these conditions: 30 °C and pH 5.0 for 24 h.

TABLE 2

FIGURE 1

3.3 Bioconversion of SCB and CB

Also, raw materials, SCB and CB, were extensively characterized hence the correct choice of any pretreatment strategy depends on knowing the fundamental biochemistry of the biomass and the desired products [21]. For that reasons, we employed a soft

233 alkaline pretreatment on SCB and CB guarantying a specific lignin removal and
234 preserving the polysaccharides into the sold fraction, a fundamental feature required
235 for the hydrolysis [22].

236 In addition to this effective pretreatment, we used the crude (home-made)
237 enzymatic cocktails of *A. niger* LBM 134 instead of purified enzymes because there are
238 clear indications that proteins with still unknown functions (present in the crude
239 cocktails) may contribute to the hydrolysis of cellulose and hemicelluloses [19, 23].
240 Furthermore, the advantages of applying the home-made enzymatic cocktails of *A.*
241 *niger* LBM 134 without purification step implies a reduction in the costs of the global
242 biotechnological application. In addition, the home-made enzymatic cocktail of *A.*
243 *niger* LBM 134 grown on SCB showed high levels of hemicellulases and cellulases and
244 the enzymatic cocktail of the fungus grown on CB presented high levels of starch-
245 degrading enzymes [4]. Therefore, these enzymatic cocktails were used for carrying out
246 the bioconversion of SCB and CB. Both wastes are complex biomass; hence, their
247 bioconversion require more than one or few enzymes. In this context, the co-action of
248 different enzymatic activities of the home-made cocktails of *A. niger* LBM 134 makes
249 the difference compared to the commercial enzymes that present only a few enzymatic

activities.

A soft alkaline pretreatment was applied on SCB and CB to remove the lignin content and make available the polysaccharides of the cell walls. This treatment was efficient to carry out the removal of the lignin content without affecting the carbohydrate fraction (no polysaccharides were detected in this fraction by DNS method). After the pretreatment, a liquid with lignin and a solid fraction with the carbohydrates were formed. The lignin was removed and discarded with the liquid fraction 88.39 ± 5.83% for SCB and 73.20 ± 0.23% for CB, from the total lignin content.

The lignin removed was also evidenced by the change of colour of the solid fraction; SCB and CB were initially brown before the alkaline treatment and after that, SCB changed to light brown and CB, to yellow cream (data not shown). In addition, there was no polysaccharides loss after the pretreatment of both bagasses due to there were no sugars detected in the liquid fraction by the DNS assay.

The enzymatic hydrolysis of the pretreated bagasses was carried out with the home-made enzymatic cocktails of *A. niger* LBM 134. The reducing and monomeric sugars from both hydrolysates and controls were shown in Table 3. The main products of the hydrolysis of SCB were in (mg mL^{-1}) 4.51 ± 1.14 glucose and 3.66 ± 1.06 xylose,

267 achieving a 28% of conversion to glucose and 42% to xylose, respectively. These
268 conversion percentages were similar to that obtained from the hydrolysis of pretreated
269 SCB using commercial enzymes: 23% conversion to glucose and 42% to xylose. The
270 hydrolysates from CB were rich in glucose, 5.12 ± 0.89 mg mL^{-1}; reaching a 16.5% of
271 conversion, three times higher than that obtained using commercial enzymes. Reducing
272 sugars were also determined to estimate the saccharification yield; hydrolyzed
273 pretreated CB with the home-made enzymatic cocktails of *A. niger* LBM 134 showed
274 the maximal saccharification yield, 80%.

275 TABLE 3

276 Changes in the structure of SCB and CB were analyzed through SEM (Figure 2).
277 Electronic microscopic photographs were taken of typical features of both bagasses
278 before any treatment; the SCB fibers were covered by lignin material (Figure 2a) and
279 the CB surface was heterogenous and porous (Figure 2b). After the alkaline
280 pretreatment, the parenchyma and conductive vessels of the SCB were altered and the
281 fibers had less cohesion due to the lignin removal (Figure 2c). On the other side, starch
282 granules could be distinguished in the pretreated CB (Figure 2d). Both bagasses were
283 also microphotographed after the hydrolysis. After the hydrolysis of SCB with the

284 enzymatic cocktails of *A. niger* LBM 134, the fibers were amorphous and disorganized
285 showing a large area exposed to the enzymatic action (Figure 2e). Regarding CB
286 hydrolysis by the enzymatic cocktails of *A. niger* LBM 134, the surface was
287 homogenous and no starch granules were shown (Figure 2f). Cellulose fibers of SCB
288 hydrolyzed by commercial enzymes showed similar changes as SCB hydrolyzed by the
289 enzymatic cocktails of *A. niger* LBM 134 (Figure 2g). CB hydrolyzed by commercial
290 enzymes showed a heterogeneous surface and the presence of starch granules (Figure
291 2h).

292 The cost of having a more or less complete commercial cocktail of cellulases and
293 xylanases is at least almost $900, more than 500 times the cost of producing the home-
294 made enzymatic cocktails by *A. niger* LBM 134, $1.90 and $1.65 when the fungus grew
295 with SCB and CB, respectively.

296 FIGURE 2

297 *3.4. Simulation model for bioethanol production from hydrolyzed SCB and CB*

298 Generic flowsheet model for bioethanol obtention was shown in Figure 3. This
299 diagram contemplates feedstock preparation (Figure 3a) and their main component
300 proportions; the enzymes production using the bagasses as carbon sources and the

301 fungus *A. niger* LBM 134 (Figure 3b) until the obtention of ethanol by a simulation
302 model.

303 Glucose and xylose yields were used to simulate the fermentation and obtention of
304 bioethanol curve-fitting model (Figure 3d). Firstly, the experimental concentrations of
305 a glucose-xylose combined fermentation reported by Kamoldeen et al. [20] were used
306 for simulating the bioethanol production model (Supplementary table 1). For a more
307 complete utilization of all fermentable sugars released in the SCB hydrolysates, the
308 yeast *Saccharomyces cerevisiae* could be used in addition to pentose-fermenting yeasts
309 like *Scheffersomyces stipitis* ATCC 5837 as indicated Kamoldeen et al. [20].

310 To obtain the apparent reaction rate constants for each component, the traditional
311 fermentation models were simplified as follows: glucose > ethanol + CO_2 x 2 and 3
312 xylose > ethanol + CO_2 x 5. Also, the experimental concentrations of glucose, xylose
313 and ethanol from Kamoldeen et al. [20] work were expressed in mol L^{-1} in a progressive
314 reaction and a first order reaction model was suggested for glucose and xylose
315 decomposition rates:

316 $$-Ln\left(\frac{C_G}{C_{Go}}\right) = k_G t;\ -Ln\left(\frac{C_X}{C_{Xo}}\right) = k_X t \qquad \text{(Eq. 3; 4)}$$

317 Experimental data from Kamoldeen et al. [20] work were adjusted to both

logarithmic expressions and k_G and k_X constants were obtained, k_G = 0.2631 and k_X = 0.0754, considering the time lag between glucose and xylose consumption start. The conversion models for glucose and xylose were as follows:

$$C_G = C_{G0}e^{-0,2631t};\ C_x = C_{x0}e^{-0,0754t} \quad \text{(Eq. 5; 6)}$$

where, G is glucose; X is xylose; t is time. These models were validated with experimental data and no statistical difference was found for P <0.05 (Supplementary Table 2). The validated conversion models and the k_G and k_X constants were used to established the bioethanol production models (Supplementary Table 3):

$$C_{EG} = 2C_{G0}\left[1 - e^{(-0,2631t)}\right];\ C_{EX} = 5/3C_{X0}\left[1 - e^{(-0,0754t)}\right] \quad \text{(Eq. 7; 8)}$$

where, *EG* is ethanol production from glucose; *G0*, glucose concentration at time 0; *EX*, ethanol production from xylose; *X0*, xylose concentration at time 0; t, time.

The validation of the models was carried out applying them to experimental and theorical data and comparing with the experimentally produced bioethanol. The model fitted well with the experimental data, there was no significance difference for P < 0.05 (Supplementary Table 4). Once the ethanol production model was validated, the curve-fitting was employed for simulating the bioethanol yield from experimental data of the saccharification of SCB and CB, achieving 4.16 mg mL^{-1} and 2.57 mg mL^{-1},

335 respectively (Figure 3e).

336 FIGURE 3

337 The successful bioconversion of both SCB and CB occurred due to the home-made
338 enzymatic cocktails were produced using the respective bagasse as substrate for the
339 fungus [8]. Moreover, as the hydrolysis was carried out using fungal enzymes, there
340 was no need to detoxify the hydrolysates since there were no formation of inhibitors
341 that can negatively influence on the fermenting microorganism [10].

342 Regarding the fermentation step, we used two yeasts enabled to simulate the
343 metabolization of hexoses such as glucose and pentoses as xylose for a more complete
344 utilization of all the sugars released during the hydrolysis of SCB [10]. On the other
345 hand, the fermentation of the hydrolysates of CB was simulated only using the glucose-
346 metabolizing yeast, *S. cerevisiae* because CB hydrolysates were mainly rich in glucose.

347 From the bioethanol model simulation, the SCB hydrolysates would reach a higher
348 bioethanol yield than the CB hydrolysates; this behavior can be explained by xylose
349 sugars present in the SCB hydrolysates. The importance of the xylose as a fermentable
350 sugar for obtaining bioethanol in higher quantities is relevant since it has been identified
351 that non or poor utilization of the xylose components of biomass is a principal factor

352 generally affecting the efficiency of lignocellulosic substrates as a renewable feedstock
353 for bioethanol generation [20].

354 Although the bioconversion of both bagasses reached low values comparing with
355 another works such as Fockink et al. [24] who yielded higher sugars values, the
356 importance of this work is that the performance of the cheap home-made enzymatic
357 cocktails of *A. niger* LBM 134 (produced from agroindustrial wastes) was higher than
358 the hydrolytic performance of commercial enzymes. The conversion to glucose of
359 pretreated SCB confirmed the good performance of cellulases, particularly BGL. This
360 is a very interesting finding due to numerous studies have described a limited
361 production of BGL for almost filamentous fungi including *Trichoderma reesei*, a well-
362 known cellulase-producer, which cocktails must be added with exogenous BGL [8].

363 Regarding the hydrolysis of SCB with commercial enzymes, the conversion to
364 glucose and xylose were similar to that obtained with the home-made enzymatic
365 cocktails of *A. niger* LBM 134. That fact evidenced the good performance of the home-
366 made enzymatic cocktails used in this work.

367 The bioconversion from glucans to glucoses in CB using the home-made enzymatic
368 cocktails was three times higher than that obtained using commercial enzymes. This

behavior could be attributed to the action of amylases present in the home-made enzymatic cocktails of *A. niger* LBM 134 [15]. The conversion in reducing sugars of CB using the home-made enzymatic cocktail was two times higher than that obtained by [25] hydrolyzing CB with *Rhizopus oligosporus* (CCT 3762). Moreover, the results in this work are similar to the maximal saccharification percentage obtained by Bayitse et al. [2] hydrolyzing cassava peel with commercial enzymes. In this sense, it is important to highlight that this bioconversion was carried out by home-made enzymatic cocktails and no commercial enzymes. This fact translates into the reduction of costs by using home-made enzymatic cocktails produced from a fungus grown on wastes (SCB and CB). Also, we must set up the potential of CB in order to generate other added-value products, a field poorly explored [25]. The use of this waste as biomass in a biorefinery concept will contribute in countries on process of development and will have a great social and economic impact at regional level through maximizing this local resource to promote industry development and added-value product generation [1, 26].

4. Conclusions

The method proposed in this article links the use of predictive model of ethanol yield to conventional biochemical techniques. The complete bioconversion of SCB

and CB to bioethanol involved complex steps to transform the carbohydrate polymers into fermentable sugars. One of the main bottlenecks of the bioethanol process is the cost of producing enzymes to be used in the hydrolysis. Therefore, we used home-made enzymatic cocktails from a native fungus, *A. niger* LBM 134 grown on agroindustrial wastes, SCB and CB. We characterized and analyzed the cost of the home-made enzymatic cocktails we produced and compare the cost with two commercial cellulolytic enzyme mixtures and a commercial xylanase enzyme. We concluded that the bioconversion of SCB and CB carried out in this work by the home-made enzymatic cocktails of *A. niger* LBM 134 was better than the hydrolytic performance using commercial enzymes and thus converting this bioprocess in a cost-effective strategy. Also, we obtained two added-value products from non-use agroindustrial wastes: enzymatic cocktails and fermentable hydrolysates. For these reasons, we believe that this process can potentially applied and adopted on sugarcane mills and starch industry.

Declarations

Availability of data and materials

All data generated or analyzed during this study are available in this study.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

GVD and SSSA conceived the present idea, participated in performing experiments, statistical analysis and the design of figures; in addition, GVD designed the study and wrote the manuscript; ROC participated in performing experiments; JEV analysed part of the results; MIF supervised the project and participated in critical revision of the manuscript and PDZ and LLV supervised the project, contributed reagents and materials and participated in critical revision of the manuscript. All authors read, provided critical feedback, and approved the final manuscript.

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Not applicable

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517

518 **Table 1** Chemical composition of raw CB and SCB used for the bioconversion. The
519 values represent the means of the triplicates ± standard deviation.

Components	Composition (%)	
	CB	SCB
Glucans	72.5 ± 0.59	43.72 ± 0.77
Hemicellulose[a]	16.6 ± 1.66	24.99 ± 8.56
Soluble lignin	6.04 ± 0.15	16.49 ± 2.55
Insoluble lignin	3.29 ± 0.9	9.37 ± 1.33

Ash	0.67 ± 0.06	1.9 ± 0.4
Extractives	1.8 ± 0.05	4.7 ± 0.08

[a]Analyzed sugars: xylose, arabinose

SCB: subarcane bagasse; CB: cassava bagasse

Table 2 Enzyme activities of home-made cocktails of *A. niger* LBM 134 grown on SCB and CB. Activity levels represented by the means of biological triplicates ± standard deviation. The cocktails of the fungus grown on SCB and CB contained total proteins 100.46 ± 27.01 μg mL^{-1} and 329.62 ± 1.17 μg mL^{-1}, respectively.

Enzyme activities (UmL^{-1})	Crude enzymatic extracts	
	SCB	CB
Filter paper activity	0.35 ± 0.00 UmL^{-1}	0.38 ± 0.00 UmL^{-1}
β-glucosidase	0.17 ± 0.00 UmL^{-1}	0.28 ± 0.00 UmL^{-1}
Endoxylanase	106 ± 14.67 UmL^{-1}	144 ± 5.65 UmL^{-1}
B-xylosidase	0.74 ± 0.05 UmL^{-1}	0.18 ± 0.03 UmL^{-1}

SCB: sugarcane bagasse; CB: cassava bagasse

Table 3 Released sugars from the enzymatic hydrolysis of SCB and CB. In this bioconversion, we used the home-made cocktails of *A. niger* LBM 134 and controls. The values represent the means (in mg mL^{-1}) of the biological triplicates ± standard deviation.

Assays	Reducing sugars	Cellobiose	Glucose	Xylose	Arabinose	Acetic acid
SCB						

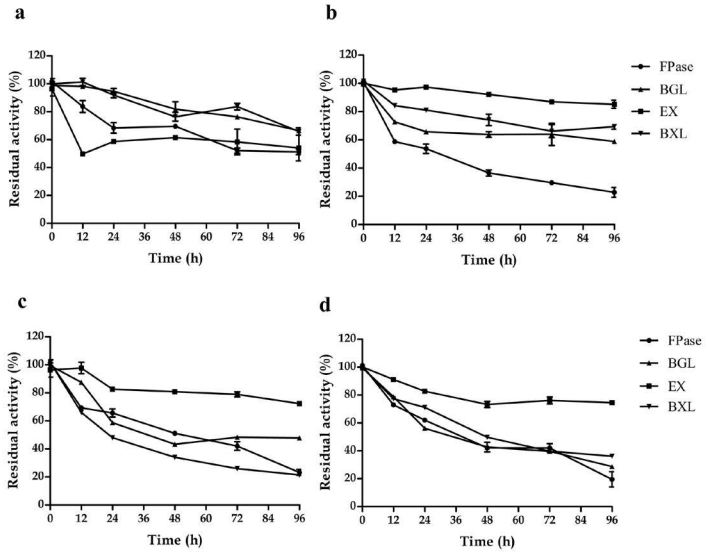
Enzymatic hydrolysis of SCB with cocktails of *A. niger* LBM 134	22.09 ± 1.26	2.48 ± 0.53	5.18 ± 1.14	4.46 ± 1.06	0.55 ± 0.13	2.56 ± 0.48
Control 1: enzymatic hydrolysis of SCB with commercial enzymes	17.41 ± 0.13	2.45 ± 0.01	4.34 ± 0.03	3.62 ± 0.09	0.07 ± 0.01	2.14 ± 0.02
Control 2: SCB + buffer	ND	0.01 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	ND	3.17 ± 0.07
Control 3: enzymatic cocktails of *A. niger* LBM 134	ND	1.00 ± 0.00	0.63 ± 0.00	ND	ND	ND
CB						
Enzymatic hydrolysis of CB with cocktails of *A. niger* LBM 134	22.81 ± 1.63	1.00 ± 0.27	5.12 ± 0.89	0.65 ± 0.06	0.11 ± 0.02	2.98 ± 0.17
Control 1: enzymatic hydrolysis with comercial enzymes	14.73 ± 2.01	0.61 ± 0.02	2.33 ± 0.14	ND	0.03 ± 0.00	2.37 ± 0.01
Control 2: CB + buffer	ND	1.10 ± 0.09	0.33 ± 0.31	0.21 ± 0.19	0.04 ± 0.00	3.22 ± 0.28
Control 3: enzymatic cocktails of *A. niger* LBM 134	ND	0.99 ± 0.00	0.38 ± 0.00	ND	ND	ND

SCB: sugarcane bagasse; CB: cassava bagasse

ND: not detected

535

536 **Figure**



537

538 **Figure 1**. Enzymatic stability of the principal enzymes in *A. niger* LBM 134 enzymatic

539 cocktails. Thermostability of FPase, BGL, EX and BXL of the fungus grown on SCB

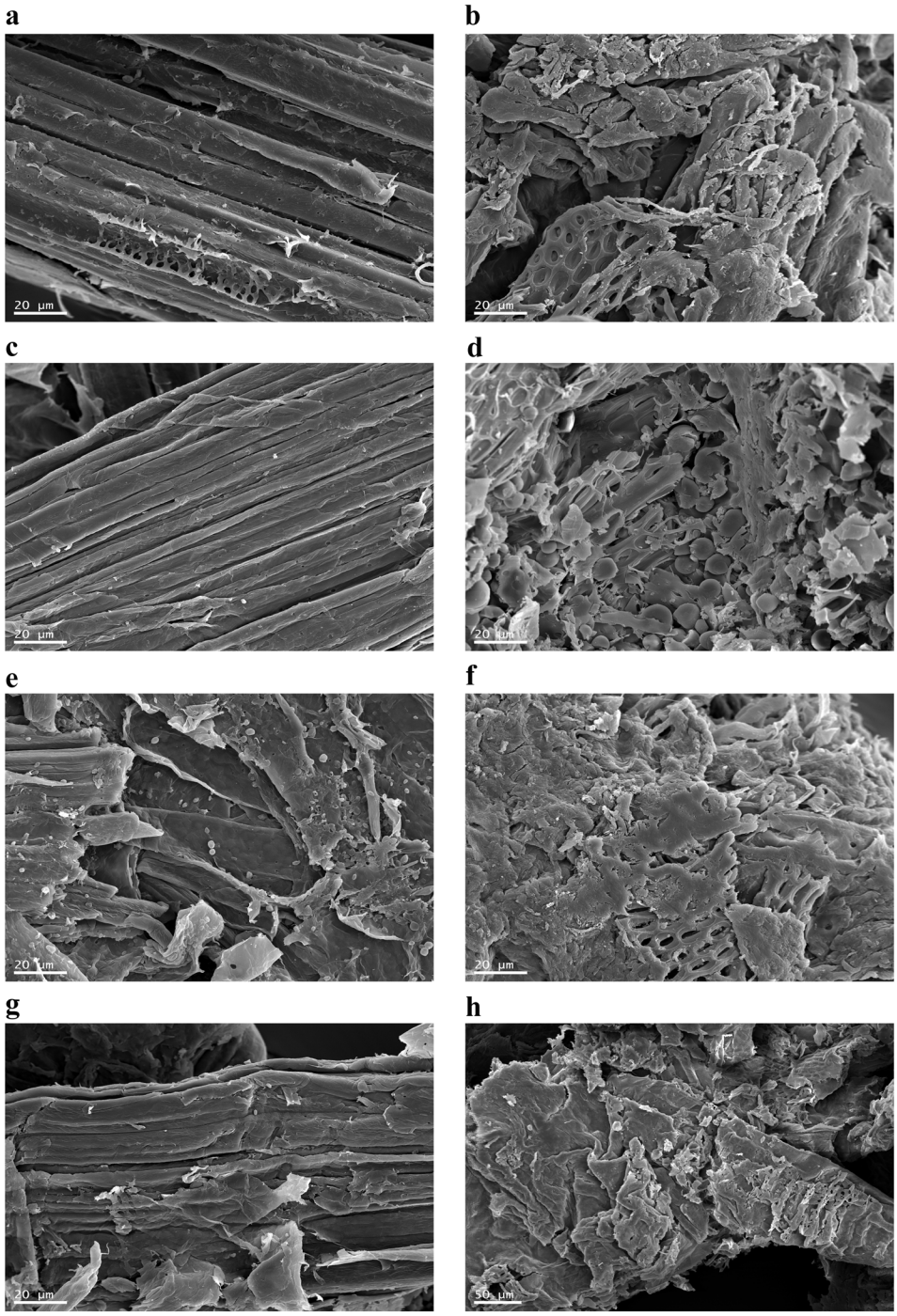
540 (**a**) and CB (**b).** pH stability of FPase, BGL, EX and BXL of the fungus grown on SCB

541 (**c**) and CB (**d**). The 100% of each enzyme activity corresponded to the levels shown in

542 Table 2. FPase, filter paper activity; BGL, β-glucosidase; EX, endoxylanase; BXL, β-

543 xylosidase; SCB, sugarcane bagasse; CB, cassava bagasse.

544



545

546 **Figure 2**. Electronic microscopic photographs of SCB and CB **a**) Structure of raw SCB

547 without any pretreatment. **b**) Structure of raw CB without any pretreatment. **c**) SCB

548 pretreated with alkaline solution Na(OH) 0.85% (w/v). **d**) CB pretreated with alkaline

549 solution Na(OH) 0.85% (w/v). **e**) SCB after alkaline pretreatment and hydrolysis with

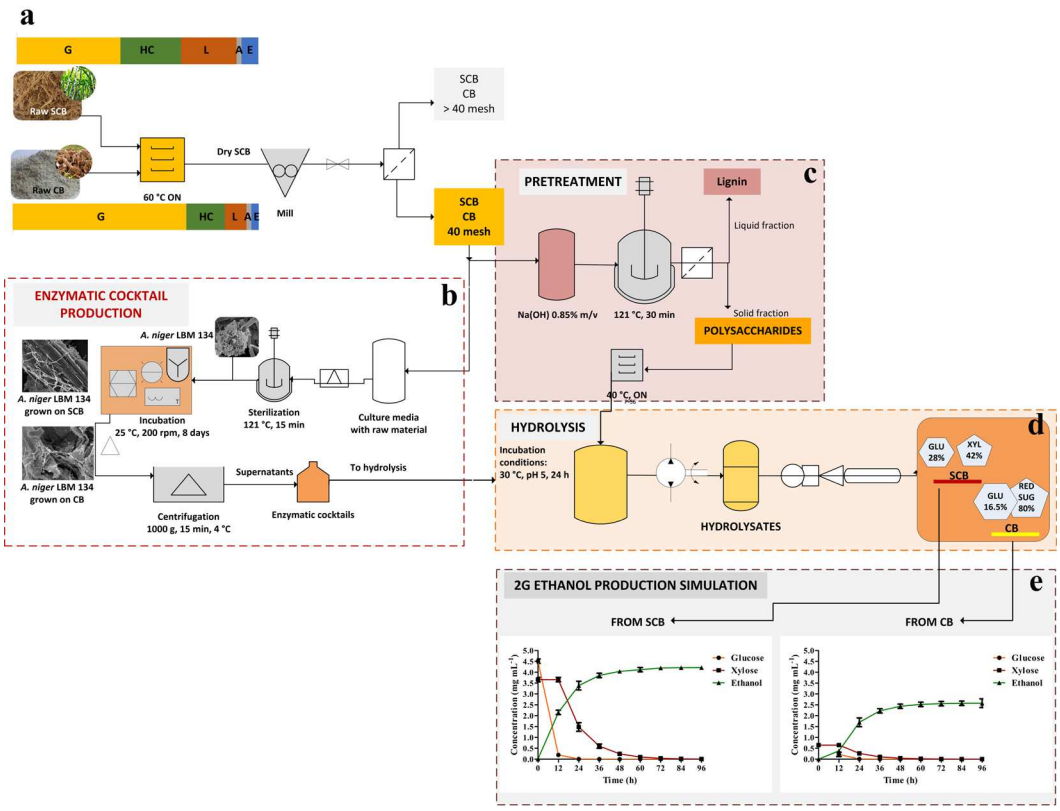
550 home-made enzymatic extract of *A. niger* LBM 134 grown on SCB. **f**) CB after alkaline

551 pretreatment hydrolysis with home-made enzymatic extract of *A. niger* LBM 134

552 grown on CB. **g**) SCB after alkaline pretreatment hydrolysis with commercial enzymes.

553 **h**) CB after alkaline pretreatment hydrolysis with commercial enzymes. SCB,

554 sugarcane bagsse; CB, cassava bagasse.



555

556 **Figure 3.** Bioprocess flowsheet of home-made enzymatic cocktails and bioethanol

557 production from SCB and CB. **a**) Main components of raw SCB and CB: G, glucans;

558 HM, hemicelluloses; L, lignin; A, ash; E, extractives. Both bagasses were prepared for

being used in the enzymatic cocktails production by *A. niger* LBM 134 (**b**) and in the pretreatment process (**c**) for removing lignin; the solid fraction, rich in polysaccharides, was selected for continuing the process. **d**) Hydrolysis of SCB and CB using the home-made enzymatic cocktails for obtaining the hydrolysates rich in monomeric sugars, GLU and XYL. **e**) Simulation model for bioethanol production from SCB and CB from the fermentable sugars obtained in this work. SCB, sugarcane bagasse; CB, cassava bagasse; GLU, glucose; XYL, xylose.

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