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Evaluation of buriti endocarp as lignocellulosic substrate for second generation ethanol production

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The production of lignocellulosic ethanol is one of the most promising alternatives to fossil fuels, however, this technology still faces many challenges related to the viability of the alcohol in the market. In this paper the endocarp of buriti fruit was assessed for ethanol production. The whole fruit was characterized physically and chemically and its endocarp submitted to acid and alkaline pre-treatments, which were optimized through the use of surface response methodology for removal of hemicellulose and lignin, respectively. Hemicellulose content was reduced by 88% after acid pretreatment. Alkaline pre-treatment reduced the lignin content in the recovered biomass from 11.8% to 4.2% and increased the concentration of the cellulosic fraction to 88.5%. The pre-treated biomass was saccharified by the action of cellulolytic enzymes and, in the optimized condition, was able to produce 110 g of glucose per L of hydrolyzate. Alcoholic fermentation of the enzymatic hydrolyzate bio-catalized by *Saccharomyces cerevisiae* resulted in a fermented medium with 4.3% ethanol and $Y_{P/S}$ of 0.33.

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1 **EVALUATION OF BURITI ENDOCARP AS LIGNOCELLULOSIC SUBSTRATE FOR**
2 **SECOND GENERATION ETHANOL PRODUCTION**

3

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14

15 **Abstract**

16 The production of lignocellulosic ethanol is one of the most promising alternatives to fossil fuels,
17 however, this technology still faces many challenges related to the viability of the alcohol in the
18 market. In this paper the endocarp of buriti fruit was assessed for ethanol production. The whole
19 fruit was characterized physically and chemically and its endocarp submitted to acid and alkaline
20 pre-treatments, which were optimized through the use of surface response methodology for
21 removal of hemicellulose and lignin, respectively. Hemicellulose content was reduced by 88%

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22 after acid pretreatment. Alkaline pre-treatment reduced the lignin content in the recovered
23 biomass from 11.8% to 4.2% and increased the concentration of the cellulosic fraction to 88.5%.
24 The pre-treated biomass was saccharified by the action of cellulolytic enzymes and, in the
25 optimized condition, was able to produce 110 g of glucose per L of hydrolyzate. Alcoholic
26 fermentation of the enzymatic hydrolyzate bio-catalized by *Saccharomyces cerevisiae* resulted in
27 a fermented medium with 4.3% ethanol and $Y_{P/S}$ of 0.33.

28

29 Keywords: *Mauritia flexuosa*; bioethanol; pre-treatment; saccharification.

30

31 **Introduction**

32 The current configuration of global economic advance has created a growing demand for
33 energy resources to support its maintenance. Additionally, the growth of the human population
34 on the planet, the depletion of fossil fuels and the growing concerns about human impacts on the
35 environment have encouraged the search for renewable sources to the development of green
36 energy production (Singh, Nigam & Murphy, 2011; Sarkar et al., 2012). In this context,
37 lignocellulosic biomasses are a promising feedstock for the production of liquid biofuels,
38 alternative to petroleum based fuels (Cherubini & Ulgiati, 2010).

39 The technology for the production of second generation (2G) bioethanol, or
40 lignocellulosic ethanol, has evolved in the last decades and functioning industrial plants already
41 exist in some parts of the world, nevertheless, this biofuel still faces the challenge of feedstock
42 access, supply chain infrastructure, and price competitiveness with the petroleum industry (UNCTAD,
43 2016).

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44 Lignocellulosic ethanol can be obtained from the fermentation of hexoses and pentoses
45 derived from the polysaccharides that constitute the plants cell wall and require additional
46 operations to those normally used to produce first generation ethanol (Mielenz, 2001). Lignin
47 removal and hemicellulose hydrolysis, followed by cellulose saccharification, are necessary steps
48 to provide the sugars to be fermented by specialized microorganisms to produce 2G ethanol
49 (Maurya, Singla & Negi 2015; Keshav, Naseeruddin, & Rao, 2016). In this sense, there is a large
50 number of biomasses being evaluated as raw materials for this nascent industry, with emphasis
51 for agro-industrial residues (Macedo et al., 2011; Hoa, Ngob & Guo 2014; Domínguez-
52 Bocanegra, Torres-Muñoz & López, 2015).

53 Buritizeiro (*Mauritia Flexuosa*) is one of the most abundant species of palm tree in
54 Brazil, its occurrence covers the Cerrado and Amazon national biomes. Its fruit (buriti) is
55 elliptical to oval in shape and comprised of pericarp (bark), mesocarp (pulp), endocarp (seed
56 shell lignocellulosic tissue), and endosperm (seed) (Sampaio & Carrazza, 2012). Buriti fruits are
57 economically exploited for a variety of purposes, such as the extraction of edible and cosmetic
58 oil, and the manufacturing of beverages, flours and ice creams (Lorenzi et al., 2004; Manzi &
59 Coomes, 2009; Gilmore, Endress & Horn, 2013), however, the endocarp fruit portion presents
60 few alternatives for commercial use, having low economic value.

61 This lignocellulosic residue (endocarp) is a potential source for the production of 2G
62 ethanol, since it is an abundant waste product of the buritizeiro palm exploitation and does not
63 directly require the availability of more cultivation lands (Van, Brose & Schenkel 2011; Bos et
64 al., 2016). Also, buriti endocarp usage presents no competition with the food market chain and
65 represents potential income generation, as its use adds value to an underutilized material (Kang
66 et al., 2014; Sawatdeenarunat et al., 2015).

67 In this paper, the buriti fruit was physically and chemically characterized and its endocarp
68 was evaluated with respect to its potential for 2G ethanol production. Pre-treatments with dilute
69 sulfuric acid and sodium hydroxide, and enzymatic saccharification were performed using
70 response surface methodology. The effects of the factors studied in the pre-treatments and in the
71 enzymatic hydrolysis were evaluated and the optimal conditions were highlighted. Ultimately,
72 the saccharified cellulose from the buriti endocarp was fermented to ethanol using
73 *Saccharomyces cerevisiae.*

74

75 **Material & methods**

76 **Physical characterization of buritizeiro fruits**

77 Twenty kilograms of fruits were collected in Três Marias city, in Minas Gerais, Brazil. The
78 physical characterization was performed on 50 randomly selected fruits. The fruits were weighed
79 in analytical balance and their longitudinal and transversal diameters were measured with the aid
80 of a digital caliper. Then, pulp, bark, endocarp, peduncle and seed of each fruit were separated
81 manually with the aid of a steel blade, and weighed. Each of the fractions of the fruit was oven
82 dried with forced air ventilation at 60°C for 24 hours, stored in polyethylene bags at room
83 temperature and protected from light. Pulp, bark and endocarp portions were ground with a
84 manual grinder (Botini® brand) and sieved for particle size standardization, between 40 and 20
85 mesh (0.42 to 0.84 mm).

86 **Chemical characterization of buriti fruit fractions**

87 Previously dried and crushed pulp, bark and endocarp were characterized in terms of total
88 moisture, ashes, proteins and lipids, according to Adolph Lutz Institute (IAL) analytical

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89 standards (IAL, 2008). Crude fiber content was determined according to Kamer and Ginkel
90 (1952). Starch and total soluble sugars (TSS) contents were determined according to
91 methodology described by McCready *et al*. (1950). The endocarp was further characterized by
92 its cellulose, hemicellulose and lignin contents, quantified by neutral detergent fiber (NDF) and
93 acid detergent fiber (FDA) methods described by Van Soest (1963,1964,1968).

94 **Pre-treatment of buriti endocarp**

95 Buriti endocarp, dried and crushed, was pre-treated with dilute sulfuric acid followed by
96 hydrolysis with alkali to remove fractions of hemicellulose and lignin, respectively.

97 ***Treatment with dilute sulfuric acid***

98 Determination of the ideal conditions for the acid pre-treatment of the biomass was
99 accomplished through the use of a Rotational Central Composite Design (RCCD) that evaluated
100 the influence of reaction time; 20 min. (-1) and 60 min. (-1), solid-liquid (S/L) ratio; 10% (-1)
101 and 20% (+1), and concentration of sulfuric acid (H_2SO_4); 2% (-1) and 7% (+1), on the removal
102 of hemicellulose contained in the buriti endocarp. In each test, carried out in a glass tube (30 x
103 2.5 cm), 1 g of sample was added with the solution of H_2SO_4 in the pre-defined concentration
104 and proportion for each of the 18 tests generated by the factorial matrix 2^3, containing four
105 central points and six axial points. The tests were performed in an autoclave (1 atm) at a fixed
106 temperature of 120°C (Corbin et al, 2015).

107 ***Determination of sugars removed by acid pre-treatment***

108 Quantification of the glucose released after acid hydrolysis was determined by the enzymatic-
109 colorimetric method described by Lloyd and Whelan (1969). The quantification of the reducing
110 sugars (RS) in the acid hydrolyzate was carried out using the dinitrosalicylic acid method

111 described by Miller (1959). The decomposition of hemicellulose was expressed in grams of
112 sugar liberated per 100 g of biomass.

113 ***Pre-treatment with sodium hydroxide***

114 Optimization of the removal of lignin present in the buriti endocarp was performed by 2^2 RCCD
115 factorial experiments, which investigated the influence of process temperature; 30°C (-1) and
116 80°C (+1), and the concentration of sodium hydroxide (NaOH); 2% (-1) and 12% (+1), in
117 addition to four central points and four axial points. In each test, carried out in glass tube (30 x
118 2.5 cm), 1 g of sample, sodium hydroxide solution at a solid-liquid ratio of 10%, were added and
119 then incubated in a water bath for the period of 12, 24, 36 and 48 hours. Lignin removal was
120 estimated indirectly by dosage of total phenolic compounds present in the hydrolyzate, according
121 to methodology described by Singleton and Rossi (1965), using gallic acid as standard.

122 **Enzymatic saccharification of pretreated endocarp**

123 For saccharification process optimization a RCCD with three factors was performed. Analyzing
124 the effect of S/L ratio; 5% (-1) and 15% (+1), time; 6 h (-1) and 24 h (+ 1), and enzyme
125 concentration (Celluclast® - Novozymes); 20 μL g^{-1} (-1) and 100 μL g^{-1} (+1), with four central
126 points and six axial points. In each condition described by the RCCD planning, the mass of 1 g
127 of pre-treated endocarp was used in a 50 mL conical flask, followed by the addition of 50 mM
128 sodium bicarbonate buffer (pH 5.0) and enzyme volume according to experimental planning. The
129 tests were incubated at 50°C with agitation of 100 rpm. At the end of each reaction, the
130 concentrations of glucose and reducing sugars (RS) were determined in the soluble fraction of
131 the hydrolyzate as described in 2.3.2. The decomposition of cellulose was expressed in grams of
132 glucose released per 100 g of biomass.

133 **Alcoholic fermentation of the enzymatic hydrolyzate**

134 Fermentation of the enzymatic hydrolyzate obtained in the optimized conditions for the
135 saccharification of the pre-treated biomass was carried out in 250 mL conical flasks coupled to
136 fermentometers, a glass system that allows carbon dioxide (CO_2) release and prevents the entry
137 of external air, at room temperature (25 ± 2°C). Dehydrated commercial baker's yeast
138 (Fleischmann®) of the *Saccharomyces cerevisiae* species was used as a fermentative agent in the
139 ratio of 1% (w/v) to the must volume. The fermentative process was monitored gravimetrically
140 for CO_2 release. The measurement of mass of gas released was used to estimate the ethanol
141 production and the consumption of the fermentable sugars every two hours until the end of the
142 fermentation. The concentration of ethanol quantified by potassium dichromate method,
143 according to methodology described by Isarankura-Na Ayudhya et al (2007), and the
144 concentrations of glucose and reducing sugars were determined at the beginning and at the end
145 of the fermentation process.

146 **Statistical analysis**

147 Modeling, graphing and analysis of the results obtained with the rotational central composite
148 designs were performed using tools available in Statistica 8.0 software (Statsoft Inc., Tulsa).
149 ANOVA with $p<0.05$ level was stipulated as a statistical parameter of significance.

150 **Results**

151 Physical characterizations of *in natura* buriti fruits are displayed in Table 1, the values are
152 disposed in percentage averages followed by their standard deviations. The integral fruits
153 presented an average mass of 38.33 ± 9.06 g, transversal diameter of 38.75 ± 3.76 mm and

154 longitudinal diameter of 48.68 ± 2.94 mm (Table 1). The endocarp mass represented, in average,
155 25.3% of the whole fruit.

156 All evaluated parts of the fruit presented fiber contents superior to 26% (Table 2). For the buriti
157 endocarp evaluated in this work, four main sugar sources that could be converted to bioethanol
158 were identified. In addition to starch, cellulose and hemicellulose, the presence of soluble sugars
159 in the biomass was also determined. Total carbohydrates portion corresponded to 44.2% of buriti
160 endocarp (Table 2). Cellulose was the main polymer in the biomass of the endocarp, with a
161 content of 22.15%, and the lignin fraction was 11.79%. The contents of the other components of
162 the fruit endocarp are organized in Table 2.

163 In this study, buriti endocarp was subjected to a sequence of acid and alkali treatments with the
164 purpose of exposing the cellulose polymers to the enzymatic hydrolysis to obtain monomers of
165 hexoses for their subsequent anaerobic fermentation bio-catalyzed by *S. cereviseae* yeast. The
166 quantities of reducing sugars and glucose removed per 100 g of endocarp subjected to acid
167 pretreatment under the different experimental design conditions are shown in Table 3. Negative
168 quadratic individual effects of H_2SO_4 and S/L ratio were also observed, however, with p values
169 of 0.114 and 0.102, respectively. The combined effects of H_2SO_4 concentration with the S/L ratio
170 factors and H_2SO_4 concentration over time on the removal of the hemicellulosic portion from the
171 buriti endocarp are presented as response surface curves in Figure 1, the coefficient of
172 determination (R^2) was 0.81.

173 There was greater release of reducing sugars in the condition of test 10 (Table 3). In this point,
174 8.75 g of reducing sugars per 100 g of biomass were removed. On the other hand, in the
175 condition of test 5 (Table 3), 8.02 g of reducing sugars per 100 g of biomass were removed, 9%
176 less than under test condition 10. The test 5 was then chosen as optimal condition for the

177 preparative test for using less acid concentration and half the reaction time of test 10. Not
178 coincidentally, the areas under the response surface curves (Figure 1) representing the regions
179 with the highest hemicellulose removal refer to the combination of factors indicated by the
180 conditions of the test 5 (Table 3). The characterization of the lignocellulosic fraction of buriti
181 endocarp recovered after acid pretreatment using the optimum condition defined, indicated
182 changes in the contents of cellulose, hemicellulose and lignin (Table 4).

183 Rotational central composite design for lignin removal from the acid pre-treated buriti endocarps
184 are presented in Table 5 in times of 12, 24, 36 and 48 hours. Regression analysis of the response
185 surfaces for the times assessed yielded squared correlation coefficients (R^2) of 0.97, 0.83, 0.94
186 and 0.93, respectively. In all caustic hydrolysis times evaluated, the alkali used (NaOH) had a
187 positive and significant effect ($p < 0.05$) on lignin removal. This effect was followed indirectly by
188 the determination of total phenolic compounds released in the hydrolyzate (Table 5). The
189 temperature also had a positive and significant effect ($p < 0.05$) on lignin removal at all evaluated
190 times.

191 The polynomial model that describes the percentage of lignin removal, expressed in the form of
192 phenolic compounds, as a function of the temperature and NaOH concentration in the time of
193 maximum lignin hydrolysis (48h) is represented by Equation (1).

194

195 $$L = 5.10 + 2.82C - 1.75C^2 + 3.43T + 0.71T^2 + 2.12CT \quad (1)$$

196 Where:

197 L = Total phenolic compounds (%);

198 C = NaOH concentration (%) (m/v);

199 T = Temperature (°C).

200

201 The graph of the projection of response surface to the time of 48 hours is seen in Figure 2. The
202 region where the maximum release of phenolic compounds can be observed is represented by a
203 temperature higher than 80°C and NaOH concentration between 10 and 14%. The highest release
204 of phenolic compounds was observed in the reaction medium with 12% NaOH and temperature
205 of 80°C (test 4, Table 5). This condition was adopted for the preparatory pretreatment of the
206 biomass previously submitted to acid pretreatment.

207 **Discussion**

208 The chemical composition of a biomass is mainly determined by its evolutionary history and
209 varies significantly with the species (Mendu et al, 2011; Dardick & Callahan, 2014). In general,
210 lignocellulosic biomass consists mainly of structural carbohydrates (cellulose and hemicellulose)
211 and lignin (Handley, Pharr & McFeeters, 1983; Humphreys & Chapple, 2002). The fraction of
212 these molecular groups varies depending on the stage of development, the anatomical part of the
213 plant and its species.

214 Cellulose, lignin and hemicellulose were, in this order, the most abundant compounds in the
215 chemical structure of the buriti endocarp (Table 2). These molecules are responsible for the
216 stiffness of the material, an important feature for seed protection (Dardick & Callahan, 2014). It
217 is important to note that most fermentable sugars in lignocellulosic biomass come from polymers
218 abundant in hexoses and pentoses, respectively cellulose and hemicellulose. However, there are
219 still no fully consolidated strategies for the production of ethanol from hemicellulose. Thus,
220 cellulose is the polymer most used as sugar source for the production of second generation
221 ethanol and, therefore, the fraction used for the production of ethanol in the present assessement
222 (Nigam 2001; Agbogbo & Coward-Kelly, 2008; Saini, Saini & Tewari, 2015).

223 Cellulose fraction of the buriti endocarp is surrounded by a matrix of hemicellulose and lignin,
224 which together promote steric hindrance on the cellulose saccharification process. The use of
225 dilute acid treatment has the purpose of solubilizing hemicellulose and increasing the exposure
226 of cellulase target sites on the cellulose homopolymer (Qian et al., 2006; Li et al., 2010). In the
227 present study, the concentration of H_2SO_4 had a significant and positive linear effect ($p < 0.05$) on
228 hemicellulose removal. The interaction of H_2SO_4 concentration over time was also significant (p
229 < 0.05), but with negative effect. Probably, such phenomenon is due to dehydration of glucose to
230 hydroxymethylfurfural promoted by the acid at the longest reaction times (Siankevich et al.,
231 2014; Woo et al., 2015). In addition, it is probable that the glucose found in the hydrolyzate was
232 the product of the hydrolysis of the starch present in the endocarp, since the *β*-1,4 glycosidic
233 bonds between the glucose residues that form the cellulose are recalcitrant to the action of dilute
234 acids. There was 88.26% reduction in the hemicellulose content in the pretreated biomass, with a
235 concomitant increase in the concentration of cellulose and lignin, polymers that were not
236 removed by acid action.

237 In her studies of dilute acid pre-treatment (1 h, 121°C, 0.5 M H_2SO_4) of red and white grape
238 marcs, Corbin et al., (2015) achieved 58% of total carbohydrates liberation from the red marc
239 and 84% from the white marc. Zhang et al., (2011), in turn, reported 74.5% release of the total
240 hemicellulose in his pretreatment assessments of cattails (*Typha* species) (15 min, 180°C, 1%
241 H_2SO_4), demonstrating inferior performances than what was accomplished in this paper.

242 Characterization of the lignocellulosic fraction of the biomass recovered after the alkaline pre-
243 treatment showed that there was a 64% reduction in the lignin concentration when compared to
244 the raw biomass (untreated) and 83% when compared to the biomass after the acid pretreatment
245 (Table 6). The cellulose concentration in the endocarp treated sequentially with H_2SO_4 and

246 NaOH was changed to 88.5%. The alkaline treatment was not effective for the removal of
247 residual hemicellulose. This result indicated that the amounts of the principal components of
248 buriti endocarps can be significantly changed by chemical treatments. Yu *et al*. (2016) published
249 a removal of 84.21% of the lignin present in sugarcane bagasse using aqueous ammonia (25%
250 ammonia, 160°C, 2 MPa, S-L ratio of 1:10, 60 min), however the final percentage of lignin on
251 the pre-treated substrate (3.9%) was not much inferior than what was achieve in this study
252 (4.2%). Azelee *et al* (2014) reported 59.25% lignin removal in a combined treatment (1 g L^{-1}
253 $Ca(OH)_2$, S-L ratio of 1:8, 50°C for 1.5 h; followed by 20% peracetic acid pretreatment at 75°C
254 for 2 h) in her studies of ethanol production from kenaf (*Hibiscus cannabinus*), a hydrolysis
255 performance less efficient than what is described in the present paper.

256 The experiments to optimize the saccharification process of the cellulose contained in the
257 pretreated biomass (Table 7) showed positive and significant linear effects ($p < 0.05$) for the
258 enzyme concentration (Celluclast-Novozyme) and for the hydrolysis time. These effects were
259 expected for a process conducted with enzymatic catalysis since the rate of catalysis is directly
260 proportional to the concentration of enzyme and the product accumulation occurs naturally with
261 the progress of time in the absence of degradation.

262 All quadratic effects were negative and significant ($p < 0.05$). The negative quadratic effects
263 indicated that there were maximum points in the hydrolytic phenomenon, probably due to the
264 exhaustion of the susceptible substrate. The linear effect of the S/L ratio was negative and
265 significant ($p < 0.05$). This observation indicates limitation in the transfer of masses with the
266 increase of the insoluble fraction, which can largely affect the enzymatic attack. The effects of
267 the interactions among the variables were not significant. The condition of the test that presented

268 the greatest release of glucose or reducing sugars was described by the central points, tests 15,
269 16, 17 and 18 (Table 7).

270 The release profiles of glucose and reducing sugars under the conditions of the RCCD can be
271 seen in Figure 3, as well as the optimal condition highlighted for saccharification of the biomass.
272 The model obtained by the experimental design had a regression coefficient (R^2) of 84.15% for
273 the glucose release and 84.42% for the release of reducing sugars. The optimum condition
274 indicated by the response surface methodology (Figure 3) showed the combined use of 74.50 μL
275 of Cellulase g^{-1} of biomass, 11.30% for the S/L ratio and 19.40 hours of reaction time.

276 After applying the optimum saccharification conditions in a preparative test with the pre-treated
277 buriti endocarp, the hydrolyzate obtained contained 129.68 ± 0.72 g L^{-1} of reducing sugars and
278 110.14 ± 0.63 g L^{-1} of glucose. Hydrolytic efficiency was 86.16%.

279 Asada *et al*., (2015) reported a saccharification slightly higher than what is seen in this paper,
280 with a glucose yield of 89% for pre-treated Beech wood using enzymatic hydrolysis (initial
281 substrate concentration of 2%, using of 0.1 g of enzyme per 1 g of substrate, at 140 strokes/min
282 for 120 h and 50°C).

283 The fermentation process was monitored gravimetrically by the evolution of CO_2 and the
284 equivalent values of glucose consumed and ethanol produced were calculated during 18 hours of
285 reaction (Figure 4). No nutrient supplementation was applied. At 16 hours the fermentation had
286 been completed, since no change in the mass of the fermentative system was observed. Once the
287 fermentation was complete, the system was opened and the glucose, reducing sugars and ethanol
288 contents were determined analytically. Thus, 43.16 g L^{-1} of ethanol was produced, with a
289 fermentative efficiency (ethanol yield) of 77% or 0.33 g EtOH g RS^{-1} (Table 8). Koti *et al*.,
290 (2016) and Jing-Ping et al., (2011) reported similar ethanol yields in fermentations of wheat

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291 straw with *Pichia stipitis* PSEB5 (0.34 g g^{-1}) and corncob (0.31 g g^{-1}) using *Candida shehatae*
292 ACCC 20335, respectively. However, the ethanol yield ($Y_{P/S}$) achieved by Ko et al. (2016) using
293 rice straw hydrolysate reached 0.46 g g^{-1}, a value significantly higher than what is detected in
294 this study.

295 Additionally, it is possible to observe that 28.99% of the reducing sugars present in the medium
296 were not consumed. Although the pretreatment processes of the lignocellulosic biomass
297 employed in this work are widely used strategies, they have the disadvantage of generating toxic
298 compounds to fermenting organisms, such as phenolic compounds, guaiacol, levulinic acid,
299 furfural and 5-hydroxymethyl furfural (Varanasi et al., 2013; Liu et al., 2016). The presence of
300 such substances may have inhibited the activity of *S. cerevisiae*, making it impossible to deplete
301 the substrate offered, since the samples were not detoxified to remove these substances.

302

303 **4. Conclusion**

304 The sequential use of diluted H_2SO_4 and NaOH under the conditions established by the process
305 optimizations contributed significantly to the reduction of hemicellulose and lignin content in the
306 pre-treated buriti endocarp, significantly changing its chemical composition. The pre-treatments
307 reverberated in the enzymatic saccharification step, in which 86% of the cellulose was converted
308 to glucose. The efficiency of the fermentative process bio-catalyzed by *Saccharomyces*
309 *cerevisiae* was comparable with literature descriptions of ethanol production using
310 lignocellulosic substrates. Furthermore, the execution of a fermentative process with a higher
311 degree of control and the detoxification of the saccharified buriti endocarp may contribute to
312 enhance ethanol yield and viability.

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313

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Figure 1

Response surface plot of the reducing sugar (RS) removed in the acid hydrolysis treatment.

Response surface plot of the reducing sugar (RS), percentage removed in the acid hydrolysis treatment as a function of (a) the combined values of the H_2SO_4 and solid-liquid ratio (S/L). (b) the combined value of the S/L ratio and time of the reaction.

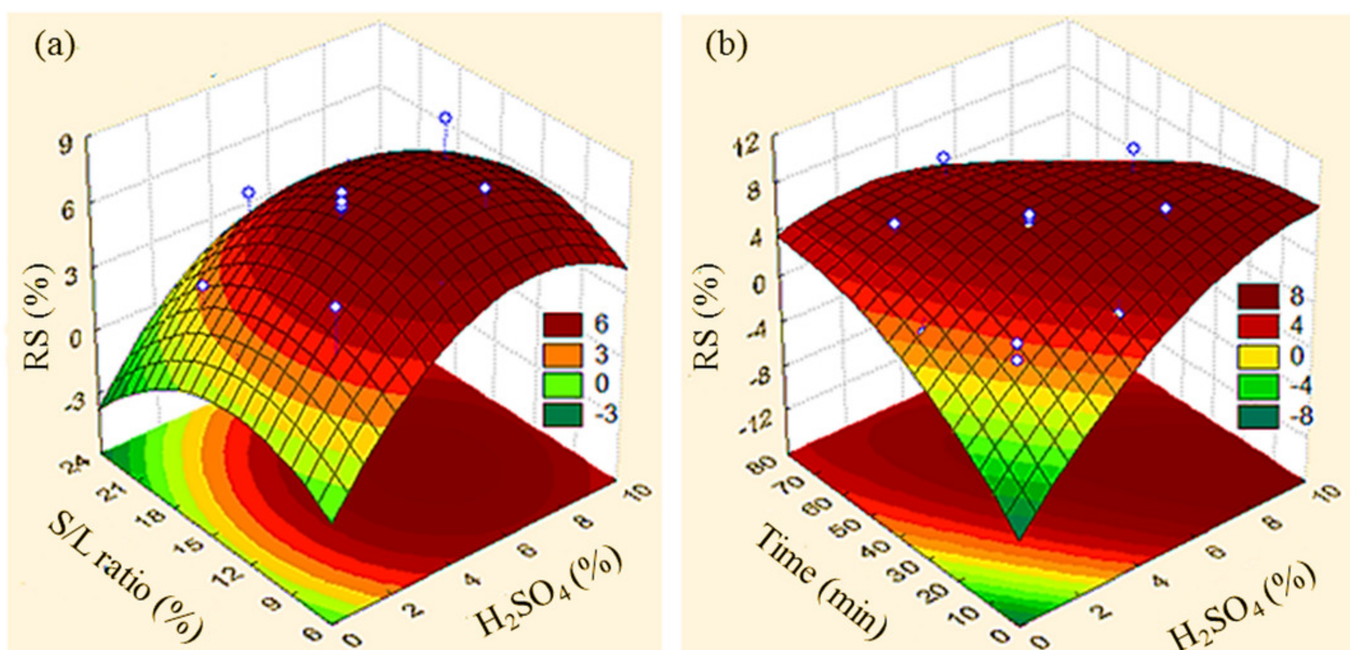

Figure 2

Response surface plot for the percentage of lignin removal expressed in the form of phenolic compounds

Lignin removal, expressed in the form of phenolic compounds released, as a function of the temperature and NaOH concentration, obtained for the maximum lignin hydrolysis, observed at 48h.

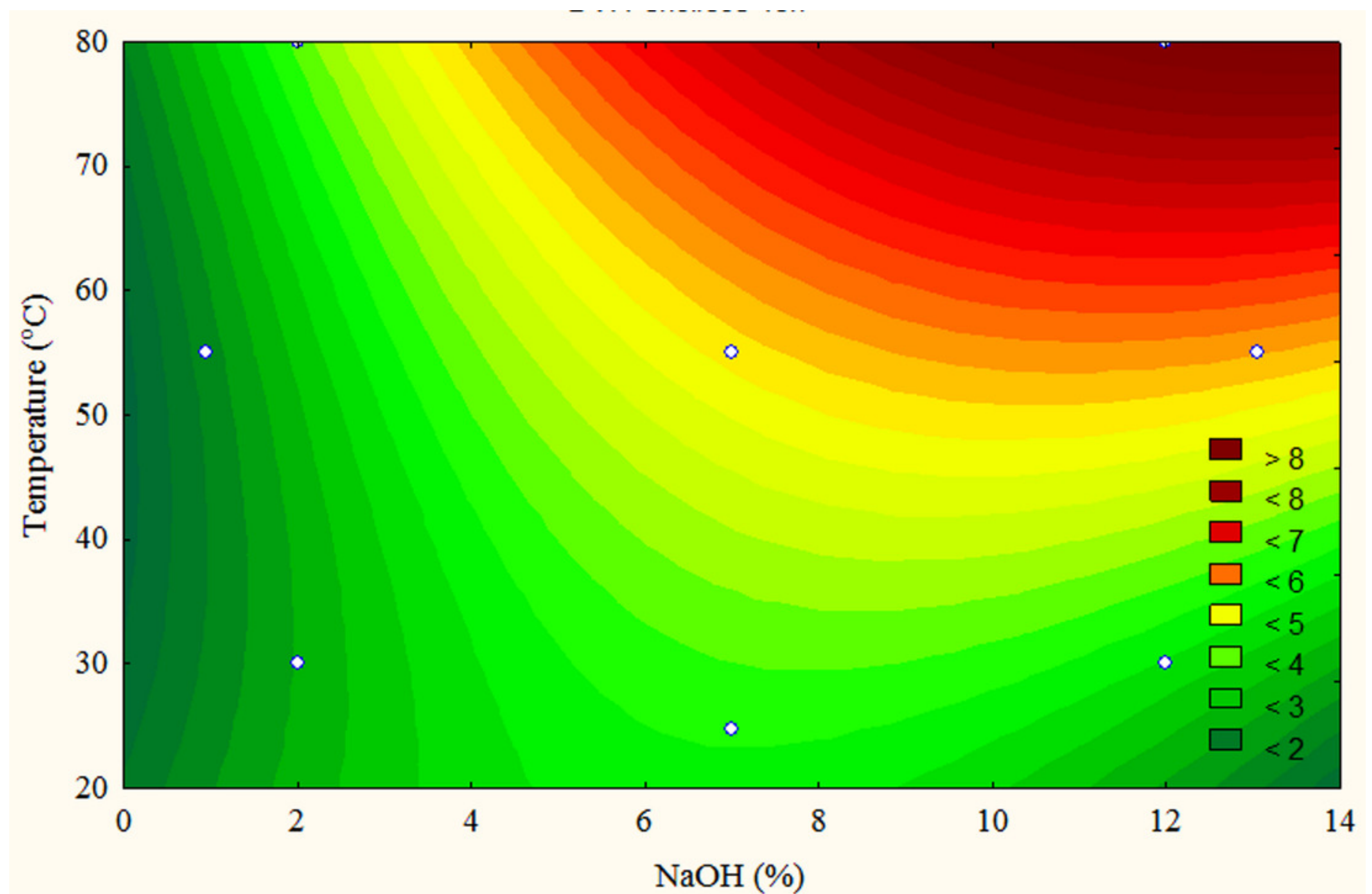

Figure 3

The optimum condition indicated by the response surface methodology for the glucose and reducing sugars release

Profile of predicted and desirable glucose and reducing sugar (RS) values (y-axes) for cellulase concentration, S/L ratio and process time factors (x-axes) used in saccharification of the cellulose contained in the pre-treated buriti endocarp.

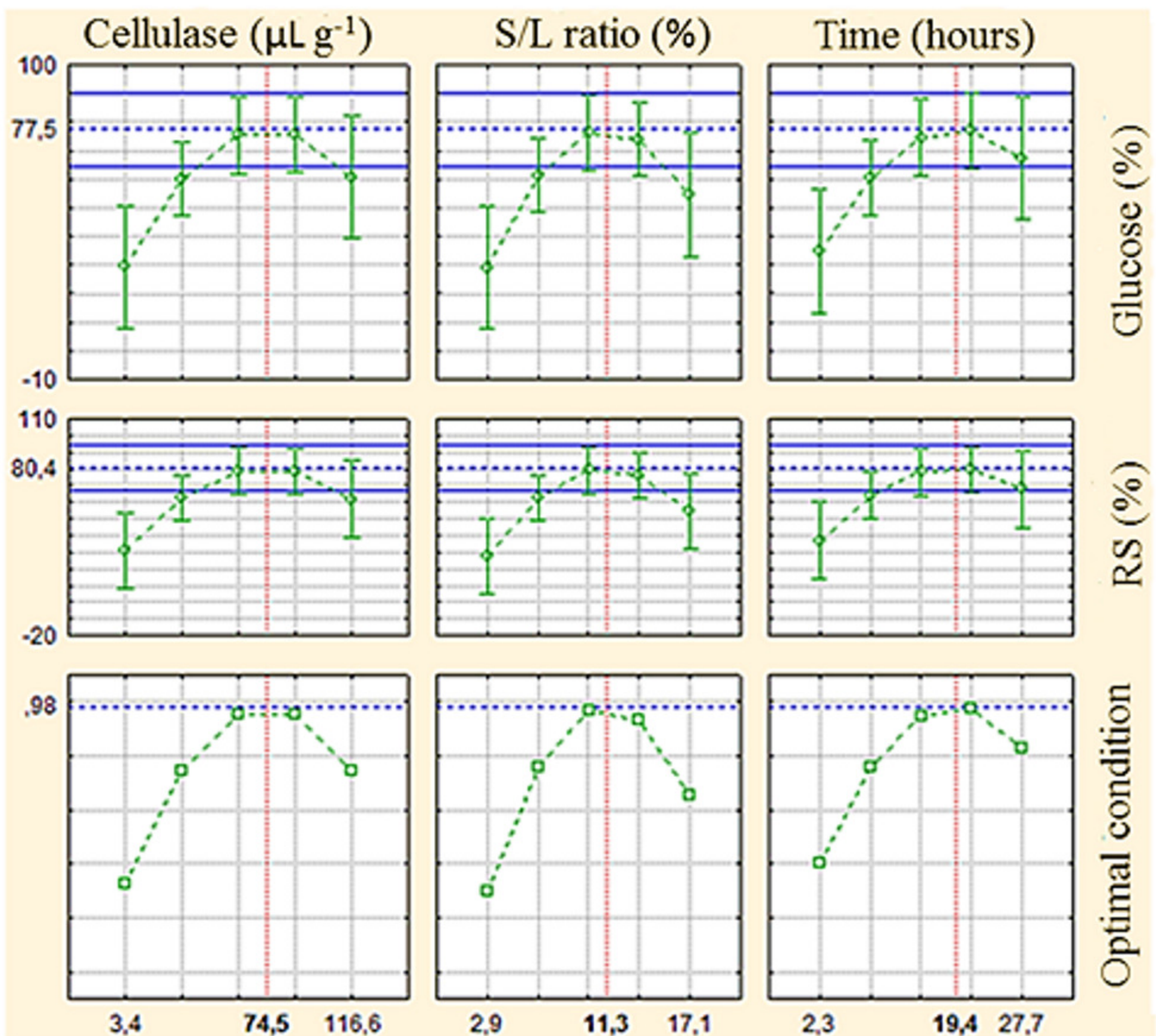

Figure 4

Ethanol production monitored gravimetrically by the evolution of CO_2 and the equivalent values of glucose consumed, calculated during 18 hours of reaction

Progress of the alcoholic fermentation of the enzymatic hydrolyzate of the pre-treated buriti endocarp, conducted in anaerobiosis with *Saccharomyces cerevisiae* yeast.

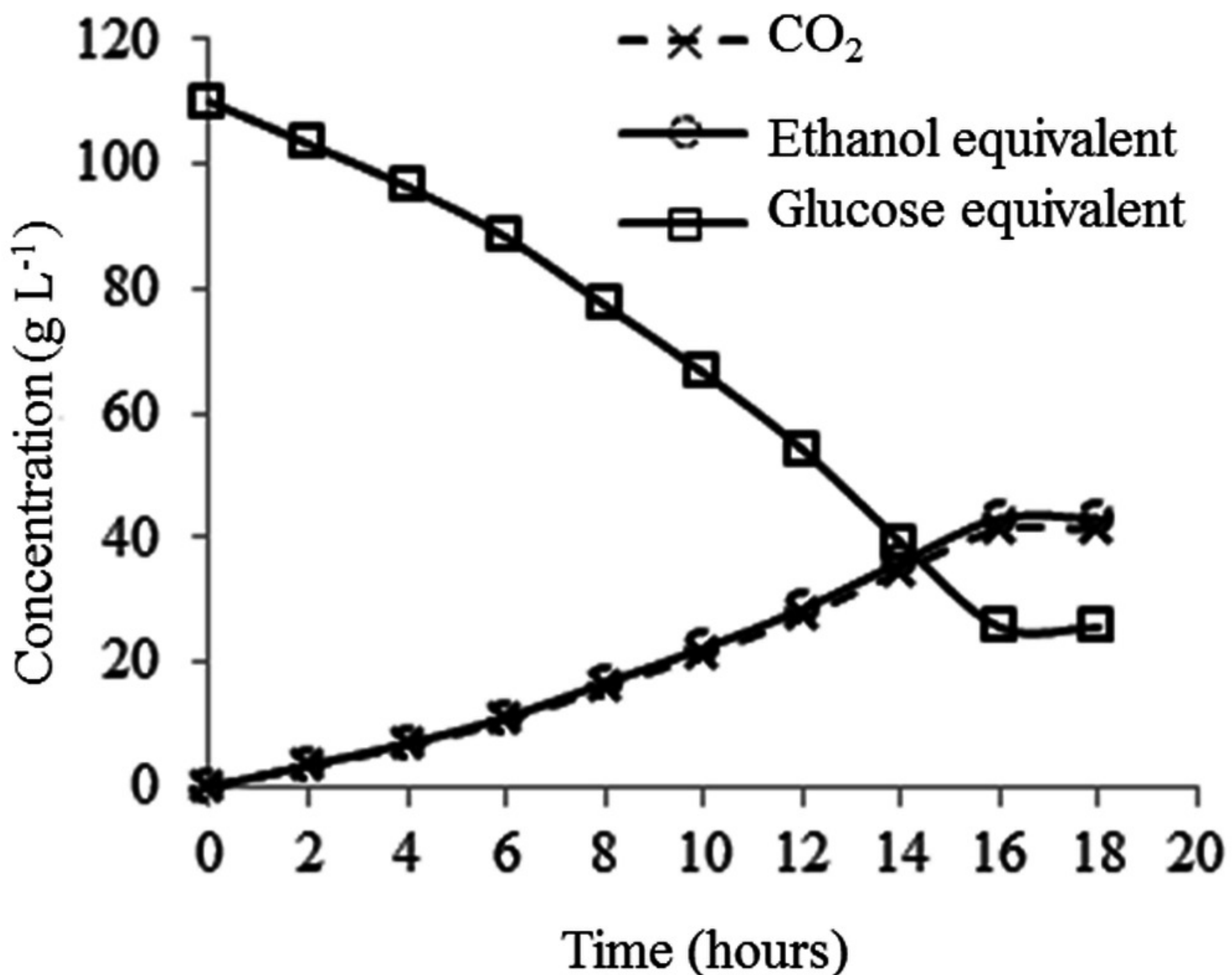

Table 1 (on next page)

Physical characterization of *in natura* buriti fruit

Physical characterizations of *in natura* buriti fruits, the values are expressed as a percentage of the averages followed by their standard deviations.

1

Parameter	Avarege
Pulp Mass (g)	9.26 ± 2.94
Bark Mass (g)	9.40 ± 2.04
Endocarp Mass (g)	9.72 ± 3.00
Peduncle Mass (g)	0.83 ± 0.26
Seed Mass (g)	6.01 ± 2.16
Transversal Diameter (mm)	38.75 ± 3.76
Longitudinal Diameter (mm)	48.68 ± 2.94

2

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Table 2 (on next page)

Chemical characterization of the different parts of the previously dried buritizeiro fruit

Percentage averages followed by their standard deviations. ND - Not Determined, TSS - Total Soluble Sugars

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1

Composition	Epicarp (Bark)	Mesocarp (Pulp)	Endocarp (Seed shell)
Moisture (%)	8.67 ± 0.56	10.29 ± 0.73	9.54 ± 0.19
Ash (%)	1.65 ± 0.04	4.45 ± 0.06	4.46 ± 0.08
Lipids (%)	2.09 ± 0.05	2.39 ± 0.21	4.39 ± 0.37
Total Proteins (%)	1.86 ± 0.04	4.28 ± 0.02	3.62 ± 0.08
Crude Fiber (%)	27.88 ± 0.67	32.58 ± 1.34	26.37 ± 0.44
TSS (%)	3.56 ± 0.01	5.12 ± 0.19	4.49 ± 0.13
Starch (%)	1.33 ± 0.09	6.12 ± 1.29	6.80 ± 0.18
Cellulose (%)	ND	ND	22.15 ± 2.43
Hemicellulose (%)	ND	ND	10.73 ± 0.79
Lignin (%)	ND	ND	11.79 ± 0.30

2

Table 3 (on next page)

Rotational central composite design for the acid pretreatment of buriti endocarp (1 atm, 120°C) with its respective response factors

S/L – Solid-Liquid ratio, RS - Reducing Sugars.

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1

Test	Independent variables			Response factors	
	S/L ratio (%)	H_2SO_4 (%)	Time (min.)	Glucose (%)	RS (%)
1	10	2.00	20.0	0.92	2.22
2	20	2.00	20.0	0.38	0.85
3	10	2.00	60.0	1.52	5.63
4	20	2.00	60.0	0.70	2.55
5	10	7.00	20.0	1.84	8.02
6	20	7.00	20.0	1.05	5.01
7	10	7.00	60.0	0.79	4.26
8	20	7.00	60.0	0.35	2.52
9	15	0.96	40.0	0.13	0.90
10	15	8.04	40.0	2.10	8.75
11	15	4.50	11.7	0.57	3.21
12	15	4.50	68.3	1.68	7.30
13	7	4.50	40.0	1.21	5.10
14	22	4.50	40.0	0.92	4.70
15	15	4.50	40.0	1.46	6.65
16	15	4.50	40.0	1.11	5.90
17	15	4.50	40.0	1.37	6.71
18	15	4.50	40.0	1.24	6.88

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Table 4 (on next page)

Lignocellulosic fraction composition of buriti endocarp samples before and after acid pretreatment

Percentage averages followed by their standard deviations.

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1

Fraction	Buriti endocarp	
	Raw (%)	Pre-treated with H_2SO_4 (%)
Cellulose	22.15 ± 2.43	43.07 ± 0.47
Hemicellulose	10.73 ± 0.79	1.29 ± 0.04
Lignin	11.79 ± 0.30	24.50 ± 0.38

2

Table 5 (on next page)

Rotational central composite design for the alkaline pre-treatment of the buriti endocarp remaining from the acid pre-treatment with its respective response factor in the times of 12h, 24h, 36h and 48h

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1

Test	Independent variables		Total phenolic compounds			
	NaOH (%)	Temperature (°C)	12h (%)	24h (%)	36h (%)	48h (%)
1	2.00	30.00	0.86	1.56	1.93	2.33
2	2.00	80.00	1.73	2.31	3.75	4.45
3	12.00	30.00	1.88	1.96	2.33	3.28
4	12.00	80.00	3.28	5.69	8.40	9.64
5	0.95	55.00	0.48	1.06	1.23	1.85
6	13.05	55.00	2.43	2.96	4.65	4.84
7	7.00	24.75	1.11	1.43	2.06	3.75
8	7.00	85.25	3.09	3.92	5.69	6.55
9	7.00	55.00	1.77	2.54	4.87	5.28
10	7.00	55.00	1.83	2.57	4.91	5.15
11	7.00	55.00	1.81	2.49	4.82	5.25
12	7.00	55.00	1.75	2.43	4.89	5.23

2

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Table 6 (on next page)

Characterization of the lignocellulosic fraction in buriti endocarp samples before and after acid and alkaline pre-treatments

Averages of percentages followed by their standard deviations

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1

Fraction	Buriti Endocarp		
	Raw (%)	Pre-treated with H_2SO_4 (%)	Pre-treated with NaOH (%)
Cellulose	22.15 ± 2.43	43.07 ± 0.47	88.54 ± 0.38
Hemicellulose	10.73 ± 0.79	1.29 ± 0.04	2.73 ± 0.16
Lignin	11.79 ± 0.30	24.50 ± 0.38	4.20 ± 0.28

2

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Table 7 (on next page)

Rotational central composite design used for the enzymatic saccharification of the buriti endocarp sequentially pretreated with acid and alkali and their respective response factors

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1

Test	Cellulase (μL g^{-1})	S/L ratio (%)	Time (h)	Glucose (%)	RS (%)
1	20.00	5.00	6.0	11.36	14.09
2	20.00	5.00	24.0	16.60	16.81
3	20.00	15.00	6.0	17.83	16.23
4	20.00	15.00	24.0	51.39	56.06
5	100.00	5.00	6.0	23.60	21.13
6	100.00	5.00	24.0	33.21	31.12
7	100.00	15.00	6.0	46.67	46.95
8	100.00	15.00	24.0	61.20	59.24
9	3.43	10.00	15.0	6.99	5.67
10	116.57	10.00	15.0	53.49	58.72
11	60.00	2.93	15.0	20.97	19.69
12	60.00	17.07	15.0	31.46	32.91
13	60.00	10.00	2.3	10.48	15.20
14	60.00	10.00	27.7	63.63	61.77
15	60.00	10.00	15.0	76.92	80.59
16	60.00	10.00	15.0	76.04	79.99
17	60.00	10.00	15.0	78.67	80.35
18	60.00	10.00	15.0	76.04	81.68

2

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Table 8(on next page)

Experimental results of buriti endocarp fermentation with *Saccharomyces cerevisiae*

1

Beginning of fermentation (g L^{-1})			End of fermentation (g L^{-1})			$Y_{P/S}$ (g g^{-1})
RS	Glucose	Ethanol	RS	Glucose	Ethanol	0.33
129.68	110.14	0.00	37.59	25.10	43.16	

2