A peer-reviewed version of this preprint was published in PeerJ on 2 August 2018.

View the peer-reviewed version (peerj.com/articles/5275), which is the preferred citable publication unless you specifically need to cite this preprint.

https://doi.org/10.7717/peerj.5275
Evaluation of buriti endocarp as lignocellulosic substrate for second generation ethanol production

Plínio R Rodrigues¹, Mateus FL Araújo¹, Tamarah L Rocha¹, Ronnie Von S VelosoCorresp.², Lilian A Pantoja¹, Alexandre S Santos³

¹ Instituto de Ciência e Tecnologia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brasil
² Programa de Pós-Graduação em Biocombustíveis, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brasil
³ Departamento de Ciências Básicas, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brasil

Corresponding Author: Ronnie Von S Veloso
Email address: ronnievond@yahoo.com.br

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-catalyzed by Saccharomyces cerevisiae resulted in a fermented medium with 4.3% ethanol and Y_{P/S} of 0.33.
EVALUATION OF BURITI ENDOCARP AS LIGNOCELLULOSIC SUBSTRATE FOR SECOND GENERATION ETHANOL PRODUCTION

Plínio Ribeiro Rodrigues a, Mateus Felipe Lourêdo Araújo a, Tamarah Lauar Rocha a, Ronnie Von dos Santos Veloso b, Lilian de Araújo Pantoja a e Alexandre Soares dos Santos c,*. 

aInstitute of Science and Technology, Federal University of Jequitinhonha and Mucuri Valleys, 39100-000, Diamantina – MG, Brazil.

bGraduate Program in Biofuels, Federal University of Jequitinhonha and Mucuri Valleys, 39100-000, Diamantina – MG, Brazil.

cDepartment of Basic Sciences, Federal University of Jequitinhonha and Mucuri Valleys, 39100-000, Diamantina – MG, Brazil.

*e-mail: alexandre.soares@ufvjm.edu.br

Abstract

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.

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

---

**Introduction**

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

The technology for the production of second generation (2G) bioethanol, or lignocellulosic ethanol, has evolved in the last decades and functioning industrial plants already exist in some parts of the world, nevertheless, this biofuel still faces the challenge of feedstock access, supply chain infrastructure, and price competitiveness with the petroleum industry (UNCTAD, 2016).
Lignocellulosic ethanol can be obtained from the fermentation of hexoses and pentoses derived from the polysaccharides that constitute the plants cell wall and require additional operations to those normally used to produce first generation ethanol (Mielenz, 2001). Lignin removal and hemicellulose hydrolysis, followed by cellulose saccharification, are necessary steps to provide the sugars to be fermented by specialized microorganisms to produce 2G ethanol (Maurya, Singla & Negi 2015; Keshav, Naseeruddin, & Rao, 2016). In this sense, there is a large number of biomasses being evaluated as raw materials for this nascent industry, with emphasis for agro-industrial residues (Macedo et al., 2011; Hoa, Ngob & Guo 2014; Domínguez-Bocanegra, Torres-Muñoz & López, 2015).

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

This lignocellulosic residue (endocarp) is a potential source for the production of 2G ethanol, since it is an abundant waste product of the buritizeiro palm exploitation and does not directly require the availability of more cultivation lands (Van, Brose & Schenkel 2011; Bos et al., 2016). Also, buriti endocarp usage presents no competition with the food market chain and represents potential income generation, as its use adds value to an underutilized material (Kang et al., 2014; Sawatdeenarunat et al., 2015).
In this paper, the buriti fruit was physically and chemically characterized and its endocarp was evaluated with respect to its potential for 2G ethanol production. Pre-treatments with dilute sulfuric acid and sodium hydroxide, and enzymatic saccharification were performed using response surface methodology. The effects of the factors studied in the pre-treatments and in the enzymatic hydrolysis were evaluated and the optimal conditions were highlighted. Ultimately, the saccharified cellulose from the buriti endocarp was fermented to ethanol using *Saccharomyces cerevisiae*.

Material & methods

Physical characterization of buritizeiro fruits

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

Chemical characterization of buriti fruit fractions

Previously dried and crushed pulp, bark and endocarp were characterized in terms of total moisture, ashes, proteins and lipids, according to Adolph Lutz Institute (IAL) analytical...
standards (IAL, 2008). Crude fiber content was determined according to Kamer and Ginkel (1952). Starch and total soluble sugars (TSS) contents were determined according to methodology described by McCready et al. (1950). The endocarp was further characterized by its cellulose, hemicellulose and lignin contents, quantified by neutral detergent fiber (NDF) and acid detergent fiber (FDA) methods described by Van Soest (1963,1964,1968).

**Pre-treatment of buriti endocarp**

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

**Treatment with dilute sulfuric acid**

Determination of the ideal conditions for the acid pre-treatment of the biomass was accomplished through the use of a Rotational Central Composite Design (RCCD) that evaluated the influence of reaction time; 20 min. (-1) and 60 min. (-1), solid-liquid (S/L) ratio; 10% (-1) and 20% (+1), and concentration of sulfuric acid (H$_2$SO$_4$); 2% (-1) and 7% (+1), on the removal of hemicellulose contained in the buriti endocarp. In each test, carried out in a glass tube (30 x 2.5 cm), 1 g of sample was added with the solution of H$_2$SO$_4$ in the pre-defined concentration and proportion for each of the 18 tests generated by the factorial matrix $2^3$, containing four central points and six axial points. The tests were performed in an autoclave (1 atm) at a fixed temperature of 120°C (Corbin et al, 2015).

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

Quantification of the glucose released after acid hydrolysis was determined by the enzymatic-colorimetric method described by Lloyd and Whelan (1969). The quantification of the reducing sugars (RS) in the acid hydrolyzate was carried out using the dinitrosalicylic acid method.
described by Miller (1959). The decomposition of hemicellulose was expressed in grams of sugar liberated per 100 g of biomass.

**Pre-treatment with sodium hydroxide**

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

**Enzymatic saccharification of pretreated endocarp**

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

Fermentation of the enzymatic hydrolyzate obtained in the optimized conditions for the saccharification of the pre-treated biomass was carried out in 250 mL conical flasks coupled to fermentometers, a glass system that allows carbon dioxide (CO$_2$) release and prevents the entry of external air, at room temperature (25 ± 2°C). Dehydrated commercial baker's yeast (Fleischmann®) of the *Saccharomyces cerevisiae* species was used as a fermentative agent in the ratio of 1% (w/v) to the must volume. The fermentative process was monitored gravimetrically for CO$_2$ release. The measurement of mass of gas released was used to estimate the ethanol production and the consumption of the fermentable sugars every two hours until the end of the fermentation. The concentration of ethanol quantified by potassium dichromate method, according to methodology described by Isarankura-Na Ayudhya et al (2007), and the concentrations of glucose and reducing sugars were determined at the beginning and at the end of the fermentation process.

Statistical analysis

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

Results

Physical characterizations of *in natura* buriti fruits are displayed in Table 1, the values are disposed in percentage averages followed by their standard deviations. The integral fruits presented an average mass of 38.33 ± 9.06 g, transversal diameter of 38.75 ± 3.76 mm and
longitudinal diameter of 48.68 ± 2.94 mm (Table 1). The endocarp mass represented, in average, 25.3% of the whole fruit.

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

In this study, buriti endocarp was subjected to a sequence of acid and alkali treatments with the purpose of exposing the cellulose polymers to the enzymatic hydrolysis to obtain monomers of hexoses for their subsequent anaerobic fermentation bio-catalyzed by *S. cerevisiae* yeast. The quantities of reducing sugars and glucose removed per 100 g of endocarp subjected to acid pretreatment under the different experimental design conditions are shown in Table 3. Negative quadratic individual effects of H$_2$SO$_4$ and S/L ratio were also observed, however, with $p$ values of 0.114 and 0.102, respectively. The combined effects of H$_2$SO$_4$ concentration with the S/L ratio factors and H$_2$SO$_4$ concentration over time on the removal of the hemicellulosic portion from the buriti endocarp are presented as response surface curves in Figure 1, the coefficient of determination ($R^2$) was 0.81.

There was greater release of reducing sugars in the condition of test 10 (Table 3). In this point, 8.75 g of reducing sugars per 100 g of biomass were removed. On the other hand, in the condition of test 5 (Table 3), 8.02 g of reducing sugars per 100 g of biomass were removed, 9% less than under test condition 10. The test 5 was then chosen as optimal condition for the
preparative test for using less acid concentration and half the reaction time of test 10. Not coincidentally, the areas under the response surface curves (Figure 1) representing the regions with the highest hemicellulose removal refer to the combination of factors indicated by the conditions of the test 5 (Table 3). The characterization of the lignocellulosic fraction of buriti endocarp recovered after acid pretreatment using the optimum condition defined, indicated changes in the contents of cellulose, hemicellulose and lignin (Table 4).

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

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

\[
L = 5.10 + 2.82C - 1.75C^2 + 3.43T + 0.71T^2 + 2.12CT
\]

(1)

Where:

$L =$ Total phenolic compounds (%);

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

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

**Discussion**

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

Cellulose, lignin and hemicellulose were, in this order, the most abundant compounds in the chemical structure of the buriti endocarp (Table 2). These molecules are responsible for the stiffness of the material, an important feature for seed protection (Dardick & Callahan, 2014). It is important to note that most fermentable sugars in lignocellulosic biomass come from polymers abundant in hexoses and pentoses, respectively cellulose and hemicellulose. However, there are still no fully consolidated strategies for the production of ethanol from hemicellulose. Thus, cellulose is the polymer most used as sugar source for the production of second generation ethanol and, therefore, the fraction used for the production of ethanol in the present assessment (Nigam 2001; Agbogbo & Coward-Kelly, 2008; Saini, Saini & Tewari, 2015).
Cellulose fraction of the buriti endocarp is surrounded by a matrix of hemicellulose and lignin, which together promote steric hindrance on the cellulose saccharification process. The use of dilute acid treatment has the purpose of solubilizing hemicellulose and increasing the exposure of cellulase target sites on the cellulose homopolymer (Qian et al., 2006; Li et al., 2010). In the present study, the concentration of H$_2$SO$_4$ had a significant and positive linear effect (p < 0.05) on hemicellulose removal. The interaction of H$_2$SO$_4$ concentration over time was also significant (p < 0.05), but with negative effect. Probably, such phenomenon is due to dehydration of glucose to hydroxymethylfurfural promoted by the acid at the longest reaction times (Siankevich et al., 2014; Woo et al., 2015). In addition, it is probable that the glucose found in the hydrolyzate was the product of the hydrolysis of the starch present in the endocarp, since the β-1,4 glycosidic bonds between the glucose residues that form the cellulose are recalcitrant to the action of dilute acids. There was 88.26% reduction in the hemicellulose content in the pretreated biomass, with a concomitant increase in the concentration of cellulose and lignin, polymers that were not removed by acid action.

In her studies of dilute acid pre-treatment (1 h, 121°C, 0.5 M H$_2$SO$_4$) of red and white grape marc, Corbin et al., (2015) achieved 58% of total carbohydrates liberation from the red marc and 84% from the white marc. Zhang et al., (2011), in turn, reported 74.5% release of the total hemicellulose in his pretreatment assessments of cattails (Typha species) (15 min, 180°C, 1% H$_2$SO$_4$), demonstrating inferior performances than what was accomplished in this paper.

Characterization of the lignocellulosic fraction of the biomass recovered after the alkaline pre-treatment showed that there was a 64% reduction in the lignin concentration when compared to the raw biomass (untreated) and 83% when compared to the biomass after the acid pretreatment (Table 6). The cellulose concentration in the endocarp treated sequentially with H$_2$SO$_4$ and
NaOH was changed to 88.5%. The alkaline treatment was not effective for the removal of residual hemicellulose. This result indicated that the amounts of the principal components of buriti endocarps can be significantly changed by chemical treatments. Yu et al. (2016) published a removal of 84.21% of the lignin present in sugarcane bagasse using aqueous ammonia (25% ammonia, 160°C, 2 MPa, S-L ratio of 1:10, 60 min), however the final percentage of lignin on the pre-treated substrate (3.9%) was not much inferior than what was achieve in this study (4.2%). Azelee et al (2014) reported 59.25% lignin removal in a combined treatment (1 g L\(^{-1}\) Ca(OH)\(_2\), S-L ratio of 1:8, 50°C for 1.5 h; followed by 20% peracetic acid pretreatment at 75°C for 2 h) in her studies of ethanol production from kenaf (Hibiscus cannabinus), a hydrolysis performance less efficient than what is described in the present paper.

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

All quadratic effects were negative and significant (p <0.05). The negative quadratic effects indicated that there were maximum points in the hydrolytic phenomenon, probably due to the exhaustion of the susceptible substrate. The linear effect of the S/L ratio was negative and significant (p <0.05). This observation indicates limitation in the transfer of masses with the increase of the insoluble fraction, which can largely affect the enzymatic attack. The effects of the interactions among the variables were not significant. The condition of the test that presented
the greatest release of glucose or reducing sugars was described by the central points, tests 15, 16, 17 and 18 (Table 7).

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

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

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

The fermentation process was monitored gravimetrically by the evolution of CO$_2$ and the equivalent values of glucose consumed and ethanol produced were calculated during 18 hours of reaction (Figure 4). No nutrient supplementation was applied. At 16 hours the fermentation had been completed, since no change in the mass of the fermentative system was observed. Once the fermentation was complete, the system was opened and the glucose, reducing sugars and ethanol contents were determined analytically. Thus, 43.16 g L$^{-1}$ of ethanol was produced, with a fermentative efficiency (ethanol yield) of 77% or 0.33 g EtOH g RS$^{-1}$ (Table 8). Koti et al., (2016) and Jing-Ping et al., (2011) reported similar ethanol yields in fermentations of wheat
straw with *Pichia stipitis* PSEB5 (0.34 g g\(^{-1}\)) and corncob (0.31 g g\(^{-1}\)) using *Candida shehatae* ACCC 20335, respectively. However, the ethanol yield (\(Y_{P/S}\)) achieved by Ko et al. (2016) using rice straw hydrolysate reached 0.46 g g\(^{-1}\), a value significantly higher than what is detected in this study.

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

### 4. Conclusion

The sequential use of diluted H\(_2\)SO\(_4\) and NaOH under the conditions established by the process optimizations contributed significantly to the reduction of hemicellulose and lignin content in the pre-treated buriti endocarp, significantly changing its chemical composition. The pre-treatments reverberated in the enzymatic saccharification step, in which 86% of the cellulose was converted to glucose. The efficiency of the fermentative process bio-catalyzed by *Saccharomyces cerevisiae* was comparable with literature descriptions of ethanol production using lignocellulosic substrates. Furthermore, the execution of a fermentative process with a higher degree of control and the detoxification of the saccharified buriti endocarp may contribute to enhance ethanol yield and viability.
5. Acknowledgments

This study was supported by the Institutional Program of Scientific Initiation Grants - PIBIC of the Federal University of the Jequitinhonha and Mucuri Valleys and financed by the Foundation for Research Support of the State of Minas Gerais.

References


Nigam JN. 2001. Ethanol production from wheat straw hemicellulose hydrolysate by Pichia stipitis. Journal of Biotechnology 87, 17-27 DOI: 10.1016/S0168-1656(00)00385-0


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 $\text{H}_2\text{SO}_4$ 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.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulp Mass (g)</td>
<td>9.26 ± 2.94</td>
</tr>
<tr>
<td>Bark Mass (g)</td>
<td>9.40 ± 2.04</td>
</tr>
<tr>
<td>Endocarp Mass (g)</td>
<td>9.72 ± 3.00</td>
</tr>
<tr>
<td>Peduncle Mass (g)</td>
<td>0.83 ± 0.26</td>
</tr>
<tr>
<td>Seed Mass (g)</td>
<td>6.01 ± 2.16</td>
</tr>
<tr>
<td>Transversal Diameter (mm)</td>
<td>38.75 ± 3.76</td>
</tr>
<tr>
<td>Longitudinal Diameter (mm)</td>
<td>48.68 ± 2.94</td>
</tr>
</tbody>
</table>
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
<table>
<thead>
<tr>
<th>Composition</th>
<th>Epicarp (Bark)</th>
<th>Mesocarp (Pulp)</th>
<th>Endocarp (Seed shell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>8.67 ± 0.56</td>
<td>10.29 ± 0.73</td>
<td>9.54 ± 0.19</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.65 ± 0.04</td>
<td>4.45 ± 0.06</td>
<td>4.46 ± 0.08</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>2.09 ± 0.05</td>
<td>2.39 ± 0.21</td>
<td>4.39 ± 0.37</td>
</tr>
<tr>
<td>Total Proteins (%)</td>
<td>1.86 ± 0.04</td>
<td>4.28 ± 0.02</td>
<td>3.62 ± 0.08</td>
</tr>
<tr>
<td>Crude Fiber (%)</td>
<td>27.88 ± 0.67</td>
<td>32.58 ± 1.34</td>
<td>26.37 ± 0.44</td>
</tr>
<tr>
<td>TSS (%)</td>
<td>3.56 ± 0.01</td>
<td>5.12 ± 0.19</td>
<td>4.49 ± 0.13</td>
</tr>
<tr>
<td>Starch (%)</td>
<td>1.33 ± 0.09</td>
<td>6.12 ± 1.29</td>
<td>6.80 ± 0.18</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>ND</td>
<td>ND</td>
<td>22.15 ± 2.43</td>
</tr>
<tr>
<td>Hemicellulose (%)</td>
<td>ND</td>
<td>ND</td>
<td>10.73 ± 0.79</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>ND</td>
<td>ND</td>
<td>11.79 ± 0.30</td>
</tr>
</tbody>
</table>
**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.
<table>
<thead>
<tr>
<th>Test</th>
<th>S/L ratio (%)</th>
<th>H$_2$SO$_4$ (%)</th>
<th>Time (min.)</th>
<th>Glucose (%)</th>
<th>RS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>2.00</td>
<td>20.0</td>
<td>0.92</td>
<td>2.22</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>2.00</td>
<td>20.0</td>
<td>0.38</td>
<td>0.85</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>2.00</td>
<td>60.0</td>
<td>1.52</td>
<td>5.63</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>2.00</td>
<td>60.0</td>
<td>0.70</td>
<td>2.55</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>7.00</td>
<td>20.0</td>
<td>1.84</td>
<td>8.02</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>7.00</td>
<td>20.0</td>
<td>1.05</td>
<td>5.01</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>7.00</td>
<td>60.0</td>
<td>0.79</td>
<td>4.26</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>7.00</td>
<td>60.0</td>
<td>0.35</td>
<td>2.52</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>0.96</td>
<td>40.0</td>
<td>0.13</td>
<td>0.90</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>8.04</td>
<td>40.0</td>
<td>2.10</td>
<td>8.75</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>4.50</td>
<td>11.7</td>
<td>0.57</td>
<td>3.21</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>4.50</td>
<td>68.3</td>
<td>1.68</td>
<td>7.30</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>4.50</td>
<td>40.0</td>
<td>1.21</td>
<td>5.10</td>
</tr>
<tr>
<td>14</td>
<td>22</td>
<td>4.50</td>
<td>40.0</td>
<td>0.92</td>
<td>4.70</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>4.50</td>
<td>40.0</td>
<td>1.46</td>
<td>6.65</td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td>4.50</td>
<td>40.0</td>
<td>1.11</td>
<td>5.90</td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>4.50</td>
<td>40.0</td>
<td>1.37</td>
<td>6.71</td>
</tr>
<tr>
<td>18</td>
<td>15</td>
<td>4.50</td>
<td>40.0</td>
<td>1.24</td>
<td>6.88</td>
</tr>
</tbody>
</table>
Table 4 (on next page)

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

Percentage averages followed by their standard deviations.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Buriti endocarp</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw (%)</td>
<td>Pre-treated with H$_2$SO$_4$ (%)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>22.15 ± 2.43</td>
<td>43.07 ± 0.47</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>10.73 ± 0.79</td>
<td>1.29 ± 0.04</td>
</tr>
<tr>
<td>Lignin</td>
<td>11.79 ± 0.30</td>
<td>24.50 ± 0.38</td>
</tr>
</tbody>
</table>
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.
<table>
<thead>
<tr>
<th>Test</th>
<th>Independent variables</th>
<th>Total phenolic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaOH (%)</td>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>1</td>
<td>2.00</td>
<td>30.00</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
<td>80.00</td>
</tr>
<tr>
<td>3</td>
<td>12.00</td>
<td>30.00</td>
</tr>
<tr>
<td>4</td>
<td>12.00</td>
<td>80.00</td>
</tr>
<tr>
<td>5</td>
<td>0.95</td>
<td>55.00</td>
</tr>
<tr>
<td>6</td>
<td>13.05</td>
<td>55.00</td>
</tr>
<tr>
<td>7</td>
<td>7.00</td>
<td>24.75</td>
</tr>
<tr>
<td>8</td>
<td>7.00</td>
<td>85.25</td>
</tr>
<tr>
<td>9</td>
<td>7.00</td>
<td>55.00</td>
</tr>
<tr>
<td>10</td>
<td>7.00</td>
<td>55.00</td>
</tr>
<tr>
<td>11</td>
<td>7.00</td>
<td>55.00</td>
</tr>
<tr>
<td>12</td>
<td>7.00</td>
<td>55.00</td>
</tr>
</tbody>
</table>
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
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Buriti Endocarp</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw (%)</td>
<td>Pre-treated with H₂SO₄ (%)</td>
<td>Pre-treated with NaOH (%)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>22.15 ± 2.43</td>
<td>43.07 ± 0.47</td>
<td>88.54 ± 0.38</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>10.73 ± 0.79</td>
<td>1.29 ± 0.04</td>
<td>2.73 ± 0.16</td>
</tr>
<tr>
<td>Lignin</td>
<td>11.79 ± 0.30</td>
<td>24.50 ± 0.38</td>
<td>4.20 ± 0.28</td>
</tr>
</tbody>
</table>
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
<table>
<thead>
<tr>
<th>Test</th>
<th>Cellulase (µL g⁻¹)</th>
<th>S/L ratio (%)</th>
<th>Time (h)</th>
<th>Glucose (%)</th>
<th>RS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.00</td>
<td>5.00</td>
<td>6.0</td>
<td>11.36</td>
<td>14.09</td>
</tr>
<tr>
<td>2</td>
<td>20.00</td>
<td>5.00</td>
<td>24.0</td>
<td>16.60</td>
<td>16.81</td>
</tr>
<tr>
<td>3</td>
<td>20.00</td>
<td>15.00</td>
<td>6.0</td>
<td>17.83</td>
<td>16.23</td>
</tr>
<tr>
<td>4</td>
<td>20.00</td>
<td>15.00</td>
<td>24.0</td>
<td>51.39</td>
<td>56.06</td>
</tr>
<tr>
<td>5</td>
<td>100.00</td>
<td>5.00</td>
<td>6.0</td>
<td>23.60</td>
<td>21.13</td>
</tr>
<tr>
<td>6</td>
<td>100.00</td>
<td>5.00</td>
<td>24.0</td>
<td>33.21</td>
<td>31.12</td>
</tr>
<tr>
<td>7</td>
<td>100.00</td>
<td>15.00</td>
<td>6.0</td>
<td>46.67</td>
<td>46.95</td>
</tr>
<tr>
<td>8</td>
<td>100.00</td>
<td>15.00</td>
<td>24.0</td>
<td>61.20</td>
<td>59.24</td>
</tr>
<tr>
<td>9</td>
<td>3.43</td>
<td>10.00</td>
<td>15.0</td>
<td>6.99</td>
<td>5.67</td>
</tr>
<tr>
<td>10</td>
<td>116.57</td>
<td>10.00</td>
<td>15.0</td>
<td>53.49</td>
<td>58.72</td>
</tr>
<tr>
<td>11</td>
<td>60.00</td>
<td>2.93</td>
<td>15.0</td>
<td>20.97</td>
<td>19.69</td>
</tr>
<tr>
<td>12</td>
<td>60.00</td>
<td>17.07</td>
<td>15.0</td>
<td>31.46</td>
<td>32.91</td>
</tr>
<tr>
<td>13</td>
<td>60.00</td>
<td>10.00</td>
<td>2.3</td>
<td>10.48</td>
<td>15.20</td>
</tr>
<tr>
<td>14</td>
<td>60.00</td>
<td>10.00</td>
<td>27.7</td>
<td>63.63</td>
<td>61.77</td>
</tr>
<tr>
<td>15</td>
<td>60.00</td>
<td>10.00</td>
<td>15.0</td>
<td>76.92</td>
<td>80.59</td>
</tr>
<tr>
<td>16</td>
<td>60.00</td>
<td>10.00</td>
<td>15.0</td>
<td>76.04</td>
<td>79.99</td>
</tr>
<tr>
<td>17</td>
<td>60.00</td>
<td>10.00</td>
<td>15.0</td>
<td>78.67</td>
<td>80.35</td>
</tr>
<tr>
<td>18</td>
<td>60.00</td>
<td>10.00</td>
<td>15.0</td>
<td>76.04</td>
<td>81.68</td>
</tr>
</tbody>
</table>
Table 8 (on next page)

Experimental results of buriti endocarp fermentation with *Saccharomyces cerevisiae*
<table>
<thead>
<tr>
<th></th>
<th>Beginning of fermentation (g L(^{-1}))</th>
<th>End of fermentation (g L(^{-1}))</th>
<th>(Y_{P/S}) (g g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RS</td>
<td>Glucose</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Beginning of</td>
<td>129.68</td>
<td>110.14</td>
<td>0.00</td>
</tr>
<tr>
<td>fermentation (g L(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>