ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 14, Issue, 07, pp. 66100-66105, July, 2024
https://doi.org/10.37118/ijdr.28365.07.2024

RESEARCH ARTICLE OPEN ACCESS

ACID HYDROLYSIS OF LIGNOCELLULOSIC MATERIALS FOR THE PRODUCTION OF SECOND GENERATION ETHANOL

***Mariane Daniella da Silva, João Pedro Cano and Crispin Humberto Garcia-Cruz**

São Paulo State Universisty, Brazil

ARTICLE INFO

Article History:
Received 03rd April, 2024
Received in revised form
24th May, 2024
Accepted 11th June, 2024
Published online 27th July, 2024

Key Words:
Waste; Biomass; Fermentable sugars; Cellulose; Hemicellulose; Lignin.

****Corresponding author:***
Mariane Daniella da Silva

ABSTRACT

Brazil is one of the countries with the largest agricultural production in the world, consequently, it generates large amounts of agro-industrial residues that are used as biomass in the production of second-generation ethanol, a renewable energy alternative capable of replacing fossil fuels. The objective of this work was to study the effect of diluted acid hydrolysis in different types of lignocellulosic residues and the production of 2G ethanol from these hydrolysates using different fermenting microorganisms. The hydrolysis experiment was carried out with different concentrations of sulfuric acid (0-5%) submitted to different heating times in an autoclave (10-15 min.). Analyzes of total reducing sugars and phenolic compounds were performed before fermentation by the microorganisms produced ethanol. Fermentation ocured for 24 hours at 30 ºC, with tests of different agitation of the fermented broth. The acid hydrolysis condition that released the highest amount of fermentable sugars was 5.0% sulfuric acid and the contact time with the biomass was 15 min. heating in an autoclave at 121 °C. The material that showed the highest release of sugars after acid hydrolysis was cassava residue, with 131.09 $g.L^{-1}$ of reducing sugars. The fermentations were carried out with microorganisms individually and in consortium. The largest production of 2G ethanol was from the hydrolyzate of soybean hulls, from 47.70 $g.L^{-1}$ of ethanol with a productivity of 5.96 $g.L^{-1}.h^{-1}$ by the consortium of *Zymomonas mobilis* and *Candida tropicalis*, during 8 h of fermentation. The hydrolyzed lignocellulosic materials released fermentable sugars that resulted in the production of second-generation ethanol.

Citation*: *Mariane Daniella da Silva, João Pedro Cano and Crispin Humberto Garcia-Cruz. 2024. *"Acid hydrolysis of lignocellulosic materials for the production of second generation ethanol". International Journal of Development Research*, 14, (07), 66100-66105.

INTRODUCTION

Brazil is one of the largest fruit producers in the world and one of the largest generators of agricultural and forestry waste, therefore, large amounts of lignocellulosic biomass is obtained, making it viable to use these renewable and sustainable materials as alternative sources for energy production (VARANDA *et al*., 2018). The inadequate disposal of these wastes causes serious environmental problems, such as soil and water contamination and atmospheric pollution (MENDES *et al*., 2015). In view of this, an alternative to minimize such damage to the environment is the use of biomass for the generation of biofuels (PONNUSAMY *et al*., 2019). Lignocellulosic biomass, an abundant source of renewable carbon, it is mainly formed by cellulose (40-60% of the total dry weight), hemicellulose (20-40%) and lignin (10-25%), it has a low cost, in addition to wide availability. It can be used for the production of second-generation ethanol (2G) without needing extra land for cultivation or generating interference in the production of food and feed (WANG *et al*., 2017; HANSSEN *et al*., 2020). The 2G ethanol is a promising option for an environmentally cleaner fuel and a renewable form of energy for the transport sector. The production of 2G ethanol from lignocellulosic biomass requires a separation of cellulose and hemicellulose and their subsequent breakdown into fermentable sugars, to be converted into alcohol by microorganisms. However, for better use of the lignocellulos material, a hydrolysis treatment step is necessary, as these residues have a rigid and complex structure, which hinders their degradation (SILVA *et al*., 2020). Hydrolysis methods can be chemical, physical, physical-chemical or biological (use of enzymes), or also a combination of these methods. Among these, acid hydrolysis is the lowest cost chemical treatment used to break the lignocellulosic matrix resulting in the release of a significant amount of glucose and xylose monomers (CANDIDO *et al*., 2019; AGUIAR *et al*., 2021). In view of the above, the objective of this work was to carry out the acid hydrolysis of cassava residues, sugarcane bagasse, grape stalks, lemon peels, banana, orange, soybean, passion fruit and green coconut followed by its use as a fermentation process for the production of 2G ethanol using different strains of microorganisms.

MATERIAL AND METHODS

Raw material: Cassava residues (tips, peels and intershells), passion fruit residues (mesocarp and peels), grape residues (stalks), soy peels and fruit peels: lemon, banana, orange and green coconut, were all used as biomass. The fruit peels were collected in the municipal market of São José do Rio Preto – SP. The other residues were

disposed of by food industries in the region of São José do Rio Preto – SP.

Preparation of raw material: The shells were cut manually with the aid of a stainless steel knife into pieces smaller than 3 cm, distributed in stainless steel trays and exposed to the sun, for approximately 24 hours, until they were hard and brittle. The other residues were crushed and dried in the sun. The dry samples were ground in a knife grinder until a powder with a particle size of 1.41 mm was obtained. After grinding, the samples were sieved up to 14 mesh, packed in glass bottles and stored at room temperature.

Acid hydrolysis of biomass: The acid hydrolysis treatments were carried out using different concentrations of sulfuric acid: 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0% (v/v), to determine the best concentration for sugar release, and with different heating times, 5, 10 and 15 min. in an autoclave at 121 °C. Afterwards, 50 ml of diluted sulfuric acid was added for each 10 g of substrate in 250 ml Erlenmeyer flasks in triplicate for each assay. At the end of the treatments, the pH of the hydrolyzed material was neutralized, up to pH 7.0, using a 50% NaOH solution (w/v). At the end of each hydrolysis test, total sugars, reducing sugars and phenolic compounds were analyzed.

Desintoxication: The detoxification process was carried out according to Mussatto and Roberto (2004). Using 2.5% (m/v) of active carbon added to all hydrolysates and the mixture stirred at 200 rpm at 30 °C for 1 h. It was then centrifuged again (3000 g, 20 min.) and filtered. The hydrolyzate was characterized regarding the concentration of total sugars, reducing sugars and phenolic compounds.

Microorganism, maintenance, inoculum preparation and fermentation medium: The microorganisms used for the fermentation were *Saccharomyces cerevisiae* ATCC 26602, *Zymomonas mobilis* CCT 4494, *Pichia stipits* CCT 2617, *Candida tropicalis* ATCC 7349 and *Pachysolen tannophilus* CCT 1891. The microorganisms were kept in tubes with media containing (g.L-1): glucose (10), peptone (5), yeast extract (3), malt extract (3) and agar (20) at pH 5.0. The strains were stored under refrigeration and periodic reactivations were carried out to maintain viability. The inoculum was prepared by adding the microorganism previously grown in 250 ml Erlenmeyer flasks containing 100 ml of the same medium, however, without agar. Which were incubated at 30 °C, in an orbital shaker for 24 h under 100 rpm shaking. The inoculum was standardized by spectrophotometry at 0.6 absorbance with wavelength at 600 nm. Ethanol production was carried out in a basal medium (pH 7) composed of ($g.L^{-1}$): yeast extract (5); KH2PO4 (1); MgSO4.7H2O (1), (NH4)2SO4 (1) and 100 mL of the carbon source (crude hydrolyzate or detoxified hydrolyzate) in 250 mL Erlenmeyer flasks, which were incubated in an orbital shaker with 0, 50 and shaking 100 rpm at constant temperature of 30 ºC, for 24 h. Every 2 h, ethanol production, cell growth, pH change of the medium and sugar consumption were analyzed.

ANALYTICAL METHODS

Total sugars (TA) were determined using the phenol-sulfuric method (DUBOIS *et al*., 1956) and reducing sugars (AR) were measured using the cuproarsenate method (NELSON, 1944; SOMOGYI, 1952). The contents of phenolic compounds were analyzed by Folin-Ciocalteu modified by Chaovanalikit; Wrolstad (2004). The final pH was determined directly in the fermented broth using the pH meter Digmed model DM20. Cell concentration was determined by turbidimetry using a Biochrom spectrophotometer, model Libra S22. Ethanol was determined by gas chromatography in cell-free fermented broth, using a Thermo Scientific Model Focus chromatograph with flame ionization detector (FID) and HP-FFAP column (25 m x 0.2 mm x 0.3 µm); oven temperature at 70 ºC (maintaining this temperature throughout the isothermal run); 5 min run time; injector temperature of 230 °C; detector temperature of 270 °C; injection of 200 µl of sample steam. The samples were left in a water bath at a temperature of 40 °C (until reaching equilibrium).

RESULTS AND DISCUSSION

The residues used for the production of second-generation ethanol present a complex lignocellulosic network formed by individual polymers of cellulose, hemicellulose and lignin, therefore, they necessarily need to be hydrolyzed or submitted to a previous pre-treatment. In the pre-treatment it is essential that there is a mechanical grinding of the biomass to reduce the size of the particles and increase the contact area of the surface that will be exposed to the hydrolysis treatment. In addition, the milling also increases the pore volume and decreases the degree of polymerization and the crystallinity of cellulose (ZABED *et al*., 2017). The materials studied in this work underwent acid hydrolysis to dissociate cellulose and hemicellulose into fermentable sugars, these sugars will be used by microorganisms in the production of cellulosic ethanol. Previous acid hydrolysis was performed with different concentrations of diluted H_2SO_4 (0.0; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4, 5 and 5.0%) and heating time (5, 10 and 15 min.), in order to determine what the most efficient concentration of H_2SO_4 and the necessary time of contact with the biomass to release the highest sugar content from the substrates, thus establishing the procedure to be used in the treatment of lignocellulosic residues. The results of the release of reducing sugars for each of the hydrolyzed residues are shown in Table 1.

Table 1. Maximum levels of total reducing sugars resulting from hydrolyzed substrates at concentrations of 0 to 5% H2SO4 heated in an autoclave at 121 °C/1.1 atm

Substrate	[H_2SO_4] (%)	Time (min.)	[AR] (mg/mL)
Cassava waste	2,0	10	131,09
Grape stalk	1,5	5	27,91
Sugar cane bagasse	2,0	15	81,66
Lemon peels	5,0	15	160,38
Banana peels	5,0	15	43,90
Orange peels	5,0	15	65,81
Soybean hulls	1,5	15	30,40
Passion fruit peels	5,0	15	26,40
Coconut shell	5,0	15	9,40

In the analysis of the hydrolysis performed with different concentrations of H2SO4 (0.0 to 5.0%), the highest levels of reducing sugars obtained for most substrates were with 5.0% of H2SO4 and 15 min. of heating in autoclave, 121 °C/1.1 atm., however, it varies according to the composition of the biomass. The concentrations used in hydrolysis are low and have advantages over higher levels, as it solubilizes hemicellulose by converting it into fermentable sugars, which rules out the need to use hemicellulose enzymes, and it also prevents the formation of a greater number of compounds that can inhibit fermentation, making the diluted concentration more interesting for the production of cellulosic ethanol. In addition to the pre-treatment with dilute acid fractionating the hemicellulose, it is also able to reduce the crystallinity and the degree of polymerization of the cellulose, where its structure is not affected and thus generates less degradation products, resulting in hydrolysates with a high content of glucose, therefore, the use of dilute acids is an effective procedure for lignocellulosic substrates (KARIMI; TAHERZADEH, 2016; BENJAMIN *et al*., 2014). Another advantage of using diluted sulfuric acid concentrations is the reduction of the risk of corrosion of fermenters, as it promotes the release of less toxic compounds. In addition, it has a low cost due to the lower amount of the reagent to be used. While lower temperatures also reduce energy costs, making the process more accessible in terms of cost-effectiveness and its high efficiency in promoting hydrolysis within the lignocellulosic raw material (CHEN *et al*., 2017; TOMÁS- PEJÓ *et al*., 2011). As in this work, other researchers also used a concentration of 5% H2SO4 and high heating to obtain reducing sugars, Loureiro *et al*. (2020), reached 44.68 ± 0.96 $g.L^{-1}$ of AR, by hydrolyzing cassava residues at 120 °C for 2 hours. Concentration lower than this research, where, for cassava residues, 134.84 $g.L^{-1}$ of AR were measured, also using 5%

H_2SO_4 and heating at 121 ºC, still with heating for only 10 min. in autoclave. In a hydrolysis of soybean residues Vedovatto *et al*. (2021) used the temperature of 220 °C for 15 min. and recovered 10.5 g of sugars/100 g of soybean, however, the hydrolysis was carried out with distilled water pumped at 25 MPa. In our study, 30.40 g.L-1 of sugars were obtained in the acid hydrolysis of soybean hulls with 1.5% H2SO4. These authors also reported that the increase in temperature (180 and 220 ºC) favored the production of fermentable sugars in the hydrolysates, because when using high pressures and temperatures it is possible to modify the physicochemical properties of the subcritical water present in the biomass, decreasing the viscosity and increasing diffusivity, thus facilitating penetration into the complex and rigid lignocellulosic chain, resulting in a rapid conversion of cellulose and hemicellulose into sugars with a shorter reaction time. The increase in temperature provides greater efficiency in the hydrolysis process (COCERO *et al*., 2018; VEDOVATTO *et al*., 2021). In lignocellulosic materials most of the cellulose is crystalline, therefore, high temperatures and acid addition are necessary to release glucose from these chains which are tightly aggregated. In addition, the yield increases with increasing temperature. These high yields are important to reduce costs, which have motivated the use of dilute sulfuric acid during hydrolysis. Since it has a lower cost when compared to the use of enzymes that degrade cellulose. On the other hand, the use of severe conditions in thermochemical treatments shows that, in addition to favoring the release of fermentable sugars, they lead to the partial breakdown of these sugars derived from the lignocellulosic matrix, and result in the formation of unwanted by-products, such as acetic acid, formic acid, levunic acid, hydroxymethyl furfural (HMF), furfural and other phenolic compounds. These compounds inhibit both fermenting microorganisms and cellulose-degrading enzymes, therefore, these degradation products need to be removed through detoxification. For this, in this work, the detoxification of hydrolysates with active charcoal was carried out, which allowed the reduction of the content of inhibitor compounds (CAVKA; JONSSON, 2013). In addition to the analysis for deciding on the best parameters for carrying out the hydrolysis, the concentrations of phenolic compounds were also determined. The results obtained before and after the detoxification process are shown in Table 2.

Table 2. Effect of detoxification on the content of phenolic compounds of lignocellulosic substrates treated with different concentrations of H2SO4 and heating time in an autoclave at 121 °C/1.1 atm

Substrate	Initial phenolic compounds (g de ácido vanílico.L^{-1})	Final phenolic compounds (g de ácido vanílico.L^{-1})
Cassava waste	0,35 ± 0,05	0,05 ± 0,02
Grape stalk	2,00 ± 0,00	nd*
Sugar cane bagasse	2,29 ± 0,10	2,10 ± 0,02
Lemon peels	0,87 ± 0,00	0,08 ± 0,00
Banana peels	0,80 ± 0,10	0,20 ± 0,10
Orange peels	0,59 ± 0,01	0,05 ± 0,01
Soy hulls	1,30 ± 0,05	1,15 ± 0,02
Passion fruit peels	1,00 ± 0,00	0,20 ± 0,00
Coconut shell	2,30 ± 0,10	0,20 ± 0,01

In Table 2 it is possible to verify that the content of phenolic compounds decreased after detoxification and between 75 and 90% of these compounds were eliminated from the hydrolysates, with the exception of sugarcane bagasse and soybean hulls that showed a lower removal of phenolic concentration. In an acid hydrolysis of olive tree residues, the researchers Mateo *et al*. (2013) also used active charcoal to reduce phenolic compounds, where they obtained a reduction of 15.9% of the compounds. This value is lower than the one obtained in this research (75 to 90%) when compared to most of the analyzed residues. Therefore, the detoxification process was efficient in removing fermentation-inhibiting compounds from the hydrolysates. Table 2 also shows that green coconut husks had the highest concentration of phenolic compounds, 2.30 g of vanillic acid.L^{-1}, compared to other lignocellulosic residues. As well as sugarcane bagasse, which also released a similar content (2.29 g of vanillic acid.L^{-1}). These higher contents may be due to the composition of these residues, since they are the materials that have the highest lignin content. According to Vaithanomsat *et al*. (2011), coconut husks are made up of 30% lignin, and in their work Candido *et al*. (2017) showed that sugarcane bagasse also has around this lignin content (30%). Table 3 presents the lignin content of these materials.

Fermentation process of hydrolyzed and detoxified waste: The nine hydrolyzed lignocellulosic materials studied in this work were used as a carbon source in the submerged fermentation process for the production of 2G ethanol. Table 4 presents each microorganism used in the fermentation of each of these hydrolysates. The detoxified hydrolysates were used as a carbon source in a salt-rich medium and were fermented by the yeasts *Saccharomyces cerevisiae* ATCC 26602, *Pachysolen tannophilus* CCT 1891, *Pichia stipits* CCT 2617, *Candida tropicalis* ATCC 7349 and by the bacterium *Zymomonas mobilis* CCT 4494. All fermentations were carried out at 30 °C for 24 hours. Sample collection was performed every 2 hours (Table 5). The hydrolyzed materials that showed the highest production of ethanol were soybean hulls (47.70 $g.L^{-1}$ of ethanol), sugarcane bagasse (42.00 $g.L^{-1}$), lemon peels (28.16 $g.L^{-1}$). 1) and cassava residues (21.23 $g.L^{-1}$) (Table 5). The hydrolyzate of cassava residues fermented by Saccharomyces cerevisiae ATCC 26602 showed its maximum ethanol production after 24 hours of the process, of 23.65 $g.L^{-1}$ for the crude hydrolyzate at pH 6.5. However, the highest productivity occurred in 8 h of fermentation under the same conditions and was 2.8 $g.L^{-1}.h^{-1}$ (Table 5). In a fermentation by S. cerevisiae GIM2.213 of cassava residue enzymatically hydrolyzed by 20 and 40 mg protein/g glucan cellulase respectively, Wang *et al*. (2020) obtained 27.29 and 30.17 $g.L^{-1}$ of glucose and a final ethanol titer of 13.74 and 15.09 $g.L^{-1}$. These ethanol contents were lower than those obtained in the present research, where acid hydrolysis, a process with a lower cost than enzymatic, released higher sugar content (134.84 $g.L^{-1}$) and consequently, higher ethanol content. (23.65 $g.L^{-1}$). The acid hydrolyzate of the grape pomace and stalk was fermented by the yeast *C. tropicalis* for 8 h. The highest ethanol production (5.89 $g.L^{-1}$) occurred after 6 hours of fermentation and initial pH 6.0.

The sugarcane bagasse hydrolyzate was used at a concentration of 15% and fermented for 6 h with the consortium *Zymomonas mobilis* CCT 4494 and Pachysolen tannophilus CCT 1891, the initial pH of the medium was 6.5, producing 42 $g.L^{-1}$ of ethanol, 3.5 $g.L^{-1}$ of biomass and a productivity of 5.4 $g.L^{-1}.h^{-1}$. The lemon peel hydrolysates were fermented with *Z. mobilis* and also with the consortium of *Z. mobilis* and *Pichia stipits*, producing 28.16 $g.L^{-1}$ and 23.8 $g.L^{-1}$ of ethanol, respectively, the fermentations were carried out for 24 h without shaking. The banana peels were fermented by *Z. mobilis* and *P. tannophilus*, inoculated sequentialy in a medium containing 15% of the hydrolyzed substrate, for 19 h with agitation at 100 rpm and initial pH 5.0, producing 11.3 $g.L^{-1}$ of ethanol and showing a productivity of 0.6 $g.L^{-1}.h^{-1}$, cell growth was 0.5 $g.L^{-1}$ for *Z. mobilis* and 1.6 $g.L^{-1}$ for *P. tannophilus*. Sequential cultivation of the two microorganisms provided better performance in fermentation. The hydrolysates obtained from orange peels were also fermented by co-culture of *Zymomonas mobilis* and *Pichia stipits*. The bacterium was inoculated first and used most of the glucose contained in the medium. The consortium generated 11.36 $g.L^{-1}$ of ethanol. This behavior may have occurred due to the limited consumption of xylose by the bacteria in the fermentation medium, since, as glucose is the substrate preferably consumed by microorganisms, it was first converted by the bacterium *Z. mobilis*, then yeast *P. tannophilus* adapted its metabolism to use xylose by increasing the final concentration of ethanol. The detoxified hydrolyzate of soybean hulls, in 12 h of fermentation by the bacteria *Z. mobilis*, produced 23.7 $g.L^{-1}$ of ethanol with a productivity of 2.0 $g.L^{-1}.h^{-1}$. When only *C. tropicalis* yeast was used, 24 h of fermentation and initial pH 5.5 without agitation were necessary to produce 30.2 $g.L^{-1}$ of ethanol. When carrying out the fermentation by the consortium of *Z. mobilis* and *C. tropicalis*, both inoculated together at the beginning of the process, the detoxified hydrolyzate of

Table 3. Lignocellulosic composition of the residues used in this work

Substrate	Lignin (%)	Hemicellulose (%)	Celullose (%)	Bibliographic reference
Cassava waste	24,41	42,18	20,48	Polachini et al. (2020)
Grape stalk	17,00	21,00	30,00	Prozil et al. (2013)
Sugar cane bagasse	22,50	20,50	39,50	Assumpção et al. (2016)
Lemon peels	7,22	6,07	18,49	Barrosa et al. (2020)
Banana peels	6,00-12,00	6,40-9,40	7,60-9,60	Mohapatra et al. (2010)
Orange peels	4,30	10,20	25,10	Kim et al. (2015)
Soy hulls	5,70	26,00	31,00	Cassales et al. (2011)
Passion fruit peels	36,18	23,01	25,90	Wijaya et al. (2017)
Coconut shell	40,10	12,26	24,70	Cabral et al. (2017)

Table 4. Hydrolyzed substrates and fermenting microorganisms used in each of the fermentations for the production of second-generation ethanol

Substrate	Micro-organism
Cassava waste	*Saccharomyces cerevisiae* ATCC 26602
Grape stalk	*Candida tropicalis* ATCC 7349
Sugar cane bagasse	*Zymomonasmobilis* CCT 4494 e *Pachysolen tannophilus* CCT 1891
Lemon peels	*Zymomonas mobilis* CCT 4494
Banana peels	*Zymomonas mobilis*CCT 4494 e *Pachysolen tannophilus* CCT 1891
Orange peels	*Zymomonas mobilis* CCT 4494 e *Pichia stipits* CCT 2617
Soy hulls	*Zymomonas mobilis* CCT 4494 e *Candida tropicalis* ATCC 7349
Passion fruit peels	*Pachysolen tannophilus* CCT 1891
Coconut shell	*Pachysolen tannophilus* CCT 1891

Table 5. Maximum production of cellulosic ethanol and cell growth of microorganisms in each hydrolyzed residue used as substrate

Materials	Initial pH	Agitation (rpm)	Time (h)	Ethanol production ($g.L^{-1}$)	Productivity ($g.L^{-1}.h^{-1}$)	Cell growth ($g.L^{-1}$)
Cassava waste	6,5	0	10	21,23	2,12	2,90
Grape stalk	6,0	0	6	5,89	0,98	0,50
Sugar cane bagasse	6,5	0	6	42,00	7,00	3,56
Lemon peels	5,5	0	24	28,16	1,17	1,82
Banana peels	5,0	100	19	11,30	0,59	2,10
Orange peels	6,5	0	24	8,22	2,05	6,04
Soy hulls	6,5	0	8	47,70	5,96	0,75
Passion fruit peels	5,0	100	19	10,00	0,52	2,00
Coconut shell	5,5	100	19	5,20	0,27	0,49

these same peels generated 47.7 $g.L^{-1}$, the productivity was 5.96 $g.L^{-1}.h^{-1}$ during 8 h of incubation. The initial pH of the medium was 6.5. These fermentations were carried out with a substrate concentration of 10%. Soybean hulls have a high content of cellulose in their composition, according to researchers De Pretto *et al*. (2018) and Barragán *et al*. (2019) is 31 to 35%; having even a content equivalent to that of sugarcane 39.5% (MELATI *et al*., 2017; ZULKANIA *et al*., 2018) which makes it a residue with prominence to be applied in the production of 2G ethanol, since, the Soybean is the most cultivated legume in Brazil and in the world (ZULKANIA *et al*., 2018). The passion fruit peels were hydrolyzed and used as a carbon source by the yeast *P. tannophilus* for 19 h at 100 rpm, with an initial pH of 4.5, it produced 10.0 $g.L^{-1}$ of ethanol, 0.52 $g.L^{-1}.h^{-1}$ productivity and 2.0 $g.L^{-1}$ cell growth. Edwards and Doran-Peterson (2012) conducted research for ethanol production using pectin-rich residues and reported that pectin-rich biomass has a low concentration of lignin, ranging from 12 to 35%.

Pectin is a complex carbohydrate that has the largest composition of galacturonic acid (70 %) and other monosaccharides such as rhamnose, xylose, arabinose and galactose. However, when using this type of residue for ethanol production, such as passion fruit, galacturonic acid and arabinose cannot be consumed by the fermenting microorganisms, which resulted in a low concentration of ethanol (EDWARDS; DORAN-PETERSON, 2012; LEIJDEKKERS *et al*., 2013). The same behavior was observed in the fermentation of the hydrolysates of green coconut husks, however, here the interference is the high concentration of lignin present in the lignocellulosic material (Table 2), since the hydrolysis is more difficult to be carried out than in the other materials with a lower lignin content. The fermentation occurred with the yeast *P. tannophilus* using 10% of the hydrolyzate of this substrate during 19 h of incubation and agitation at 100 rpm, at an initial pH of 5.5, resulting in 5.2 $g.L^{-1}$ of ethanol, 0.27 $g.L^{-1}.h^{-1}$ of productivity and 0.49 $g.L^{-1}$of cell growth. In general, the results obtained allowed us to verify that all hydrolyzed lignocellulosic materials were able to serve as a substrate for ethanol-producing microorganisms, becoming alternatives of renewable and sustainable sources against the use of the traditional energy source obtained from petroleum.

CONCLUSION

The nine lignocellulosic materials studied showed good performance in hydrolysis, with the release of sugars used for the production of second-generation ethanol. The acid concentration that generated the highest fermentable sugar content was 5.0% and the contact time with the biomass was 15 min. heating in an autoclave. The material that showed the highest release of sugars after acid hydrolysis were cassava residues, 131.09 $g.L^{-1}$ of reducing sugars. All fermentations were carried out with microorganisms individually and in consortium. The highest production of 2G ethanol was 47.70 $g.L^{-1}$ using soybean hulls. The hydrolyzed substrate that presented the highest productivity was sugarcane bagasse, with 7.00 $g.h^{-1}.L^{-1}$, followed by soybean hulls with 5.96 $g.h^{-1}.L^{-1}$.

Funding Sources: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with Ethical Standards: The authors declare that they have no conflict of interest in this publication. This research does not involving human participants and/or animals. All authors agreed with the content and all gave explicit consent to submit this paper.

REFERENCES

AGBOGBO, F. K.; *et al*. 2006. Fermentation of glucose/xylose mixtures using *Pichia stipits*. *Process Biochemistry*. v. 41, n. 11. Pages 2333-2336.

AGUIAR, A. *et al*. 2021. Sugarcane straw as a potential second generation feedstock for biorefinery and white biotechnology applications. Biomass and Bioenergy, v. 144.

ASSUMPÇÃO, S. M. N.; *et al*. (2016) Combined Pre-Treatment H2SO4/H2O2/NaOH to Obtain Lignocellulosic Fractions from Sugarcane Bagasse. Rev. Virtual Quim., v. 8, n. 3. 803-822.

BARRAGÁN, E. U. P.; GUERRERO, C. F. C.; ZAMUDIO, A. M.; CEPEDA, A. B. M.; HEINZE, T.; KOSCHELLA, A. (2019) Isolation of cellulose nanocrystals fromTyphadomingensis named southern cattail using a batch reactor. *Fibers and Polymers*, v. 20, n. 6, p. 1136-114.

BARROSA, A. H. *et al* (2020) Nickel adsorption from aqueous solution using lemon peel biomass chemically modified with TiO2 nanoparticles. *Sustainable Chemistry and Pharmacy*. v, 17.

BENJAMIN *et al*. (2014) Y.H. Benjamin, H. Cheng, J.F. Görgens. Optimization of dilute sulfuric acid pretreatment to maximize combined sugar yield from sugarcane bagasse for ethanol production. *Appl. Biochem. Biotechnol*., v. 172, pp. 610-630 https://doi.org/10.1007/s12010-013-0545-z

BHARATHIRAJA, B. *et al*. (2017) Biobutanol – An impending biofuel for future: A review on upstream and downstream processing tecniques. *Renewable and Sustainable Energy Reviews,* v. 68, Part 1, p. 788–807.

CABRAL, M. M. S. *et al*. (2017) Composição da fibra da casca de coco verde in natura e após pré-tratamentos químicos. Engevista. v. 19, n.1 , p. 99-108.

CANDIDO, R. G.; GODOY, G. G.; GONÇALVES, A. R. (2017) Characterization and application of cellulose acetate synthesized from sugarcane bagasse. *Carbohydrate Polymers*, v. 167, p. 280–289.

CANDIDO, R. G.; MORI, N. R.; GONÇALVES, A. R. (2019) Sugarcane straw as feedstock for 2G ethanol: Evaluation of pretreatments and enzymatic hydrolysis. *Industrial Crops and Products,* v. 142.

CANILHA, L.; MILAGRES, A. M. F.; SILVA, S. S.; SILVA, J. B. A. E.; FELIPE, M. G. A.; ROCHA G. J. M.; FERRAZ, A. E.; CARVALHO, W. (2010) Sacarificação da biomassa lignocelulósica através de pré-hidrólise ácida seguida por hidrólise enzimática: uma estratégia de "desconstrução" da fibra vegetal. São Paulo, *Rev. Analytica*, 44.

CASSALES, A. R *et al*. 2016 Optimization of soybean hull acid hydrolysis and its characterization as a potential substrate for bioprocessing. *Biomass & Bioenergy, Oxford,* v. 35, n. 11, p. 4675-4683, 2011.

KARIMI, K., TAHERZADEH, M. J., A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. Bioresource Technology. 348–356.

KIM, B. *et al*. (2015) Pyrolysis and catalytic upgrading of Citrus unshiu peel. *Bioresource Technology*, 194. 312-319

CAVKA, A.; JÖNSSON, L. J. (2013) Detoxification of lignocellulosic hydrolysates using sodium borohydride. *Bioresource Technology*. v. 136, 368-376.

CHAOVANALIKIT, A.; WROLSTAD, R. E. (2004) Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Journal Food Chemistry and Toxicology,* v. 69, p. 67-72.

DE PRETTO, C.; GIORDANO, R. D. L. C.; TARDIOLI, P. W.; COSTA, C. B. B. (2018) Possibilities for producing energy, fuels, and chemicals from soybean: a biorefineryconcept. *Waste and Biomass Valorization,* v. 9, n. 10, p. 1703-1730.

EDWARDS, M. C.; DORAN-PETERSON, J. 2012. Pectin-rich biomass as feedstock for fuel ethanol production. *Applied Microbiology and Biotechnology.* v. 95, pages565–575(2012).

GÜNAN YÜCEL, H. G.; AKSU, Z. (2015) Ethanol fermentation characteristics of *Pichia stipits* yeast from sugar beet pulp hydrolysate: Use of new detoxification methods. Fuel, v. 158, n. 15. Pages 793-799.

HANSSEN, S. V., DAIOGLOU, V., STEINMANN, Z. J. N. *et al*. (2020) Biomass residues as twenty-first century bioenergy feedstock—a comparison of eight integrated assessment models. Climatic Change, v. 163, 1569–1586. https://doi.org/10.1007/s10584-019-02539-x

KUMAR, A. K.; SHARMA, S. (2017) Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresour. Bioprocess, v.4, n.7.

LEIJDEKKERS, A.G.M.; BINK, J.P.M.; GEUTJES, S.; SCHOLS, H.A.; GRUPPEN, H. (2013) Enzymatic saccharification of sugar beet pulp for the production of galacturonic acid and arabinose: A study on the impact of the formation of recalcitrant oligosaccharides. Bioresource Technology, v. 128, p. 518–525.

LOUREIRO *et al*. (2020) Avaliação do potencial dos resíduos (casca, entrecasca e pontas) do beneficiamento da mandioca (Manihot esculenta Crantz) para a produção de bioetanol utilizando hidrólise ácida. Braz. Ap. Sci. Rev., v. 4, n. 2, p. 606-620.

MATEO, S. *et al*. (2013) Detoxification of hemicellulosic hydrolyzate from olive tree pruning residue. Industrial Crops and Products. v. 49. Pages 196-203.

MELATI, R. B.; SHMATZ, A.; PAGNOCCA, F. C.; CONTIERO, J.; BRIENZO, M. (2017) Sugarcane bagasse: production, composition, properties, and feedstock potential. In: Rachel Murphy. (Org.). Sugarcane: Production systems, uses and economic importance. 1ed. Nova York: Nova Science Publishers, v. 1, p. 1-38.

MENDES, F. M. T.; MARQUES, A. C. C.; MENDONÇA, D. L.; OLIVEIRA, M. S.; MOUTTA, R. O.; FERREIRA-LEITÃO, V. S. (2015) High Surface Area Activated Carbon from Sugar Cane Straw. Waste and Biomass Valorization, v. 6, p. 433-440.

MJ COCERO , Á. CABEZA , N. ABAD , T. ADAMOVIC , L. VAQUERIZO , CM MARTÍNEZ , MV PAZO-CEPEDA. (2018) Compreendendo o fracionamento de biomassa em água subcrítica e supercrítica. J. Supercrit. Fluidos, v. 133. 550 – 565.

MOHAPATRA, D.; MISHRA, S.; SUTAR, N. Banana and its by-product utilisation: an overview. Journal of Scientific and Industrial Research, New Delhi, v. 69, n. 5, p. 323–329, 2010.

MUSSATTO, S. I.; ROBERTO, I. C. (2004) Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: a review. Bioresource Technology, v. 93, n. 1, p. 1–10.

NELSON, N. (1944) A photometric adaptation of Somogy method for determination of glucose. Biochemistry, v. 153, n. 2, p. 375–380.

PEDROSO, G. B. *et al*. (2019) Strategies for fermentable sugar production by using pressurized acid hydrolysis for rice husks. Rice Sci., v. 26, n. 5. 319-330.

POLACHINI, T. C. *et al*. (2020). Composição química de biomassa lignocelulósica de bagaço de mandioca e de casca de mandioca: influência da distribuição do tamanho de partícula. Avanços das pesquisas e inovações na engenharia química 1 [recurso eletrônico] / Organizadora Jéssica Verger Nardeli. – Ponta Grossa, PR: Atena.

PONNUSAMY, V. K.; NGUYEN, D. D.; DHARMARAJA, J.; SHOBANA, S.; BANU, J. R.; SARATALE, R. G.; KUMAR, G. (2019) A review on lignin structure, pretreatments, fermentation reactions and biorefinery potential. *Bioresource Technology*, v. 271, p. 462-472.

PROZIL, S. *et al*. (2013) Caracterização do Engaço da Uva e Avaliação do seu Potencial como Matéria-Prima Lenhocelulósica. Millenium, 44 (janeiro/junho). Pp. 23-40.

SILVA, M. D. *et al*. (2020) Acid Hydrolysis of corn COB for the production of second generation ethanol by *Saccharomyces cerevisiae* ATCC 26602. *International Journal of Development Research*, v. 10, n. 8, p. 38871-38878.

SOMOGYI, M. (1952) Notes on sugar determination. Journal of Biologycal Chemistry, v. 195, n. 1, p. 19–23.

TOMÁS-PEJÓ, E. *et al*. (2011) Pretreatment Technologies for Lignocellulose-to-Bioethanol Conversion. In: Biofuels: Alternative Feedstocks and Conversion Processes. Madrid: Elsevier, p. 149-176.

VAITHANOMSAT, P. *et al*. (2011) The Potential of Coconut Husk Utilization for Bioethanol Production. *Kasetsart Journal, Natural Sciences*. v. 45, n.1, pp.159-164.

VARANDA, L. D. *et al*. (2018). Physical performance of particleboards using Castor oil-based adhesive. *Revista Brasileira de Engenharia Agrícola e Ambiental.* v.22, n.10, p.707-712, 2018.

VEDOVATTO, F. *et al*. (2021). Subcritical water hydrolysis of soybean residues for obtaining fermentable sugars. *The Journal of Supercritical Fluids.* v., 167, 2021.

WANG, *et al*. (2020). Processo de etanol de mandioca adicionado por celulase aumenta o titulador do etanol e reduz a produção de glicerol. *Industrial Crops and Products*. V 148, 2020.

WANG, X., TSANG, Y.F., LI, Y., MA, X., CUI, S., ZHANG, T.-A.A., HU, J., GAO, M.-T.T. (2017) Inhibitory effects of phenolic compounds of rice straw formed by saccharification during ethanol fermentation by *Pichia stipits*. *Bioresource Technology*, v. 244, n 1, 1059-1067.

WIJAYA, C. J. *et al*. (2017) Cellulose nanocrystals from passion fruit peels waste as antibiotic drug carrier, Carbohydrate Polymers, v. 175. 370-376.

ZABED, H. *et al*. Bioethanol production from renewable sources: Current perspectives and technological progress. *Renewable and Sustainable Energy Reviews,* v. 71, 475-501. 2017.

ZULKANIA A, SETYANINGSIH L, CHAFIDZ A, ALEL AE (2018) Influence of temperature pyrolysis and biomass size particles on characterization of bio oil derived fromPinus Merkusii flower. IOP Conference Series: Earth and Environmental Science 212(1):12080.

****