Acid Hydrolysis of Lignocellulosic Materials for The Production of Second Generation Ethanol

Mariane Daniella Silva (marianedaniella@hotmail.com)
Universidade Estadual Paulista Júlio de Mesquita Filho https://orcid.org/0000-0002-2900-9741

João Pedro Cano
UNESP IBILCESJRP: Universidade Estadual Paulista Julio de Mesquita Filho Instituto de Biociencias Letras e Ciencias Exatas Campus de Sao Jose do Rio Preto

Fernanda Maria Pagane Guereschi Ernandes
UNIP: Universidade Paulista

Crispin Humberto Garcia-Cruz
UNESP IBILCESJRP: Universidade Estadual Paulista Julio de Mesquita Filho Instituto de Biociencias Letras e Ciencias Exatas Campus de Sao Jose do Rio Preto

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Abstract

Brazil is one of the countries with the largest agricultural production in the world. Therefore, it is capable of generating large amounts of agro-industrial waste that can be used as biomass for the production of biofuels. Second generation ethanol is a renewable energy alternative, capable of replacing fossil fuels. Within this context, the objective of the present work was to study the effect of diluted acid hydrolysis in different types of lignocellulosic residues and the consequent production of 2G ethanol from these hydrolysates using different fermenting microorganisms. The acid concentration that released the highest content of fermentable sugars from the acid hydrolysis of lignocellulosic materials was 5.0% of sulfuric acid and the contact time with the biomass was 15 min. while heating in autoclave. The material that showed the highest sugar release after acid hydrolysis was cassava residues, with 131.09 $g.L^{-1}$ of reducing sugars. The fermentations were carried out with microorganisms alone and also in consortium. The largest production of 2G ethanol was from the hydrolyzate of soybean hulls, of 47.70 $g.L^{-1}$ of ethanol by the consortium of *Zymomonasmobilis* and *Candida tropicalis*, during 8 h of fermentation and showed productivity of 5.96 $g.L^{-1}.h^{-1}$.

1 Introduction

Brazil is one of the largest generators of agricultural and forestry residues in the world, being one of the largest fruit producers, consequently, large amounts of lignocellulosic biomass are obtained, making it viable to use these renewable and sustainable materials as an energy source [1].

The inadequate disposal of these residues causes serious environmental problems, such as soil and water contamination and also air pollution [2]. In view of this, an alternative to minimize such damage to the environment is the use of biomass in the generation of fuels [3].

Lignocellulosic biomass is 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 (2G) ethanol without needing extra land or generating interference in the production of food and feed [4].

The 2G ethanol is a promising option for an environmentally cleaner fuel. Biofuels are a renewable form of energy for the transport sector [5].

The production of 2G ethanol from biomass requires the conversion of cellulose and hemicellulose into monomeric sugars, to be converted into ethanol by microorganisms. For the best use of the lignocellulosic material, a treatment step called hydrolysis is necessary, as these residues have a rigid and complex structure, which hinders their degradation [6].

Hydrolysis methods can be chemical, physical, physical-chemical or biological (use of enzymes), but also, a combination of 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 [7].

In view of all these factors, the main objective of this work was to enable the acid hydrolysis of cassava and passion fruit residues, sugarcane bagasse, grapes, lemon peels, banana, orange, soy, passion fruit and green coconut and then the fermentation of hydrolysates for the production of 2G ethanol.

2 Material And Methods

2.1 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.

2.2 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 maximum 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.

2.3 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. 50 ml of diluted sulfuric acid was added for each 10 g of substrate.

At the end of the treatments, the pH of the hydrolyzed material was neutralized, up to pH 7.0, using a 50% NaOH solution (m/v). All hydrolysis tests were performed in triplicate and 250 mL Erlenmeyer flasks were used.

At the end of each hydrolysis test, total sugars, reducing sugars and phenolic compounds were analyzed.

2.4 Detoxification

The detoxification process was carried out in all hydrolysates according to [8], by the addition of 2.5% (m/v) of active carbon, the mixture was subjected to stirring at 200 rpm, at 30º C, for 1 h. The mixture

was then centrifuged again (3000 g, 20 min) and filtered. The treated hydrolyzate was characterized as to the concentration of total sugars, reducing sugars and phenolic compounds.

2.5 Microorganism, maintenance, inoculum preparation and fermentation medium

The microorganisms used for the fermentation were *Saccharomyces cerevisiae* ATCC 26602, *Zymomonas mobilis* CCT 4494, *Pichia stipitis* 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); KH_2PO_4 (1); $MgSO_4.7H_2O$ (1), $(NH_4)\ 2SO_4$ (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.

2.6 Analytical methods

Total sugars (TA) were determined using the phenol-sulfuric method [9] and reducing sugars (AR) were measured using the cuproarsenate method [10, 11].

The contents of phenolic compounds were analyzed by Folin-Ciocalteu modified by [12].

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).

3 Results And Discussion

The residues used for the production of second generation ethanol have in their structure a complex lignocellulosic network formed by individual polymers of cellulose, hemicellulose and lignin, therefore, they necessarily need to have their hydrolyzed lignocellulosic network, through a 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. The grinding also increases the volume of the pores and decreases the degree of polymerization and the crystallinity of the cellulose [13].

The materials studied in this work underwent acid hydrolysis so that cellulose and hemicellulose dissociate into fermentable sugars. Previous acid hydrolysis were carried out in order to determine what the most efficient concentration of H_2SO_4 is and how long it takes to contact biomass to release the highest sugar content from the substrates. So that the procedure to be used in the treatment of lignocellulosic waste was already established.

Among the 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.) Used in the treatment, the best results of the release of reducing sugars for each hydrolyzed residue are shown in Table 1.

Table 1
Maximum levels of reducing sugars resulting from hydrolyzed substrates, with H_2SO_4 (0 to 5%) and 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
Soy hulls	1,5	15	30,40
Passion fruit peels	5,0	15	26,40
Coconut shell	5,0	15	9,40

Table 1 shows the diluted acid content and the heating time required for the release of reducing sugars, which are fermentable sugars from hydrolysates that can be used by microorganisms in the production

of cellulosic ethanol. Among the hydrolysis analyzes carried out with 0.0 to 5.0% H_2SO_4, the highest levels of reducing sugars obtained for most substrates, resulted from hydrolysis with 5.0% H_2SO_4, for 15 min. heating in an autoclave, 121 ° C / 1.1 atm, however, varies according to the composition of the biomass. The materials that present in their composition lower levels of lignin use lower concentrations of H_2SO_4, because with less lignin they are easier to be hydrolyzed, such as cassava residues, grape stalks and soy husks.

The concentrations used (from 1.5 to 5% of H_2SO_4) are diluted concentrations 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 the use of diluted acid to solubilize the hemicellulose, it is able to reduce the crystallinity and the degree of polymerization of the cellulose, where the cellulose structure is not affected and generates less degradation products, resulting in hydrolysates with high xylose content, therefore, the use of diluted acids is an effective procedure for lignocellulosic substrates [14, 15].

Another advantage of using diluted sulfuric acid concentrations is the reduction of the risk of corrosion of fermenters, releases less toxic compounds, in addition to reducing costs due to the lower amount of reagent to be used, while lower temperatures also decrease costs with energy, making the process more accessible in relation to cost-benefit [16, 17].

As observed in this work, other researchers also used a concentration of 5% H_2SO_4 and high heating to obtain reducing sugars, [18], 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, however, with heating of only 10 min. in autoclave. The autors [19] hydrolyzed rice husks, with 1.5% H_2SO_4 at 128 ºC, obtaining 18.20 $g.L^{-1}$ of sugars, also a lower level than those found in this research.

In a hydrolysis of soy residues [20] used the temperature of 220 ºC for 15 min. and recovered 10.5 g of sugars/100 g of soy, 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 husks with 1.5% H_2SO_4. These authors also report that the increase in temperature (180 and 220 ºC) favored the production of fermentable sugars in hydrolysates, since, by using high pressures and temperatures, it is possible to modify the physical-chemical 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 [20, 21].

Most cellulose is crystalline, so high temperatures and high concentrations of acid are necessary to release glucose from these chains that are strongly aggregated. In addition, the yield increases with increasing temperature. These high yields are important to obtain low costs and have motivated the use of diluted sulfuric acid in acid hydrolysis. Because, the use of sulfuric acid has a lower cost when compared to the use of enzymes that degrade cellulose.

In addition to promoting the release of fermentable sugars, the use of severe conditions and diluted sulfuric acid in thermochemical treatments, lead to the partial breakdown of sugars derived from the lignocellulosic matrix, and result in the formation of unwanted by-products, such as acetic acid, formic acid, acid levúnico, hydroxymethyl furfural (HMF), furfural and 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 carbon was carried out, which allowed the reduction of the content of inhibitory compounds and other factors that may interfere in the development of microorganisms [22].

From the treatments to decide the best parameters for the hydrolysis, the concentrations of phenolic compounds obtained during the hydrolysis were also determined. All analyzes were performed in triplicate. The results of the analysis of phenolic compounds released before and after the detoxification process are shown in Table 2.

Table 2
Effects of detoxification on the levels of phenolic compounds in lignocellulosic substrates

Substrate	**Initial phenolic compounds (g de ácido vanílico.L^{-})**	**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
* no detoxification analysis was performed for this lignocellulosic residue		

In Table 2 it is possible to see that the content of phenolic compounds decreased after detoxification and this revealed that between 75 and 90 % of the phenolic compounds were eliminated from the hydrolysates, with the exception of sugarcane bagasse and soy husks that showed less removal of the concentration of the inhibitory compounds.

The data in Table 2 show that the green coconut shells had the highest concentration of phenolic compounds, 2.30 g vanillic acid.L^{-1}, compared to the other shells. Sugarcane bagasse also released this content (2.29 g of vanylicacid.L^{-1}). These higher levels may be due to the composition of these residues, they are the materials that have the highest lignin content. According to [23], coconut shells are made up of 30 % lignin, and in their work [25] showed that sugarcane bagasse also presents around this lignin content (30 %).

The researchers [24] also used active charcoal to reduce phenolic compounds in the acid hydrolyzate of olive residues. They obtained a reduction of 15.90 % of the phenolic compounds. This value is less than that obtained in this research (75 to 90 %) when compared to most of the analyzed residues. [26] quantified the removal of phenolic compounds in a content similar to our work, it was 71.1 % after the detoxification process of beet bagasse hydrolyzate, using commercial active charcoal. With this, it is possible to observe that the detoxification process was efficient in removing the fermentation inhibiting compounds in the hydrolysates.

The hydrolysates of the nine lignocellulosic materials studied in this work were used as a carbon source in the submerged fermentation process for the production of second generation ethanol. Table 3 shows each microorganism used in the fermentation in each of these hydrolysates.

Table 3
Hydrolyzed substrates and fermenting microorganisms used for the production of second generation ethanol

Substrate	**MO**
Cassava waste	*Saccharomyces cerevisiae* ATCC 26602
Grape stalk	*Candidatropicalis*ATCC 7349
Sugar cane bagasse	*Zymomonasmobilis*CCT 4494 e *Pachysolentannophilus* CCT 1891
Lemon peels	*Zymomonasmobilis* CCT 4494
Banana peels	*Zymomonasmobilis*CCT 4494 e *Pachysolentannophilus* CCT 1891
Orange peels	*Zymomonasmobilis* CCT 4494 e *Pichiastipitis*CCT 2617
Soy hulls	*Zymomonasmobilis*CCT 4494 e *Candidatropicalis*ATCC 7349
Passion fruit peels	*Pachysolentannophilus* CCT 1891
Coconut shell	*Pachysolentannophilus* CCT 1891

The hydrolysates were used as a carbon source in a medium rich in salts and were fermented by the yeasts *Saccharomyces cerevisiae* ATCC 26602, *Pachysolen tannophilus* CCT 1891, *Pichia stipitis* CCT 2617, *Candida tropicalis* ATCC 7349 and by the bacterium *Zymomonas mobilis* CCT 4494. In order to produce 2G ethanol, the maximum obtained for each hydrolyzate is shown in Table 4.

Table 4
Maximum cellulosic ethanol production and cell growth of microorganisms in each hydrolyzed residue used as a substrate

Substrate	Time (h)	Ethanol production ($g.L^{-1}$)	Productivity ($g.L^{-1}.h^{-1}$)	Cell growth ($g.L^{-1}$)
Cassava waste	10	21,23	2,13	2,90
Grape stalk	2	5,89	2,94	0,50
Sugar cane bagasse	6	42,00	7,00	3,56
Lemon peels	24	28,16	1,17	1,82
Banana peels	19	11,30	0,59	2,10
Orange peels	24	8,22	0,34	6,04
Soy hulls	8	47,70	5,96	0,75
Passion fruit peels	19	10,00	0,52	2,00
Coconut shell	19	5,20	0,27	0,49

For the hydrolyzate of cassava residues, fermented by *Saccharomyces cerevisiae* ATCC 26602, the largest ethanol production occurred after 24 hours of the process without stirring, which had an initial concentration of reducing sugar of 50 $g.L^{-1}$, the maximum value of ethanol production was 23.65 $g.L^{-1}$ for the crude hydrolyzate at pH 6.5 at 30 ºC. However, the highest productivity occurred in 8 h of fermentation under the same conditions and was 2.8 $g.L^{-1}.h^{-1}$.

When using cassava residue applied for separate hydrolysis and fermentation (SHF) by *S. cerevisiae* GIM2.213, [27] obtained 27.29 and 30.17 $g.L^{-1}$ of glucose and a final ethanol titer of 13.74 and 15.09 $g.L^{-1}$ from the enzymatically hydrolyzed cassava residue per 20 and 40 mg of protein/g glucan cellulase respectively. These ethanol contents were lower than those obtained in the present study, where acid hydrolysis, a process with a lower cost than the enzyme, released a higher sugar content (134.84 $g.L^{-1}$) and, consequently, a higher ethanol content (23.65 $g.L^{-1}$).

The acid hydrolyzate of the grape stalk was fermented by the yeast *C. tropicalis* for 8 h without stirring. The highest ethanol production, of 5.89 $g.L^{-1}$, occurred in 6 hours of fermentation, 30 ºC, initial pH 6.0.

The concentration use of 15% of the hydrolyzate obtained with the sugarcane bagasse substrate submitted to a submerged fermentation with the initial consortium *Zymomonas mobilis* and *P. tannophilus* during 6 h of fermentation without agitation, the hydrolyzed medium detoxified with initial pH 6, 5 and 30 ºC, generated 42.0 $g.L^{-1}$ of ethanol, 3.5 $g.L^{-1}$ of biomass and a productivity of 5.4 $g.L^{-1}.h^{-1}$.

In a research for ethanol production from sugarcane bagasse by Z. mobilis using SSF process, [28] obtained the highest values of SSF initial glucose concentration of 76 g $.L^{-1}$, final ethanol concentration of 60 $g.L^{-1}$ and volumetric productivity of 1.5 $g.L^{-1}$.h, obtained with the conditions 1% of H2SO4 for acid hydrolysis at 121 ºC for 30 min., Followed by basic hydrolysis with 4% NaOH (121 ºC / 30 min.) and further enzymatic hydrolysis for 12 h at 50 ºC. In our research, the acid hydrolysis of sugarcane bagasse released 81.66 g / L of sugars with a faster process of just 10 min., the fermentation carried out in SHF resulted in 42 $g.L^{-1}$, promising results when compared to the authors [28], with greater economic benefits, due to the lower use of chemical reagents, energy and lower processing time.

The lemon peel hydrolysates were fermented with only *Z. mobilis* and also with the consortium of *Z. mobilis* and *P. striptis*, producing 28.16 gL-1 and 23.8 gL-1 of ethanol, respectively, fermentations were carried out during 24 h without shaking.

The banana peels were fermented by *Z. mobilis* and *P. tannophilus*, separated and in co-culture. The fermentation was carried out with 15 % of initial substrate at 30 ºC for 19 h with agitation of 100 rpm and initial pH 5.0. The sequential cultivation of the two microorganisms, first the inoculum of the bacterium and then the yeast, provided better performance than the co-cultivation in which the two microorganisms started fermentation together in the culture medium, being 11.30 $g.L^{-1}$ and 4.90 $g.L^{-1}$, respectively.

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 [40].

The hydrolysates obtained from the orange peels were fermented by co-culture of *Zymomonas mobilis* and *Pichia stipitis* inoculated together at the beginning of the fermentation without agitation. The consortium generated 11.36 $g.L^{-1}$ of ethanol.

In 12 h of fermentation of the detoxified hydrolyzate of soy husks by the bacteria *Z. mobilis*, 23.70 $g.L^{-1}$ was quantified with a productivity of 2.0 $g.L^{-1}.h^{-1}$. When carrying out a fermentation by the consortium of *Z. mobilis* and *C. tropicalis* the two inoculated together at the beginning of the process, the detoxified hydrolyzate, produced 47.70 $g.L^{-1}$, in a medium with an initial pH of 6.5 during 8 h of fermentation productivity was 5.96 $g.L^{-1}.h^{-1}$. Only the yeast C. tropicalis in the detoxified hydrolyzate, for 24 h, 25 ºC, initial pH 5.5 without stirring was recorded 30.20 $g.L^{-1}$. These fermentations were carried out with a concentration of 10% hydrolyzed substrate.

The passion fruit peels were hydrolyzed and then used by the microorganisms *Z. mobilis* and *P. tannophilus* alone and in a consortium inoculated together at the beginning of fermentation. The two fermentations were carried out for 19 h at 30 ºC and 100 rpm, with 10 and 15 % of the hydrolyzate. Only the P. tannophilus yeast, with an initial pH of 4.5, generated 10.0 $g.L^{-1}$ of ethanol, 0.52 $g.L^{-1}.h^{-1}$ productivity, 2.0 $g.L^{-1}$ cell growth. The consortium with an initial pH of 5.5, resulted in 7.50 $g.L^{-1}$ of ethanol, 0.39 $g.L^{-1}.h^{-1}$ productivity and 1.70 $g.L^{-1}$ cell growth.

In a review presenting the main residues rich in pectin used for ethanol production, Edwards; Doran-Peterson (2012) report that biomass rich in pectin 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 may also contain rhamnose, xylose, arabinose and galactose. However, when using this type of residue, such as passion fruit peels, for the production of cellulosic ethanol, galacturonic acid and arabinose cannot be consumed by fermenting microorganisms [29, 30]. As a result, in our work, the use of *Z. mobilis* showed a low ethanol production when compared to other lignocellulosic materials (Table 4). Since, in addition to not fermenting pentoses, *Z. mobilis* also does not ferment galacturonic acid, released in the hydrolysis of passion fruit peels.

The same can be observed in the fermentation of the hydrolysates of green coconut shells, however, the interference is the high concentration of lignin present in the lignocellulosic material (Table 5), because hydrolysis is more difficult to be performed than in other materials with lower lignin content.

The fermentation of the coconut shells occurred with the yeast *P. tannophilus* using 10 % of the hydrolyzate of this substrate during 19 h of incubation at 30 ºC and agitation of 100 rpm, at an initial pH of 5.5 resulting in 5.2 $g.L^{-1}$ of ethanol, 0.27 $g.L^{-1}.h^{-1}$ productivity and cell growth of 0.49 $g.L^{-1}$.

Table 5
Lignocellulosic composition of the residues used in this work

Substrate	Lignin (%)	Hemicellulose (%)	Celullose (%)	Bibliographic reference
Cassava waste	24,41	42,18	20,48	[32]
Grape stalk	17,00	21,00	30,00	[31]
Sugar cane bagasse	22,50	20,50	39,50	[39]
Lemon peels	7,22	6,07	18,49	[38]
Banana peels	6,00–12,00	6,40 – 9,40	7,60 – 9,60	[33]
Orange peels	4,30	10,20	25,10	[35]
Soy hulls	5,70	26,00	31,00	[36]
Passion fruit peels	36,18	23,01	28,58	[34]
Coconut shell	40,10	12,26	24,70	[37]

From this it was possible to verify that the studied lignocellulosic materials produced second generation ethanol being renewable and sustainable alternatives in face of the use of fossil energy sources.

4 Conclusion

The nine lignocellulosic materials studied showed good performance in hydrolysis with the release of sugars and were able to produce second generation ethanol.

The acid concentration that generated the highest fermentable sugar content was 5.0 % and the contact time with biomass was 15 min. heating in autoclave. The material that showed the greatest release of sugars after acid hydrolysis were cassava residues, 131.09 $g.L^{-1}$ of reducing sugars. All fermentations were carried out with microorganisms in consortium. The largest production of 2G ethanol was from soy husks, 47.70 $g.L^{-1}$ of ethanol.

5 Declarations

Ethical Approval

Not applicable.

Consent to Participate

All autors are consent to participate.

Consent to Publish

All autors are consent to the publish.

Authors Contributions

M. D. Da Silva: Author responsible for preparing the research project and carrying out the experiments.

J. P. Cano: Responsible for data analysis and translation of the manuscript text.

F. M. P. G. Ernandes: Author responsible for revising and editing the manuscript.

C. H. Garcia-Cruz: Responsible for the production of the project and revision of the manuscript.

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Competing Interests

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Availability of data and materials

Not applicable.

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