Optimizing the process of extraction and acid hydrolysis for Amazon and Cerrado biomass

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Abstract — *We investigated the effect of the reaction variable (time) on the extraction and hydrolysis process. The data showed that the methodologies of the National Renewable Energy Laboratory (NREL) must be adequate for each type of biomass and each regional reality. For the contents of total sugars in the biomass of green coconut, babassu and Brazil nut shell epicarp, the highest yields were found under different conditions from those proposed by NREL. In the total lignin content, the highest yields obtained were those proposed by the NREL method for all biomasses. In the content of extracts for coconut and babassu, the use of longer extraction time provided greater removal. For the chestnut fractions, shorter times led to higher yields. It is important to note that, with the optimized results, these biomasses can be used in the production of high value-added bioproducts with higher yields.*

Keywords — *NREL, babassu, Brazil nut, green coconut.*

I. INTRODUCTION

Lignocellulosic biomass is one of the most abundant renewable resources in the world and it is an economical and environmentally friendly alternative for the production of bio-derived chemical products [1,2]. Due to its high composition of carbohydrates, this type of biomass has great potential for the production of biofuels and is considered one of the potential substitutes for fossil fuels in the chemical industry [3,4].

Several pretreatment methods have been studied such as physical, chemical, physical-chemical, biological, and combined pre-treatments to obtain a greater recovery of fermentable carbohydrates from sugar degradation [5,6]. The procedures for implementing these methods are mostly based on methodologies established by the National Renewable Energy Laboratory.

According to the National Renewable Energy Laboratory [7], the acid hydrolysis occurs in two-step to fractionate biomass components. The samples were kept at 30 °C for 1 h in the first step and autoclaved for 1 h at 120 ºC in the second step. For the extractives analysis, [8] the

reflux for 8-24 hours in 95% ethanol using the Soxhlet apparatus is recommended.

The extraction procedure is important by removes possible interferents, that can may affect the hydrolysis performances [8]. The acid hydrolysis is used to convert the polymers into polymeric carbohydrates and lignin (acid-insoluble lignin and acid-soluble lignin), that can be used for biorefining industries. Thus, both concentrated and diluted acid hydrolysis has been used to obtain sugars free from highly complex pre-treated lignocellulosic biomass [9].

However, these methodologies are used for standard biomasses from Europe (miscanthus), the United States (corn straw) or sugarcane bagasse from Brazil [10,11,12].

New biomasses from the Amazon region and the Cerrado have shown potential in the context of biorefineries, both for their chemical composition and for the amount of waste generated and discarded in the environment [13,14]. Many of these biomasses, such as coconut husk, Brazil nut husk, and outer shell, babassu endocarp and mesocarp have high levels of carbohydrates

and lignin, which can be converted into high value-added products [15,16,17,18]

Therefore, this study aims *to optimize the best conditions to maximize yield in extraction and hydrolysis processes concerning Brazilian biomasses from Cerrado and Amazon regions,* mainly of their major constituents (cellulose, hemicellulose, and lignin), which have been increasingly employed in biorefining processes.

II. MATERIALS AND METHODS

2.1 Samples

Three typical biomasses from the Cerrado and Amazon regions of Brazil were properly collected between 2018 and 2019. The evaluated feedstocks were the following: *Cocos nucifera* (green coconut, whose mesocarp was used), *Orbygnia phalerata* (babassu, whose mesocarp was used) and *Berthollitia excelsa* H.B.K. (Brazilian nut, whose epicarp and endocarp was used). These samples were kept frozen in plastic bags awaiting the next steps of the process. After dried at room temperature, they were ground in a cutting mill (MA 580, Marconi) and sieved in an automatic sieve shaker (VP-01, Bertel), until particles of 355 µm in diameter (45-mesh sieve).

2.2 Soxhlet extraction

A Soxhlet extractor was used, and extraction cartridges received 3g of each biomass. After that, they were covered with cotton wool and then taken to the extractor, with 190 mL of ethanol (90%), for 8, 12, and 24 hours in reflux [8].

After the end of the reflux, the cartridges were taken and placed on Petri dishes on the counter for 48 hours to be dried. After 48 hours, the moisture content of the extracted sample was determined again, so that the extractives content was calculated according to the weight loss after the extraction, deducting the moisture.

2.3 Acid hydrolysis

The acid hydrolysis step was performed according to the methodology of the National Renewable Energy Laboratory [7] for the determination of structural carbohydrates and lignin in biomass. Pressure tubes were used, in which 300 mg of the extracted biomass were placed, and 3.0 mL (4.91 g) of H_2SO_4 at 72% (m/m); then, the tubes were taken to a water bath (TE 056, Technal) for 120 min at 30 ºC and shaken every 10 min. This is the primary hydrolysis step. Subsequently, 84 mL of deionized water was added so that the concentration of sulfuric acid was reduced to 4% (m/m); the tubes were then taken to an autoclave (AV 18, Phoenix) for 1 hour at 120 ºC (secondary hydrolysis step).

In our conditions, we tested two different methodologies: primary hydrolysis with 60 min and secondary hydrolysis with 1h, and also primary hydrolysis with 180 min and secondary hydrolysis with 1.5h.

After this step, the hydrolyzed solution was filtered in crucibles of medium porosity (10 to 15 µm) using a vacuum pump (NOF-650). The solids retained in the filter crucibles were taken to an oven at 105 ºC so that the content of acid-insoluble residue (AIR) was determined, and then kept in a muffle furnace for 4 hours at 575 ºC to obtain the acid-insoluble ash (AIA). From the difference between AIR and AIA, the content of insoluble lignin – also known as Klason Lignin (KL) – was determined. The filtrate (hydrolysate) contains the acid-soluble lignin (ASL) and any other soluble acid components of the biomass, such as the hydrolyzed sugars.
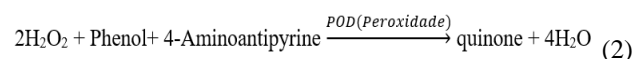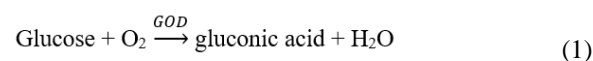
2.4 UV spectroscopy

The ASL is determined using a UV-Vis spectrophotometer (DR 5000, HACH). The hydrolysate was placed in a quartz cuvette and diluted when necessary, and the UV spectra were collected using a wavelength of 205 nm at molar absorptivity of 113 $M^{-1}cm^{-1}$ [19]. The blank was a solution of H_2SO_4 at 4% (m/m).

2.5 Carbohydrate content

The absorbance of reducing sugars at 540 nm was measured in a UV–Vis spectrophotometer (Varian, Cary 4000) to quantify the sugar content. Total reducing sugar (ART) concentrations were calculated based on the standard curve of D-glucose. The hydrolysate was boiled in the 3,5-dinitrosalicylic acid (DNS) solution for 5 min and cooled in an ice-water bath.

A test kit (LAB TEST) was used to measure the enzymatic activity by the amount of glucose formed glucose oxidase enzyme (GOD/POD) is a flavoprotein used in these kits, was used for glucose determination. The reaction is:

$$Glucose + O_2 \xrightarrow{GOD} gluconic\ acid + H_2O \qquad (1)$$

$$2H_2O_2 + Phenol + 4\text{-}Aminoantipyrine \xrightarrow{POD(Peroxidade)} quinone + 4H_2O \qquad (2)$$

The analytical procedure was to add to 1.0 mL of the hydrolyzed sample and 1.0 ml of the enzyme glucose reagent solution. The aliquots formed were taken to a water bath (37 °C) for 15 min [20]. Afterward, the absorbance of the samples at 500 nm was measured in a spectrophotometer (Varian, Cary 4000).

2.6 Cellulose, hemicellulose and lignin analysis

The acid detergent fiber (ADF) and cold neutral detergent (FDN) according to Trujillo, Marichal, and

Carriquiry [21], were used to determine hemicellulose content, while the lignin was determined by NREL methodologies and cellulose by difference.

2.7 X-ray diffraction

Through x-ray diffraction, the crystallinity reference values were determined. The diffractograms were recorded on the XRD-7000 Shimadzu diffractometer, with Cu Kα radiation, a voltage of 30 kV, and a current of 10 mA. The scan was carried out in the angle range of 5º <2Ɵ> 99.98º.

III. RESULTS AND DISCUSSIONS

Table 1 shows the values of the approximate analysis. The biomass of babassu and coconut showed percentages considered low in moisture (<10%). The fractions of Brazil nuts had higher percentages and may interfere in future raw material handling and transport processes [22], also, to affect combustion processes [23].

The ash percentages were all low, which is essential in processes involving biorefineries. Both volatile matter and fixed carbon are in line with what is expected for lignocellulosic biomass [16].

Table.1: Approximate analysis of lignocellulosic biomass.

Analysis (%)	Raw sample			
	Babassu mesocarp	**Green Coconut mesocarp**	**Brazil nut shell epicarp**	**Brazil nut shell endocarp**
Moisture	7,25 ± 0,07	5,22 ± 0,02	13,9 ± 0,00	11,35 ± 0,70
Ashes	1,70 ± 0,14	2,82 ± 0,04	6,75 ± 0,49	0,9 ± 0,28
Volatile Matter	86,7 ± 0,14	90,78 ± 0,28	75,02 ± 0,71	77,45 ± 0,35
Fixed Carbon	4,35 ± 0,01	1,18 ± 0,01	4,33 ± 0,01	10,3 ± 0,01

As for the extraction process (table 2), longer extraction times (24h) favored the removal of coconut and babassu constituents, while in the Brazil nut fractions, shorter times were more efficient in removing possible interferents. High levels of extractives were found for the green coconut epicarp (average value of 15.3%), while for the other biomasses, low values were determined (average value <3.5%). These values are the following data found by other authors [24,25].

Table 2: Extractive yield at different times.

Analysis (%)	Babassu mesocarp	Green Coconut mesocarp	Brazil nut shell epicarp	Brazil nut shell endocarp
Extractives (8h)	2,12	12,16	3,43	3,33
Extractives (12h)	1,32	14,13	4,13	1,53
Extractives (24h)	2,37	19,83	2,59	2,64

The three different primary and secondary hydrolysis methodologies for the content of polysaccharides and their respective monosaccharides were tested and figure 1 shows their results.

Intermediate times in both stages showed the maximum cellulose yields, except for the Brazil nut shell epicarp. In the same way for ART, except for the nut shell with the highest percentage of ART in 1h for both primary and secondary hydrolysis. Glucose also showed higher yields in intermediate times, except for the Brazil nut shell epicarp, with higher levels (5.81%) in more drastic conditions (2h and 1.5h).
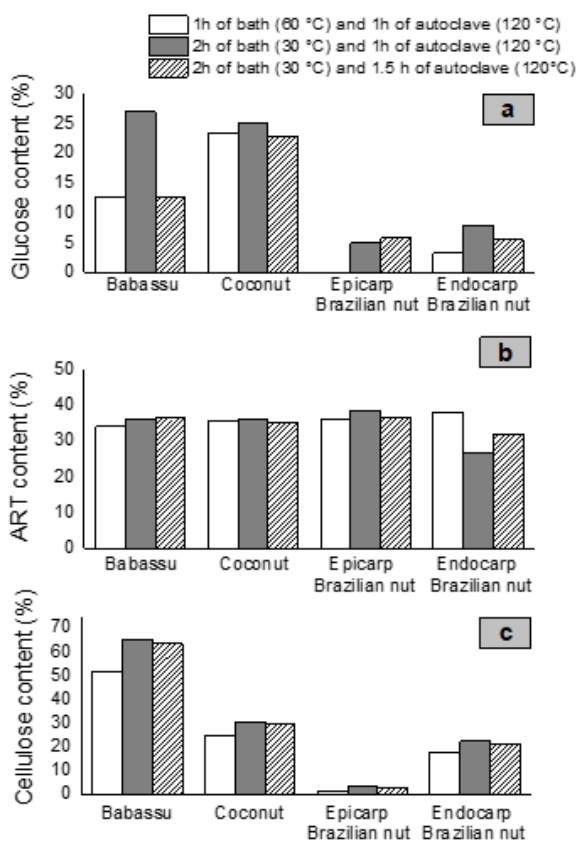
Fig. 1: Content of glucose, ART e cellulose.

Figure 2 shows the results of soluble, insoluble, and total lignin after different hydrolysis conditions. Unlike carbohydrates, for soluble lignin, the shortest reaction times favor the best results, except for the Brazil nut shell epicarp. Also, the trend followed for insoluble lignin (Klason), in which the highest yields were in less severe conditions. The total lignin that is the sum of the soluble and insoluble lignin obtained the same result.
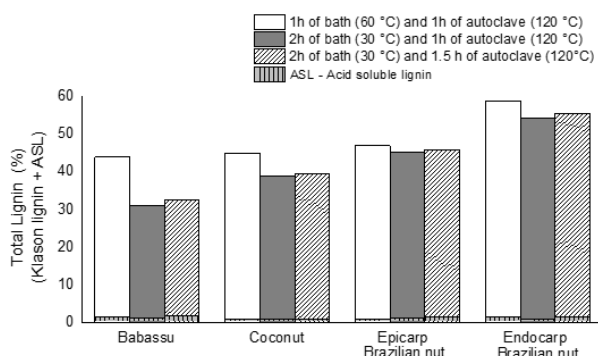

Fig. 2: Content of total lignin after different conditions.

These results show the high potential of these biomasses, as levels above 28% of ART were found and

above 30% for lignin, which strongly suggests that they can be used in biorefineries industries.

Figure 3 shows the X-ray diffractograms of the raw samples and the samples extracted at different extraction times. No major changes were observed between samples. We observed that, in general, after extraction some peaks became more prominent, increasing the crystallinity content of the samples.

The data obtained show that the biomasses presented crystalline cellulose percentages that varied between 25 and 40.0%; associated respectively with Brazil nut endocarp and with babassu extracted (24 and 12h). Has been reported a relation between a higher level of crystallinity and hydrolysis yields, which is given by the content of carbohydrates (ART); this indicates that the variation range of crystallinity affects the yield of hydrolyses. For example, Brazil nut endocarp, with less crystallinity, also had lower levels of ART and glucose. On the other hand, babassu, with higher levels of crystallinity, also had the highest values of glucose and ART.
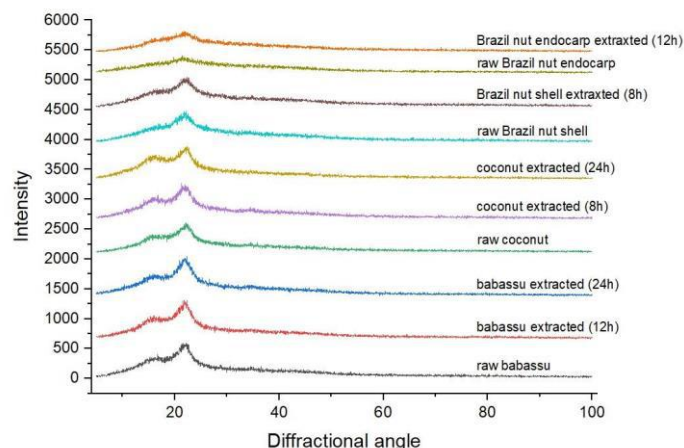

Fig. 3: Raios-x diffractograms.

IV. CONCLUSION

Given these results, we conclude that the NREL methodology needs to be adapted for each lignocellulosic biomass, as a considerable difference was found in optimizing primary and secondary hydrolysis. Extractive content also needs to be corrected for each specific biomass. High levels of polysaccharides were found in all fractions (except for the Brazil nut shell epicarp), showing that the residual biomasses of the Amazon region and the Brazilian Cerrado can provide a range of valuable products.

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