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Obtainment and Characterization of Lignin from Enzymatic Hydrolysis of Sugarcane Bagasse of 2G Ethanol Process in Pilot Scale

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A promising use of sugarcane bagasse is for cellulosic ethanol production. One step of this production is the enzymatic hydrolysis process. A residual stream from this process is mainly composed of lignin and some non-hydrolysed cellulose fibers. In order to extend the application of the biorefinery concept to second generation (2G) ethanol production, the residue generated in this process could be used as raw materials for new processes to obtain value-added products. Besides, fully utilization of the residues produced in the enzymatic hydrolysis process is a feasible method to reduce cost. Lignin has a potential to replace petroleum-based materials, which are increasingly scarce and expensive, in many industrial applications, such as phenolic resins. The original source, the extraction method used and the process by which lignin was stemmed change its physicochemical characteristics. Thus, the suitability of lignin as raw materials into value-added products can vary widely. Bearing all these in mind, it is necessary to characterize the material to be able to evaluate it in formulations of value-added products. In this work, a chemical characterization of Enzymatic Hydrolysis Residue Lignin (EHRL) was carried out aiming the use for resins. The EHRL was obtained from the sugarcane bagasse pretreated by hydrothermal process to 190 °C by 10 min with solid-liquid ratio of 1:10. Then, the enzymatic hydrolysis process was carried out using the 15 FPU/g dry biomass of cellulolytic complex (Celluclast® 1.5 L) and 10 UI/g dry lignocellulose of β-glucosidase (Novozym® 188). The pretreatment and the enzymatic hydrolysis processes were carried out in batch reactor (Pope Scientific 350 L) pilot scale using the facilities of Brazilian Bioethanol Science and Technology Laboratory (CTBE). A chemical characterization of the EHRL allowed knowing better its features, before being applied in manufacturing of value-added products. By chemical characterization method, we found that the total lignin content is 47.3 %, cellulose content is 39.8 %, hemicelluloses content is 4.5 % and ash content is 8.4 % (w/w on dry matter) in the EHRL. An alternative to use the EHRL for manufacturing of composites has been considered. Since it could be used to partially replace petroleum-based phenol in synthesis of phenolic resins, it is interesting to evaluate the effect of the remaining cellulose fibers in the EHRL and as they could act as reinforcement in phenolic composites.

1. Introduction

Lignin has a potential to replace petroleum-based materials, since it is abundant, relatively cheap and renewable (Ragauskas et al., 2014). The lignin macromolecule contains various functional groups that can be used to obtain some lignin-based polymers, such as polyurethane, phenol formaldehyde, phenol epoxy, polyester, polyolefin, vinyl polymer and others (Chung and Washburn, 2012).

Lignin can be obtained from processes which use polysaccharides as raw material. The cellulosic ethanol production from sugarcane bagasse is a mill that generate lignin (Dias et al., 2012). Cellulosic ethanol (2G ethanol) industry does not produces much lignin nowadays. However, it is expected that the expansion of cellulosic ethanol industry generates relatively large amounts of lignin.

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Among the major steps to produce cellulosic ethanol, the enzymatic hydrolysis of sugarcane bagasse generates a residual stream that is mainly composed of lignin and some non-hydrolysed cellulose fibers. Besides optimizing the cellulosic ethanol processes, the full use of by-products is a feasible method to contribute with economic viability, especially residues produced in the enzymatic bagasse hydrolysis.

Alternatives to use the Enzymatic Hydrolysis Residue Lignin (EHRL) not only for energy co-generation but also for other purposes have to be considered. However, the physicochemical characteristics of lignin depend on its original source, the applied extraction method and the process by which it was stemmed. Thus, the suitability of lignin as raw materials into value-added products can vary widely. Because of this, it is important to study it before employing it in formulations of added-value products.

The lignin employed in this work was the sugarcane bagasse lignin from the enzymatic hydrolysis of a second-generation ethanol process in a pilot scale (the EHRL). The lignin, cellulose, hemicellulose and ash contents of the EHRL were determined by chemical characterization. Besides, the following analyses were performed: elemental analysis (CHNSO), ^{1}H NMR spectroscopy, Higher Heating Value (HHV) determination, particle size distribution by sieving and by Light-Scattering (LS) and Scanning Electron Microscopy (SEM). In this context, the current work aims to obtain the EHRL in pilot scale, as well as to perform the physicochemical characterization of the EHRL in order to assess its potential in the manufacturing of phenolic composites.

2. Material and methods

2.1 Obtainment of the Enzymatic Hydrolysis Residual Lignin - EHRL

The EHRL was obtained from a process where the sugarcane bagasse was pretreated in a 350 L steel reactor (Hastelloy C276) built by Pope Scientific. The pretreatment occurred at 190 °C for 10 min and the solid/liquid ratio of 1:10. This process aims to hydrolyse the hemicelluloses, decreasing the recalcitrance of the biomass to enzymatic hydrolysis step from 2G ethanol process. Then, the enzymatic hydrolysis process was carried out by using the 15 FPU/g dry biomass of cellulolytic complex (Celluclast® 1.5 L) and 10 UI/g dry lignocellulose of β-glucosidase (Novozym® 188). The enzymatic hydrolysis process was carried out in a 350 L steel reactor, Hastelloy C276 (Pope Scientific). Before processes were performed in pilot scale, their conditions were optimized at laboratory scale.

2.2 Chemical characterization

Total lignin (acid-insoluble and soluble lignin), carbohydrate (cellulose and hemicelluloses) and ash contents in the EHRL were determined according to Rocha et al. (2015).

2.3 ^{1}H NMR spectroscopy

The EHRL was submitted to acetylation reaction with anhydrous pyridine (10:1 v/w of EHRL) and acetic anhydride (10:1 v/w of EHRL) for 12 h with stirring at room temperature. Acetylated and dried EHRL was submitted to the analysis of solution ^{1}H NMR. The sample (25 mg/1 mL of deuterated chloroform (Aldrich, 99.8 atom % D, containing 0.03 % v/v TMS)) was placed in a 5 mm NMR tube into the NMR equipment (Agilent DD2 500 MHz with inverse z-gradient triple resonance probe). The spectra were obtained with following conditions: acquisition time of 2.0 s, relaxation delay of 1.0 s, spectral width of 8000 Hz and 256 scans. The software Mnova NMR was used for visualization, processing, analyses and reporting of the 1D NMR data.

2.4 Elemental analysis

Carbon, hydrogen, nitrogen and sulphur contents were determined by using the Perkin Elmer® 2400 Series II CHNS/O Elemental Analyser. The oxygen content was calculated by difference.

2.5 Determination of Higher Heating Value (HHV)

Experimental HHVs were measured by using a bomb calorimeter (IKA® C200). Theoretical HHVs (MJ Kg^{-1}) were estimated as a function of the contents of C, H, O and N to Demirbas (1997) using the Eq (1):

$$HHV = (33.5C + 142.3H - 15.4O - 14.5N) \times 10^{-2} \tag{1}$$

where: C (carbon content), H (hydrogen content), O (oxygen content) and N (nitrogen content).

2.6 Particle size distribution by sieving

Initially, the EHRL were well mixed and the agglomerates contained in EHRL were undone. The sampling was performed by quartering to obtain samples with better representativeness. Four EHRL samples (≈ 60 g) were forwarded to particle size distribution analysis. A vibratory sieve shaker (Analysette 3, Fritsch®) with a sieve stack (sieve opening sizes from 4.75 mm to 0.15 mm) was used in this analysis. The sieving time was set to 15 min and the selected amplitude of vibration of the sieve stack was 3.0 mm.

2.7 Particle size distribution by Light-Scattering (LS)

Particle size distributions were evaluated by LS to two samples: EHRL from bottom of sieve set (< 0.15 mm) and the milled EHRL. The milling process of EHRL was done in a variable-speed-rotor-mill at 11000 rpm using a 0.08 mm sieve (Pulverisette 14, Fritsch®). The samples were suspended in water before the analysis. The laser diffraction particle size analyser used was the Beckman Coulter LS 13 320 instrument with Liquid Universal module. The instrument was operated with 780 nm light source, 8 % obscuration and Fraunhofer light-scattering model (Driemeier et al., 2011).

2.8 Scanning Electron Microscopy (SEM)

Only SEM images of the EHRL from bottom of sieve set (< 0.15 mm) and the milled EHRL, as described in section 2.7, were obtained. SEM images were acquired with a Scanning Electron Microscope (FEI® Quanta 650 FEG) with ETD secondary electron detector (high vacuum). The beam conditions were 5.0-15.0 kV with a spot size of 3.0 and a working distance of 8.0-10.0 mm. The samples were mounted on stubs and sputter-coated with gold using a BAL-TEC SCD 050 sputter coater (40 µA, 60 s).

3. Results and discussion

The EHRL was obtained as described in section 2.1. Results and discussion of the physicochemical characterization are described in the following.

3.1 Chemical characterization

Table 1 shows the results of moisture, acid-soluble lignin (ASL), acid-insoluble lignin (AIL), total lignin, cellulose, hemicelluloses, ash and elemental sugars contents in the EHRL.

Table 1 - Analysis of moisture, total lignin (acid-insoluble lignin-AIL and acid-soluble lignin-ASL), cellulose, hemicelluloses and ash contents and carbohydrates composition in the EHRL. The standard deviation is shown in parentheses.

Content (% (w/w) on dry matter)		Carbohydrates composition (g/L)	
Moisture	6.83	Glucose	1.688
AIS	36.94 (0.07)	Xylose	0.160
ASL	10.37 (0.15)	Formic acid	0.016
Total lignin	47.31 (0.08)	Acetic acid	0.026
Cellulose	39.79 (1.34)	Furfural	0.011
Hemicellulose	4.48 (0.13)	Hydroxymethylfurfural	0.012
Total ashes	8.4 (0.06)		

The EHRL is mainly composed of lignin (≈ 47 %) and cellulose fibers not hydrolysed (≈ 40 %) as observed in Table 1. Rocha et al. (2015) performed the chemical characterization of 60 samples of sugarcane bagasse. Cellulose, hemicellulose and lignin contents of these samples ranged from 36.9-45.7 %, 25.6-29.6 % and 18.9-26.1 %, respectively. It is observed that the lignin content is almost twice as in the EHRL in relation to lignin content in bagasse sugarcane. The hemicelluloses content in the EHRL is about 70-80% less than in sugarcane bagasse, while, the cellulose contents are almost the same in both. Regarding to the ash content, the EHRL has about 8 %, while in the sugarcane bagasse has between 1.1-6.0 % of ashes.

Since the EHRL has high content of lignin and cellulose, it could be used to produce lignin-phenol-formaldehyde matrix composites reinforced with cellulose microfibers. The total lignin content, ASL and AIL, in the EHRL could react with formaldehyde when it is used to synthesize phenolic resins. On the other hand, the cellulose fibers not hydrolysed could act as structural material (reinforce) to phenolic resins. In literature, it is reported that cellulose from sugarcane bagasse has a tensile strength of 222-290 MPa and the Young's modulus of 17.0-27.1 GPa, having the possibility for its use as structural components (Loh et al., 2013; Saba et al., 2015).

3.2 ^{1}H NMR spectroscopy

The major functional groups of lignin affecting its reactivity for the synthesis of phenolic resins are phenolic hydroxyl and methoxyl. The acetylated EHRL was analysed by ^{1}H-NMR in order to determine aliphatic and phenolic hydroxyls and methoxyl groups. In the acetylation reaction of the EHRL, the hydroxyl group has been converted into acyl group. So, in fact what is observed in spectra is the acyl group. The signal acyl group refers to three hydrogen atoms. To estimate the area corresponding the relative amount of the hydroxyl group, it is necessary to divide by three. Table 2 shows the relative amount of hydroxyl and methoxyl groups present in the EHRL.

It can be observed (Table 2) that the aliphatic hydroxyl content is more than double in relation to the phenolic hydroxyl content. This high relative amount of aliphatic hydroxyl is arising from its high carbohydrate content. The presence of phenolic hydroxyl group in lignin structure allows activating the free aromatic ring positions making it reactive toward formaldehyde (Qiao et al., 2015). It can also be observed that the methoxyl group content is about 19 %. The lower the methoxyl content is, the more unblocked the ortho positions on the phenyl rings are. This could lead to a higher reactivity of the phenol towards the formaldehyde.

Table 2: ^{1}H NMR results of the major functional groups in the EHRL.

Functional groups	δ (ppm)	$\%_{relative}$
Aliphatic hydroxyl	2.0	8.97
Phenolic hydroxyl	2.3	3.90
Methoxyl (OCH_3)	3.7	18.85

3.3 Elemental analysis and HHV

In Table 3, it is showed the elemental composition and experimental and estimated HHVs of the EHRL. The element with higher content in the EHRL is carbon, following by oxygen. The EHRL has about 5 % more of carbon content, 53 % more of nitrogen content and 7 % less of oxygen content than the average contents of the sugarcane bagasse samples evaluated by Rocha et al. (2015). They estimated the HHVs for their samples by Equation (1). The average of HHVs that they estimated was 16.1 MJ/kg. Both, the estimated and the real HHVs of ERHL, were higher than that estimated by them. This occurs because the obtainment process of the EHRL enabled higher lignin content to be produced. The HHV of lignin is about 25-26 MJ/kg, while both cellulose and hemicellulose have HHVs in the range 16-18 MJ/kg (Demirbas, 2004). Higher carbon and lower oxygen contents lead to a higher HHV.

Table 3: Elemental composition of the EHRL (% (w/w) on dry matter) and experimental and estimated HHVs (kJ/kg). The standard deviation is shown in parentheses.

Carbon	47.46 (0.09)
Hydrogen	6.28 (0.01)
Nitrogen	0.57 (0.04)
Sulphur	0.59 (0.01)
Oxygen	45.11 (0.13)
$HHV_{experimental}$	19347
$HHV_{estimated}$	17806

3.4 Particle size distribution by sieving and by Light-Scattering (LS)

A set of ten sieves with opening size ranging from 4.75 to 0.15 mm was used in the particle size distribution by sieving. Table 4 shows the opening diameter of each sieve, the average diameter and the retained mass fraction in each sieve. It was seen mainly fiber clusters in the sieves with opening diameter of 4.57 and 4.05 mm. In other sieves, it was visibly observed that the fibers of the EHRL were released. The particle size distribution of the EHRL presented profile of polydispersity material, since it had two regions with a higher retained mass fraction. About 46 % of the EHRL was comprised in the set of sieves with average diameter of ≤ 1.0 mm. Size and morphology of fibers influence directly in determining the mechanical properties of composites. Figure 1 shows particle sizing distributions by LS of the EHRL from bottom of sieve set (< 0.15 mm) and the milled EHRL (as described 2.7 in section). Both samples showed similar and polydisperse profiles. The EHRL from bottom ranged approximately from 0.4-820 μm. About 5 % of this sample is between 0.4-50 μm and 50 % is ≤ 170 μm. Only 44 % of the EHRL from bottom is ≤ 0.15 mm. This fact can be explained due to the fibers diameter is much smaller than its length, so it is possible that longer fiber can pass through sieves with smaller diameter opening. The milled EHRL ranged between approximately 0.4-990 μm. About 5 % of the milled EHRL is 6.45-0.4 μm and 50 % it is ≤ 70 μm.

By the means of the particle size distribution analysis, it can be observed that the size particles of the EHRL covers a wide range of values. Based on the literature, it is known that the fiber size has a great impact on the properties of composites (Stark and Rowlands, 2003). Joseph et al. (2002) noted that longer fibers resulted in the higher tensile strength for phenol formaldehyde/banana fiber composites. However, Cândido et al. (2014) analysed the sugarcane bagasse fibers with diameter range of 0.11-0.47 mm and they observed an inverse correlation between tensile strength values and their equivalent diameter. In addition, the smaller size of the particles, the larger its contact surface, thus, providing a better interaction between lignin deposited on the fibers and formaldehyde reagent. Further studies are required in order to find the optimum length of fibers to obtain the maximum tensile strength in the phenolic composites, without lowering the reactivity of the EHRL towards formaldehyde.

Table 4: Particle size distribution by sieving of the EHRL. The standard deviation is shown in parentheses.

Opening diameter of sieve (mm)	Average diameter (mm)	Retained mass fraction (%)
Bottom	< 0.15	4.0 (0.83)
0.15	0.20	5.7 (0.46)
0.25	0.34	11.6 (0.29)
0.43	0.52	7.4 (0.30)
0.60	0.73	8.1 (0.35)
0.85	1.02	9.6 (0.50)
1.18	1.44	10.8 (0.58)
1.70	2.03	10.8 (0.40)
2.36	2.86	14.4 (0.59)
3.35	4.05	11.8 (1.03)
4.75	> 4.75	5.7 (0.62)

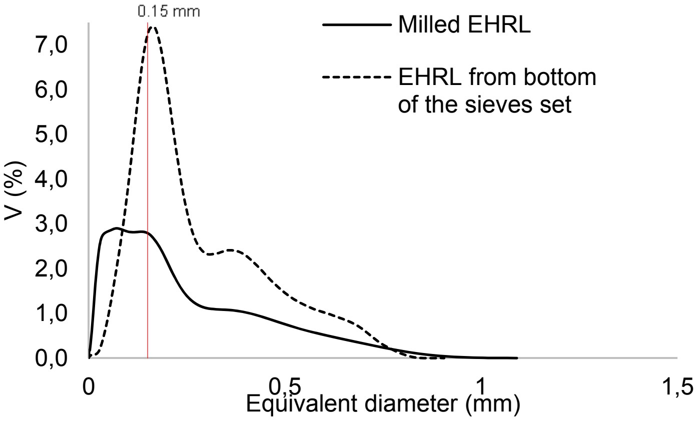

Figure 1: The particle size distributions by LS of the EHRL from bottom of the sieves set and the milled EHRL.

3.5 Scanning Electron Microscopy (SEM)

Morphological studies of the fibers of both samples (the EHRL from bottom and the milled EHRL) showed different sizes and arrangement from them, since fibers are not spherical and dispersively arranged. By means of the SEM technique, it was possible to view the distribution of fibers and lignin in the EHRL. In Figure 2 it is observed the EHRL and its fibers are well distributed each other in all region. In addition, it could be noted that there is lignin deposited on the fibers of both samples. This probably will provide an easier reaction between lignin and formaldehyde, since the EHRL is more susceptible to interact with formaldehyde.

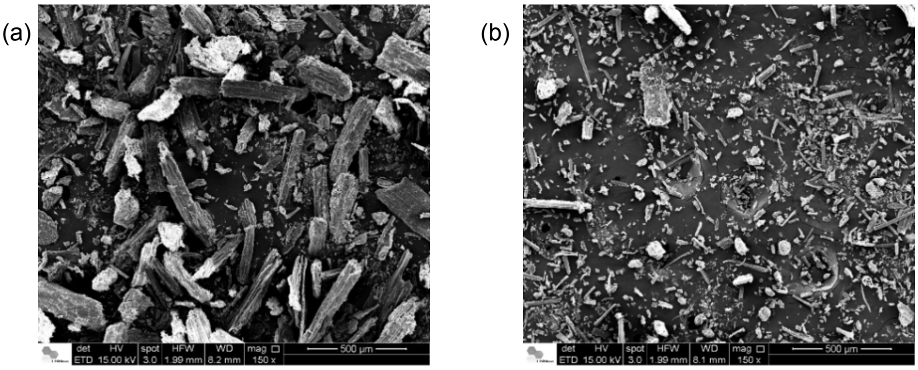

Figure 2: SEM images of (a) the EHRL from bottom of sieve set and (b) the milled EHRL (150x).

4. Conclusions

The obtainment process of the EHRL generated a material with more than double of lignin content in relation the lignin content in the bagasse sugarcane reported in the literature, while the cellulose content remained high. The ^{1}H-NMR showed the aliphatic hydroxyl content is higher than phenolic hydroxyl in the EHRL. This is due to the contribution of the aliphatic hydroxyl group originating from the high amount of cellulose present in EHRL. The EHRL showed higher carbon content and higher HHV than bagasse sugarcane. The particle size distribution by sieving and by LS allowed to observe a wide range of particle sizes in the EHRL. It is important to choose a suitable particle size of the EHRL in order to obtain adequate tensile strength in phenolic composites without impairing the reactivity of lignin towards formaldehyde. By the means of SEM analysis, it was possible to observe that lignin is deposited on the fibers of the EHRL, thus, showing a higher susceptibility of the EHRL to interact with formaldehyde.

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