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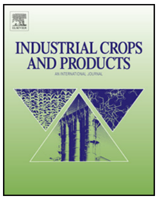

Physicochemical characterization of residue from the enzymatic hydrolysis of sugarcane bagasse in a cellulosic ethanol process at pilot scale

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ABSTRACT

One of the major obstacles to use lignin as feedstock for value-added products at industrial scale is that its physicochemical characteristics can widely vary. Thus, it is necessary to know such characteristics before applying in the synthesis of products. In this study, we produce a lignocellulosic biomass in pilot scale composed of lignin (47% w/w) and cellulose fibers (40% w/w). This biomass was obtained from the enzymatic hydrolysis of sugarcane bagasse that was pretreated by a hydrothermal process (190 °C/10 min). After obtaining this residue, which is listed here as “Enzymatic Hydrolysis Residue Lignin” (EHRL), its physicochemical and thermal characterization was performed. A highly concentrated lignin from the LignoBoost process in Kraft pulp mill, lignin LignoBoost (LBL), was also evaluated in order to compare it with the EHRL. The C9-formulae of the lignin extracted from the EHRL is $C_{9.0}H_{11.2}O_{4.3}S_{0.08}N_{0.17}(OCH_3)_{0.9}(OH_{phenolic})_{0.7}(OH_{aliphatic})_{0.6}$ and the C9-formulae of the LBL is $C_{9.0}H_{7.9}O_{1.4}S_{0.16}N_{0.01}(OCH_3)_{1.1}(OH_{phenolic})_{0.7}(OH_{aliphatic})_{0.5}$. By the 2D Heteronuclear Single-Quantum Correlation Spectroscopy (HSQC) technique, syringyl/guaiacyl ratios for the EHRL and the LBL are 1.8 and 4.8, respectively. Thus, the EHRL appears to be promising for phenolic composite, for instance, since it has lower methoxyl content. The EHRL showed larger range of fiber particle sizes (<4.05 mm–0.4 μm) than the LBL (0.4–1090 μm).

1. Introduction

Lignin is a complex macromolecule that contains three main units: *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S). The units differ from each other by the presence or absence of methoxyl (OCH_3) groups (Monlaua et al., 2013). In addition, lignin contains various functional groups that allow it to be used to obtain different polymers (such as phenolic resin), and for other applications, including bio-jet fuels (Bi et al., 2015) green composite hydrogels, lignosulfonates, cement and concrete additives, and as binder, for instance (Agrawal et al., 2014; Bi et al., 2015; Chung and Washburn, 2012; Thakur and Thakur, 2015). In many industrial operations, such as the pulp and paper industry and sugar/bioethanol production, lignin is not the main commercial product, and is a raw material with potential to be better explored than it usually is.

According to the Food and Agriculture Organization, the quantity of world sugarcane production in 2014 was about 1.9 Gt (FAOstat, 2014). This biomass is one of the main raw materials used in ethanol production. About 23.4 Mm^3 of ethanol was produced in Brazil in 2014 (RFA, 2014). Sugarcane juice is extracted by crushing process in order to produce sugar and/or ethanol. The fibrous residue of sugarcane which remains after the juice extraction is named bagasse. Currently, the large amount of sugarcane bagasse produced is mostly used for generating heat and elec-

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tricity. However, this material is an attractive feedstock for the production of other products, including the production of second-generation bioethanol, bio-jet fuels, and composite materials, for instance (Huang et al., 2016; Loh et al., 2013; Zhang and Lynd, 2004). Among the major steps to produce cellulosic ethanol, enzymatic hydrolysis of sugarcane bagasse generates a residual stream that is mainly composed of lignin and some non-hydrolyzed cellulose fibers, called Enzymatic Hydrolysis Residue Lignin (EHRL) (Galbe and Zacchi, 2010). Besides optimizing the cellulosic ethanol process, full use of byproducts is a feasible method to reduce costs and residue generation in enzymatic hydrolysis bagasse, as well as to contribute to the financial income of the process.

Another important industrial activity that leads to lignocellulosic streams is the Kraft and LignoBoost processes. According to the Brazilian Pulp and Paper Association, Brazil was the world's fourth largest producer of pulp in 2013, producing about 14 Mt of it (Bracelpa, 2014). Every year, the Kraft pulp mills produce large amounts of lignin. This lignin is mainly burnt to produce energy that supply the pulp mill and is sold to grid. However, modern pulp industries aim to expand the use of lignin to value-added products (Benali et al., 2014). An alternative for this is to obtain a highly pure lignin. The LignoBoost lignin (LBL) is a relatively recent process that consists of concentrating lignin from the Kraft lignin precipitated by black liquor (Tomani, 2010).

Currently, cellulosic ethanol industries do not produce as much lignin as Kraft pulp mills. However, it is expected that relatively large amounts of lignin will be generated with the expansion of cellulosic ethanol production (Maniatis et al., 2012). Therefore, it is important to seek others alternatives for using the EHRL and LBL. It is known that the suitability of lignin as feedstock for value-added products can vary widely according of its physicochemical characteristics. These characteristics of lignin depend on the original source, the applied extraction method, and the process by which lignin was stemmed.

Bearing all this in mind, in this study we performed a physicochemical characterization of the EHRL and LBL in order to understand details about their chemical and structural characteristics, enabling future processes and product designs. In addition, we investigated the reactivity of the EHRL and LBL as replacements for phenol in phenol-formaldehyde resin. Besides the chemical characterization and elemental analysis, these raw materials (EHRL and LBL) were characterized by the following methods/analyses: proton nuclear magnetic resonance spectroscopy (^{1}H NMR), and 2D Heteronuclear Single-Quantum Correlation Spectroscopy (HSQC) to determine their functional and structural groups; Higher Heating Value (HHV) determination, and thermogravimetric analysis (TGA) for their thermal analysis and stability; particle size distributions by sieving and by laser scattering (LS) for their morphological analysis.

2. Experimental section

2.1. Raw materials

One of the lignocellulosic materials characterized in this study was the residue from the enzymatic hydrolysis of pretreated sugarcane bagasse in a pilot plant of cellulosic ethanol, referred to here as EHRL. The procedure to obtain the EHRL is as follow. 20 kg (on dry weight basis) of sugarcane bagasse was transferred to a 350 L steel reactor (Hastelloy C276) built by Pope Scientific. Then, 200 L of water was added into the reactor in order to perform the hydrothermal pretreatment at 190 °C for 10 min. The reactor was gradually depressurized to reach 100 °C and atmospheric pressure, and then discharged. After a filtration step, the pretreated bagasse (the solid stream) was washed and dried at room temperature. A sample of this pretreated bagasse was withdrawn to determinate its chemical composition. The purpose of this process was to hydrolyze the hemicelluloses, decreasing the recalcitrance of the biomass for the enzymatic hydrolysis step from the cellulosic ethanol process. Then, the enzymatic hydrolysis process of the pretreated bagasse was undertaken using the 15 FPU/g of dry biomass of cellulolytic complex (Celluclast® 1.5 L) and 10 IU/g dry lignocellulose of β-glucosidase (Novozym® 188). The enzymatic hydrolysis process was carried out in a 350 L Hastelloy C276 (Pope Scientific) steel reactor. After the filtration step, a liquid stream enriched in glucose and a solid stream enriched in lignin were obtained. The former was conducted to the fermentation step in order to produce ethanol and the latter was washed with water and dried at room temperature, obtaining the EHRL. The conditions of pretreatment and enzymatic hydrolysis were previously optimized at laboratory scale.

Another feedstock used in this work was the LBL. It is an industrial lignin that was supplied by Fibria, a Brazilian pulp and paper company. The LBL originated from a Kraft pulping of Eucalyptus and obtained through the LignoBoost process.

In order to estimate the C9-formulae and the Syringyl/Guaiacyl ratio of the EHRL lignin, 100 g of the EHRL was submitted to an alkaline delignification process using a NaOH solution (1.5%). This process was performed in a Parr Stirred Vertical Reactor (2 L). The solid/liquid ratio was 1:10. The reaction conditions were the following: 100 °C for 1 h with stirring at 300 rpm, and afterwards, it was filtrated. To precipitate the lignin from liquor, H_2SO_4 (98%) solution was slowly added until pH 2 was reached. After storing it overnight in a refrigerator, the vacuum filtration was conducted. The extracted lignin from the EHRL (EL-EHRL) was washed until pH 5 was reached. Lastly, it was dried overnight using a Thermo Scientific™ SpeedVac centrifuge.

2.2. Chemical characterization

The EHRL and the LBL were chemically characterized according to the methodology described by (Rocha et al., 2015). The acid-insoluble and soluble lignin, carbohydrates and ash content, and moisture in the EHRL and the LBL were determined.

2.3. NMR spectroscopy analyses

All samples were submitted to an acetylation reaction with anhydrous (Menezes et al., 2016). Acetylated and dried samples were submitted to analysis of solution ^{1}H NMR and 2D HSQC. Samples with a concentration of 25 mg/mL of deuterated chloroform (Aldrich, 99.8 atom% D, containing 0.03% v/v TMS) were placed in a 5 mm NMR tube into the NMR equipment (Agilent DD2 500 MHz with inverse z-gradient triple resonance probe). ^{1}H NMR spectra were obtained with conditions previously described (Menezes et al., 2016). HSQC contour maps were obtained with the following conditions: the HSQC adiabatic pulse program was selected, acquisition time of 0.26 s, relaxation delay of 1.5 s, spectral widths of 8000 Hz and 25,000 Hz for the ^{1}H and ^{13}C dimensions, respectively. The total increments were of 1024 with 32 scans each. The free NMRPipe software was used for processing the 2D HSQC data. Prior to Fourier transformation, 2D data were zero-filled to obtain a matrix size of 4K × 1 K points. Additionally, a Gaussian and a shifted sine-bell window were applied in f_2 and f_1, respectively. Finally, a polynomial baseline correction was performed.

2.4. Elemental analysis

Carbon, hydrogen, nitrogen and sulfur contents from the EHRL and the LBL were determined by using in the PerkinElmer® 2400 Series II CHNS/O Elemental Analyzer. The oxygen content