Structural Characteristics of Bagasse Furfural Residue and its Lignin Component. A NMR, Py-GC/MS, and FTIR Study.

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ABSTRACT

Commercial furfural, an important platform chemical, is produced from acid hydrolysis of lignocellulosic biomass. The manufacturing processes are inherently inefficient, and so it is necessary to value add to substantial amounts of residue obtained. The structural features of bagasse furfural residue and the lignins extracted from it by three NaOH treatments have been studied in order to understand the transformations that occurred by these treatments. 2D-NMR and Py-GC/MS of the furfural residue revealed that it contains mostly lignin and depolymerized cellulose moieties and the complete absence of xylans as a result of their hydrolysis during the furfural production process. In addition, the analyses revealed that the furfural residue contains 44% of H-type lignin units, in comparison to 11% for bagasse, and most of the lignin inter-unit linkages present in bagasse have disappeared. The pyrograms show that the furfural residue produced unusually high phenol content which was attributed to the high levels of 'H-type' units present in this lignin. The proportion of functional groups, particularly total OH aliphatic groups, where significantly lower in the extracted lignins compared to soda lignin obtained by the normal pulping process. The highest severity of NaOH extraction process reduced the amount of reactive functional groups present in the lignin, though the S/G ratios of ~1.1were independent of the extraction method. The three lignins have high proportions of "H-units" (around 36–37%), which gives them special properties for different applications, particularly in the production of phenolic resins.

SYNOPSIS

The structural features of the bagasse furfural residue and its lignin component was elucidated

INTRODUCTION

Furfural, a versatile industrial solvent, has been identified as a key, renewable platform chemical that is used to produce fuels, solvents, polymers, pharmaceuticals, and agrochemicals.[1-2] For the last century, the most common industrial method to produce furfural has been the acid-catalyzed dehydration of pentose sugars (*e.g.*, from C5 of hemicelluloses) from agricultural residues, a process that has not been improved significantly since the 1980s.[3-4] All commercial furfural production process using this method requires mineral acid catalysts and relatively high steam consumption for furfural recovery, but only achieves relatively low furfural yields because of by-product formation. Furthermore, the corrosiveness of the acid catalyst and the low yields result in both relatively high production and environmental compliance costs.[4] As such, most of the current world market for furfural (>600,000 t) is produced in developing countries where low labor costs, cheap feedstocks and lesser environmental regulations prevail [5-6]. Notably, these commercial plants want to supplement the production of furfural with the sale of higher value by-products to achieve a profitable outcome highlighting that an integrated production strategy is required to recover value from a relatively inefficient process[7].

Commercial furfural is typically produced from lignocellulose to give a yield of ~9.6 wt%% and a cellulose- and lignin-rich residue of up to 70 wt% of the original mass of the starting material. The poor yield of furfural is due to losses from condensation and the formation humic-like substances. Despite recent developments where side reactions have been minimized using biphasic systems with over 65 wt% furfural yield from xylose[8], the highest achievable yield say with sugarcane bagasse containing 24 wt% hemicelluloses is less than 19wt% . The very high proportion of the furfural residue is burned to generate the steam for furfural recovery.[9-10] However, valorization of the cellulose- and lignin-rich residue would provide both environmental and economic benefits.[11] Valorization of the lignin-based materials is

technically challenging because of their structures and associated chemistries. Lignin is an amorphous, high molecular-weight, highly branched and substituted polymer of phenylpropane units. There are three major phenylpropane units, *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) which differ in O-methyl substitution of the aromatic ring. While the lignin in the furfural residue has the potential to be used for the production of fuels, materials (such as carbon fibers, polymers, adhesives, and resins), BTX chemicals, phenol, and their derivatives, the optimal choice of product and production process are dependent on a clear understanding of the structure and chemistry of the residual lignin.[12-14] Recently, Katahira et al. [15] performed a base-catalyzed depolymerization of biorefinery lignins derived from enzymatic hydrolysis and found that the lignin structure and composition affected product yield and type. Ronnols et al.[16] showed that the severity of acid treatment also affected the C-C and β-*O*-4′ linkages in lignin. When the heating time at 160 °C was increased from 2 h to 4 h the signals corresponding to coniferyl alcohol units were absent and those due to β-O-4 ethers and secoisolariciresinol were further reduced after 24 h. Further heating at 240°C, the compound secoisolariciresinol disappeared. So it would be expected that the products derived from lignin valorization will depend on lignin structure.

In this study, the effect of commercial furfural production from sugarcane bagasse on the structure and chemistry of the lignin therein was investigated. This complements previous studies on bagasse lignin isolated by various workers using sodium hydroxide, acid-alkali treatment, and acidified aqueous ethylene glycol and ionic liquids.[16-18] The effect of alkaline treatment on the properties of the lignin extracted from the furfural production residue was also investigated to examine impact of alkali severity. The structural properties (and associated linkages) and the proportions of hydroxyl functional groups of bagasse furfural residue, extracted lignins and bagasse soda lignin (as control as it used in commercial applications) were determined using elemental analysis, size exclusion chromatography (SEC), Fourier

transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy (proton-, carbon-, phosphorous-, and two dimensional heteronuclear single quantum coherence; ^{1}H, ^{13}C, ^{31}P and 2D-HSQC) and pyrolysis coupled to gas chromatography-mass spectrometry (Py-GC/MS).

EXPERIMENTAL SECTION

Materials. Raw sugarcane bagasse was collected from Racecourse Sugar Mill (Mackay Sugar Limited) in Mackay, Australia. Pith was removed by hand-sieving (aperture size of 1.0 cm) and depithed bagasse was air-dried at room temperature (25 ºC) for 12 h. Residue from furfural production was collected from the Proserpine Furfural Plant (Proserpine, Australia) when it was operated at 200 °C and 18 bar with ~1% H_2SO_4 on dry bagasse.

Chloroform, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP), chromium acetylacetonate, cyclohexanol, deuterated chloroform, dimethyl sulfoxide-d_6 (DMSO-d_6), dioxane, paraformaldehyde, pyridine, polystyrene sulfonate standards, and sodium hydroxide were purchased from Sigma-Aldrich company (US). Hydrochloric acid (mass fraction of 32%) and sulfuric acid (mass fraction of 98%) and were purchased from Chem-Supply Ltd, Australia.

Sodium hydroxide treatment of furfural production residues. The residue from commercial furfural production was treated in a 1 L Parr reactor (Parr Instruments, USA) with aqueous NaOH solution for 1 h under three conditions. In each case, the aqueous NaOH solution to furfural residue weight ratio was 20:1 given reaction mixture of 0.5 kg. The conditions for the treatments were as follows: Treatment 1 - 145 °C, 0.35 M NaOH; Treatment 2 - 100 °C, 0.35 M NaOH; Treatment 3 - 100 °C, 0.175 M NaOH. The reactor was cooled to less than 90 °C (~45 min) and the treatment solution was filtered (Whatman no. 541 filter paper).

Sodium hydroxide pretreatment of sugarcane bagasse – soda pulping. Depithed bagasse (1 kg dry weight) was mixed with 10.5 L of aqueous sodium hydroxide (1 M NaOH) solution

and pretreated at 170 °C for 90 min (~30 min heating time) in a 20 L Parr reactor (Parr Instrument, US). The reactor was cooled to <90 °C (~45 min) and the pretreatment hydrolysate containing carbohydrates, organic acids, and lignin (also called 'black liquor') was filtered (Whatman no. 541 filter paper).

Lignin recovery. Lignin was recovered from pretreatment hydrolysate and treatment solutions by lowering the pH in two-stages.[19] The pH was reduced to 4.5 by the gradual addition of aqueous H_2SO_4 (2M) with gentle stirring and the solution was stirred for a further 15 min at room temperature. The pH was then reduced to 3.0 by the gradually addition of aqueous H_2SO_4 (2 M) with gentle stirring and the solution was incubated at 65 °C for 30 min with stirring. The solutions were filtered (Whatman no. 541 filter paper) and the precipitated lignin was washed several times with hot (70 – 80 °C) water. Washed, precipitated lignin was dried at 40 °C for 72 h in a vacuum oven.

Biomass characterization. The biomass composition (ash, cellulose, hemicellulose and lignin) of sugarcane bagasse and recovered lignins was determined using the method developed by the National Renewable Energy Laboratory (NREL) USA.[20-21] The maximum standard deviation was no more 3% using this method.

Elemental analysis. Elemental analyses of bagasse, furfural residue, and recovered lignins were performed using a FlashEA 1112 Organic Analyser (Thermo Scientific, US). All samples were dried overnight at 105 °C prior to analysis. Samples (2 – 4 mg) were encapsulated in a tin container for the measurement of carbon, hydrogen, and nitrogen content. Oxygen content was determined using samples encapsulated in a silver container. The maximum error of analysis for oxygen was 2.8%. The maximum error for the other elements was 0.2%.

Average molecular weight. SEC was used to determine the molecular weights of recovered lignins. Lignin samples (10 mg) were dissolved in 0.1 M NaOH and filtered (0.45 μm syringe filter) prior to SEC analysis. SEC analysis was undertaken using a Shodex Asahipak GS-320

HQ column and a Waters 2487 UV detector (280 nm) at a flow rate of 0.5 mL min^{-1}. The mobile phase was 0.1 M NaOH (adjusted to pH 12 by the addition of dilute HCl) and column temperature was maintained at 30 °C. Sodium polystyrene sulfonates with a molecular weight range from 1530 g mol^{-1} to 34 700 g mol^{-1} were used as standards and their mobility was used to determine the M_w and M_n of the lignin extracted from the solid residues. It should be noted that the results should be considered as only qualitative because lignin which is highly branched has a very different charge density than the poly(styrene sulfonate) standard.

Attenuated total reflectance-FTIR spectroscopy. The infra-red spectra of bagasse, furfural residue and recovered lignin were collected using a Nicolet 870 Nexus FTIR spectrometer equipped with a Smart Endurance single bounce diamond attenuated total reflectance (ATR) accessory (Nicolet Instrument Corp., US). The spectrometer incorporated a KBr beam splitter and a deuterated triglycine sulfate room temperature detector. Spectra were collected from 4000 cm^{-1} to 525 cm^{-1} using 64 scans at 4 cm^{-1} resolution and a mirror velocity of 0.6329 cm s^{-1}. The measurement time for each spectrum was ~60 s.

2D HSQC NMR. 2D heteronuclear single quantum coherence (HSQC) NMR spectra were collected to provide more detailed chemical information about the lignin in the solid residues. Lignin samples (20 – 30 mg) were dissolved in 1 mL of DMSO-d_6. ^{1}H–^{13}C correlation 2D HSQC NMR spectra were recorded at room temperature on a XL-300 spectrometer (Varian, US). The spectral widths were 5000 and 20 000 kHz in the ^{1}H and ^{13}C dimensions, respectively. A total of 1024 complex points were collected for the ^{1}H dimension with 1.5 s recycle delay. A total of 64 transients at 256 time increments were recorded in ^{13}C dimension. The central solvent (DMSO-d_6) peak was used as an internal chemical shift reference point (δ_C/δ_H 39.5/2.49).

"Gel-state" NMR analysis was performed on ~80 mg samples of finely divided (ball-milled) bagasse and furfural residue swelled in 0.75 mL of DMSO-d_6 according to the method

described.[22-23] 2D HSQC NMR spectra were acquired at 25 ºC on a Bruker AVANCE III 500 MHz spectrometer fitted with a cryogenically cooled 5 mm TCI gradient probe with inverse geometry (proton coils closest to the sample) equipped with a cryogenically-cooled z-gradient triple-resonance probe at the NMR facilities of the General Research Services of the University of Seville. 2D ^{13}C–^{1}H correlation spectra were acquired out using an adiabatic HSQC pulse program (Bruker standard pulse sequence 'hsqcetgpsisp2.2') under the following conditions: spectra were acquired from 10 to 0 ppm in F2 (^{1}H) using 1000 data points, acquisition time of 140 ms, and an interscan delay of 1 s, and from 165 to 0 ppm in F1 (^{13}C) using 256 increments of 32 scans with a total acquisition time of 2 h 40 min. The $^{1}J_{CH}$ used was 145 Hz. Processing used typical matched Gaussian apodization in ^{1}H and a squared cosine bell in ^{13}C. The central solvent peak was used as an internal reference (δ_C/δ_H 39.5/2.49). 2D NMR cross-signals were assigned according to the literature.[22-24]

^{31}P NMR. The hydroxyl groups in lignin were quantitated by ^{31}P NMR. A mixture of pyridine and chloroform-d_6 (1.6:1, v/v) was prepared (Solution 1), protected from moisture with molecular sieve (3A), and kept in a sealed container under nitrogen. Solution 2 was prepared by dissolving chromium (III) acetylacetonate (5.0 mg mL^{-1}) and cyclohexanol (10.85 mg mL^{-1}) in Solution 1. Chromium (III) acetylacetonate and cyclohexanol serve as a relaxation agent and internal standard, respectively. Samples (30 mg) of lignin were dissolved in 0.5 mL of Solution 1. An aliquot (100 µL) of the resulting mixture was mixed with 100 µL TMDP for 5 min, diluted to 1 mL with Solution 1 and thoroughly mixed.^{31}P-NMR spectra were obtained using a, XL-300 spectrometer (Varian, US). Each ^{31}P-NMR acquisition was performed with a 25 s delays between 90° pulses and 1000 scans. An inverse gated decoupling pulse sequence was used to obtain quantitative spectra. Spectra were acquired at 24 °C. The concentrations of the different hydroxyl groups were calculated based upon the intensity of the internal standard (cyclohexanol, 144.5 – 144.0 ppm).

Py-GC/MS. Py-GC/MS was used to further characterize the chemical composition of the lignin in the solid residues. Pyrolysis of samples (bagasse, furfural residue, and lignin) (ca. 0.1 mg) was performed at 500 ºC in an EGA/PY-3030D micro-furnace pyrolyzer (Frontier Laboratories Ltd., Fukushima, Japan) connected to a GC 7820A (Agilent Technologies, Inc., Santa Clara, CA) and an Agilent 5975 mass-selective detector (EI at 70 eV). The column used was a 30 m x 0.25 mm i.d., 0.25 μm film thickness, DB-1701 (J&W Scientific, Folsom, CA). The oven was heated to 50 °C and held at that temperature for 1 min before increasing the temperature to 100 °C at 20 °C min^{-1}. Oven temperature was then increased to 280 °C at 6 °C min^{-1} and then held at this temperature for 5 min. Helium, at a flow rate of 1 mL min^{-1}, was the carrier gas. Released compounds were identified by comparison of their mass spectra with those contained within the Wiley and NIST libraries, those reported in the literature[25-26] and, whenever possible, the retention times and mass spectra of standards. Molar peak areas for each lignin degradation product released were calculated, the summed areas were normalized, and the data for two replicates were averaged and expressed as percentages.

RESULTS AND DISCUSSION

Compositional analysis. The elemental, empirical, and C_9 formulae of bagasse, commercial furfural residue, and extracted lignins are summarized in Table S1. The C_9 formula of the lignin was calculated as a hypothetical hydroxyphenyl structural unit (*i.e.*, representing the six carbon atoms in a benzene ring and an additional three carbon atoms in a propyl side-chain).[18] The chemical composition of bagasse, commercial furfural residue, and extracted lignins are summarized in Table 1. The results show that commercial conversion of sugarcane bagasse into furfural produces a residue with substantially less hydrogen and oxygen than the substrate, as a majority of the hemicelluloses were converted to furfural.

NaOH extraction of the lignin from the furfural residue results in the production of solids with reduced oxygen content compared to the source material. Treatment 1 (145 °C, 0.35 M NaOH) produces a lignin-rich residue with less carbon and oxygen than the original furfural residue. On the other hand, Treatments 2 (100 °C, 0.35 M NaOH) and 3 (100 °C, 0.175 M NaOH) both increased the hydrogen content in the residue compared to the original furfural residue.

The lignin recovered from the furfural residue by Treatment 3 as well as the soda lignin contain lower glucan contents compared to the other recovered lignin samples. This might explain the lower proportion of oxygen obtained from elemental analysis. Slightly higher proportion of acid insoluble lignin was detected in soda lignin. This is because the soda lignin was not subjected to the harsher conditions used to isolate the lignins from the furfural residue, nor did it undergo an acid pretreatment used to generate the furfural residue. As a consequence, the soda lignin did not break down to smaller molecular weight fragments. The molecular weight of the lignin samples was in the following order Treatment 1 (M_w = 9692; M_n = 4625) > Treatment 2 (M_w = 6990; M_n = 3957) > Treatment 3 (M_w = 6746; M_n = 3884). Soda lignin

has an M_w value of 7019 and M_n of 3812. The harshest alkaline treatment condition produced the highest molecular weight lignin.

FTIR-ATR spectroscopy. In order to understand the changes in the functional groups after furfural production, FTIR spectra (Figure 1) of the raw bagasse and furfural residue were obtained. The peak assignments were conducted according to literature and Table S2 summarizes the main functional groups found in the raw bagasse and furfural residue.[27-29] The band at 1627 cm^{-1} which may be due to water absorbance [27] was not detected in the furfural residue. The bands at 1372 and 1317 cm^{-1} were only found in bagasse and are related to the bending of O–H bonding in polysaccharides, including cellulose and hemicellulose. [27, 30-31] The intensity of 1236 cm^{-1} band which is related to C–O stretching of acetyl ester units in hemicellulose [27 32-33] was decreased as expected in the furfural residue. The band detected at 896 cm^{-1} is assigned to C_1 group frequency, representative of β-glucosidic linkage between the xylopyranose unites in the xylan chains.[27, 32] The band was not detected in the furfural residue because of the removal of hemicellulose by the acid-cata;yzed processremoval.

The functional groups in the lignin extracted from the furfural residue and soda lignin were also analyzed by ATR-FTIR (Figure 2). The position and intensity of the peaks in the spectra obtained from these lignins are similar. A comparison between the soda lignin and the extracted lignins indicated that the CH_2 and CH_3 stretching peaks have the highest intensities in soda lignin and therefore the highest proportion of total CH groups.

The peak at 1695 cm^{-1} is attributed to unconjugated ketones or carboxyl group stretching.[34-35] The intensity of this peak in the lignin isolated from furfural residue is higher than soda lignin. This might be due to oxidation and degradation of lignin.[36] The peak values at 1601 cm^{-1} and 1514 cm^{-1}, combined with the intensity of the peaks at 1460 cm^{-1} and 1425 cm^{-1}, are attributed to the characteristic vibrations of aromatic structures and C–H deformation of the lignin, respectively.[37-39] A reduction in the intensity of these peaks in furfural residue lignin

indicates reduced aromatic ring abundance relative to that of lignin obtained after soda pulping of sugarcane bagasse.[38] The appearance of the bands at 1326 cm^{-1} and 1261 cm^{-1} are attributed to syringyl ring breathing with C–O stretching and guaiacyl ring breathing with C=O stretching, respectively.[40-41] These syringyl group peaks are more intense in soda lignin than furfural residue lignin. This appeared only as a peak shoulder in the furfural residue extracted lignin, indicating a higher content of syringyl group in the soda lignin. The band at 1220 cm^{-1} in soda lignin is related to ring breathing with C–O stretching of both syringyl and guaiacyl structure.[41-42] This band shifted to a lower wavenumber (1210 cm^{-1}) suggesting greater hydrogen bond interactions in the furfural residue lignins in comparison to soda lignin.[43] The peak related to the CH=CH bending was only observed in soda lignin spectrum at 983 cm^{-1}.[44] The intensity of the 832 cm^{-1} peak in soda lignin is much higher in comparison with the lignin isolated from furfural residues. This indicates that the soda lignin contains more guaiacyl units.

2D-HSQC-NMR spectroscopy. The bagasse and furfural residue samples were analyzed by 2D-NMR-HSQC at the gel-state according to the methods previously published [22-23] (Figure 3). The main lignin and carbohydrate correlation signals assigned in the HSQC spectra are listed in Table S3 and the main substructures found are also depicted in Figure 3. The assignments of the peaks are based on previous publications.[18, 24, 41]

The HSQC spectrum of bagasse indicates that the most important hemicellulosic carbohydrate in bagasse is xylan. The main carbohydrate signals in the spectra of bagasse corresponded to C_2/H_2, C_3/H_3, C_4/H_4, and C_5/H_5 correlations of xylans (X_2, X_3, X_4, X_5). Signals from *O*-acetylated xylans (3-*O*-acetyl-β-D-xylopyranoside, X'_3, and 2-*O*-acetyl-β-D-xylopyranoside, X'_2) were also observed in this spectrum, confirming that xylans in bagasse are partially acetylated at C-2 and C-3. Other carbohydrate signals included the C_1/H_1 correlations for the anomeric carbons of β-D-xylopyranoside (X_1) and 2- and 3-*O*-acetyl-β-D-

xylopyranosides (X'_1), together with signals from the hemicellulosic glucans (1→3)- and (1→6)-D-glucopyranosides ($Gl_{1(hem)}$). It is important to note that crystalline cellulose is practically invisible in the NMR spectra due to its reduced mobility. The carbohydrate signals observed in the HSQC spectrum of the furfural residue are completely different to those observed in the spectrum of bagasse. Interestingly, all the signals from xylans (X_1, X_2, X_3, X_4, X_5), including those from acetylated xylans (X'_1, X'_2 and X'_3), and which were present in important amounts in the spectrum of bagasse, are now completely absent in the HSQC spectrum of the furfural residue as a result of the hydrolysis of xylans during the furfural production process. The only carbohydrate signals that appear in the spectrum of furfural residue correspond to depolymerized cellulose. The presence of signals for cellulose (G_I), and particularly those signals for the reducing ($G_{I(R)}$) and non-reducing ($G_{I(NR)}$) ends, indicates that cellulose has been largely modified and depolymerized during the furfural production process. These data correlated well with the FTIR analysis.

Lignin signals were also observed in the HSQC spectra of the bagasse and furfural residue samples. The main cross-signals in the aromatic regions of the HSQC spectra corresponded to the aromatic rings of the different lignin units (*p*-hydroxyphenyl, H, guaiacyl, G and syringyl, S) and to the *p*-hydroxycinnamates (ferulates, FA and *p*-coumarates, PCA). In the HSQC spectrum of bagasse, the signals for H-lignin units were barely observed. However, the intensity of the PCA signals is lower in the HSQC spectrum of furfural residue, indicating a partial removal and/or modification of PCA moieties during the furfural manufacturing process. Interestingly, the most important feature in the spectrum of the furfural residue was the presence of strong signals for H-lignin units, indicating a significant enrichment of H-lignin units by acid treatment of bagasse. Similar increase in the proportion of H-lignin units in furfural residues has also been reported by other authors.[45] Lignin inter-unit linkages, such as β–*O*–4' alkyl-aryl ethers (A), were observed in the spectrum of bagasse but were absent in the

spectrum of the furfural residue. In addition, the occurrence of signals in the range δ_C/δ_H 62.7/3.83−4.30 (partially overlapped with carbohydrate signals) from the C_γ/H_γ correlations of γ-acylated units (A′), were evident in the bagasse, and revealed that this lignin is extensively acylated at the γ-OH of the lignin side-chain, mostly with *p*-coumarates, as already published.[24] These signals, however, were absent in the spectrum of the furfural residue, probably as a result of the partial hydrolysis and/or removal of *p*-coumarates.

The 2D-HSQC-NMR spectra of the lignins extracted from the furfural residue are shown in Figure 4. Cross-signals of methoxyl group and β–*O*–4′ substructures are present in all lignins, in agreement with the FTIR data. The C_γ/H_γ correlation signal of β–*O*–4′ substructures (A) were detected in all three lignins extracted from the furfural residue; however, the signal for C_α/H_α correlations of β–*O*–4′ structures were only observed in the lignins isolated from furfural residue by Treatments 2 and 3, which are in agreement with the lowest severity of these extractions. Soda lignin also showed these signals.[18] Carbohydrate signals were barely discerned in these spectra. The main cross-signals observed in the aromatic region are assigned to H, G and S lignin units, as well as to PCA (Figure 4). A weak signal was also observed in all lignins at δ_C/δ_H 106.2/7.23 for the $C_{2,6}/H_{2,6}$ correlations of α-oxidized S-units (S′), which indicate that these lignins were not oxidised significantly. The strong signal at δ_C/δ_H 114.9/6.77 corresponds mostly to the $C_{3,5}/H_{3,5}$ correlations of H-lignin units (together with the C_5/H_5 correlations of G units and the $C_{3,5}/H_{3,5}$ correlations of PCA). A strong signal for $C_{2,6}/H_{2,6}$ correlations of H-units were observed at δ_C/δ_H 127.9/7.19 in all lignins, including soda lignin, and indicates the enrichment in H-lignin units in the lignins extracted from the furfural residue. We could think on two mechanisms leading to the formation of high levels of H-lignin units. A first mechanism could be the demethoxylation of S- and G-units due to the harsh chemical conditions used for furfural production. It has been reported that pyrolysis of lignin at high temperatures result in the demethoxylation of S- and G-units increasing the H-unit content.[46]

Another reasonable explanation could involve condensation reactions of the free *p*-coumaric acids released during the process with the core lignin network through C-C bonds, leading to 'H-type' structures. Additional studies, however, are still needed to confirm the definitive mechanism.

^{31}P NMR. The results of quantitative NMR analysis of TMDP phosphitylated lignin samples are summarized in Table 2 (Figure S1). The hydroxyl groups (particularly the aliphatic OH groups, δ at 150.0 – 145.5) content are significantly lower in the extracted lignins compared to soda lignin. The lignin obtained with Treatment 1 has the lowest proportion of the total OH groups, an indication of the harsher conditions used in the extraction process. Carboxylic acid OH signal was the dominant OH group in the lignins, more so with the lignins obtained from Treatments 2 and 3. The OH signal for carboxylic acid may have originated from lignin oxidation during furfural production or from the fatty acid impurity component of the lignin.[47] As a consequence of the very low proportion of total OH groups in these lignins (unlike soda lignin) they will not be readily amenable to derivatization or as suitable reinforcement materials in composite materials[19].

Py-GC/MS. The bagasse, furfural residue and the isolated lignins from the furfural residue were analyzed by pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC/MS). The pyrograms of the bagasse and furfural residue as well as the lignin isolated from a representative treatment (Treatment 2) are shown in Figure S2. The identities and relative molar abundances of the lignin-derived compounds released are listed in Table 3. The identities of the carbohydrate-derived compounds are detailed in the legend of Figure S2.

Pyrolysis of bagasse (Figure S2A) and furfural residue (Figure S2B) released compounds derived from carbohydrate as well as phenolic compounds released from lignin moieties and

p- hydroxycinnamates (PCA and FA). In contrast, pyrolysis of lignin samples only released phenolic compounds derived from lignin and *p*- hydroxycinnamates, with the absence of carbohydrate compounds.

Most of the carbohydrate-derived compounds cannot be used as diagnostic compounds as they can derive from both the cellulose and the hemicelluloses, such as (3*H*)-furan-2-one (a), (2*H*)-furan-3-one (**b**), furfural (**c**), 2,3-dihydro-5-methylfuran-2-one (**g**), 5-methyl-2-furfuraldehyde (**h**) and 2-hydroxy-3-methyl-2-cyclopenten-1-one (**k**). However, other some compounds are diagnostic for the occurrence of cellulose, such as 2-hydroxymethylfuran (d), 2-acetylfuran (e), cyclopenten-1-ene-3,4-dione (f), 5-hydroxymethylfurfural (o), 1,4-dideoxy-D-glycerohex-1-enopyranos-3-ulose (**p**) and levoglucosan (q), whereas other compounds are characteristic of the hemicelluloses, particularly 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one (j).[26]. Hence, the pyrogram of bagasse showed compounds derived from both cellulose and hemicelluloses, whereas the pyrogram of furfural residue only shows compounds derived from cellulose., as the diagnostic compound 4-hydroxy- 5,6-dihydro-(2*H*)-pyran-2-one (j), which was present in significant amounts in the pyrogram of bagasse, was absent in the pyrogram of the furfural residue indicating the removal of the hemicelluloses and the enrichment in cellulose in the furfural residue.

Among the lignin- and *p*-hydroxycinnamate-derived compounds, the pyrograms showed compounds derived from the H, G and S lignin units, such as phenol (1), guaiacol (2), 4-methylphenol (4), 4-methylguaiacol (5), 4-ethylphenol (6), 4-ethylguaiacol (7), 4-vinylphenol (8), 4-vinylguaiacol (9), syringol (13), 4-methylsyringol (18), vanillin (19), 4-vinylsyringol (24), and acetosyringone (32), among others. The relative abundances of the lignin-phenolic compounds, together with relative abundance of the H, G and S-lignin units and the S/G ratios, are shown in Table 3. High amounts of 4-vinylphenol (8) (and to a lesser extent 4-vinylguaiacol, 9) were released from all these samples, being particularly abundant in the case

of bagasse (45.1% of all phenolic compounds released), and which are responsible of the large percentage of "H-type units" observed in these samples (up to 48.9% in bagasse and 58.9% in furfural residue). However, it is important to note that 4-vinylphenol (8) mostly arise from PCA moieties after decarboxylation upon pyrolysis (and 4-vinylguaiacol, 9, is similarly produced from FA after decarboxylation), as usually happens in the pyrolysis of grasses.[24, 48-50] Therefore, these vinyl compounds cannot be used to calculate the lignin H:G:S composition by Py-GC/MS since they are mostly derived from *p*-hydroxycinnamates and not from core lignin structural units. A more accurate estimation of the H, G and S-lignin composition of the samples was calculated without taking into account 4-vinylphenol (8) and 4-vinylguaiacol (9), and the respective 4-vinylsyringol (25), as shown in Table 3.

The Py-GC/MS analysis revealed important differences in the lignin composition among the different samples (Table 1). Although, bagasse and furfural residue seem to contain the highest amounts of "H-type units" (around 49–59%); however, after the correction by ignoring the vinyl compounds, it is interesting to note the much lower abundance of H-units in bagasse (11.1%) which confirms that most of the 4-vinylphenol released from this sample does not arise from actual H-units but rather from PCA. However, the furfural residue still contains high amounts of H-units (44.4%) after the correction, clearly indicating an enrichment of H-lignin units in this residue (as has been confirmed by 2D-NMR).

Pyrolysis of the lignins isolated upon the different treatments also released phenolic compounds derived from lignin units and associated *p*-hydroxycinnamates, with the complete absence of compounds derived from carbohydrates (Figure 2S, Table3). The pyrograms showed compounds derived from H, G and S lignin units. Minor amounts of the 3-methoxycatechol (10) were also present in lignin isolated from Treatments 1 and 3 while it was almost 3.5 time more in the lignin from Treatment 2. The Py-GC/MS analysis indicated that

all three lignins have rather similar composition, with S/G ratios around 1.1, and high proportions of "H-units" (around 36–37%).

It is worth noting the high proportions of phenol (1) and other compounds derived from H-type units, in the furfural residue and its extracted lignins, in comparison with bagasse (Table 3), and which are associated with the high amounts of 'H-type' units observed by HSQC. Previous work have shown that the bagasse lignins derived from the cellulosic ethanol production do not have such high phenol content in the pyrograms,[51] a confirmation that the different reactions taken place during the different industrial chemical processings play an important role in the products derived from thermochemical processes.

As said above, demethoxylation of S- and G-lignin units and/or condensation reactions of *p*-coumaric acid with the core lignin network could be plausible mechanisms for the formation of 'H-type' units, which in turn will be responsible for the formation of the high levels of phenol released under Py-GC/MS. [46, 52]

Finally, it is important to note that the potential applications of the lignin obtained from furfural residue production can be considered based on its chemical characteristics. Lignins with high amounts of H-units could be of interest for the synthesis of products that need *ortho* positions of phenyl rings unblocked by methoxyl groups. The lignin from the furfural residue, with high content of H-units, would provide sufficient reactivity for the synthesis of phenolic resins.[53-54 55]

CONCLUSIONS

This study has provided insightful information on the composition and structural characteristics of the furfural bagasse residue and its lignin component which will assist in valorization studies. Acid hydrolysis conditions used in the conversion of bagasse to furfural favored the increased proportion of H-lignin units in the residue, as seen by 2D-NMR and Py-GC/MS. The alkaline conditions (particularly temperature) used to extract lignin from the

furfural residue has some effects, though not significant, on the lignin structure, lignin inter-unit linkages, and the degree of condensation of the lignin. The high levels of 'H-type' units present in these lignins make them suitable for selected applications, such as the synthesis of phenolic resins.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

Figure Legends

Figure 1. FTIR spectra of (a) bagasse, and (b) furfural solid residue

Figure 2. FTIR spectra of isolated lignins

Figure 3. 2D HSQC NMR spectra of (A) bagasse, and (B) furfural residue. The main lignin and carbohydrate structures identified are also depicted, A: β–*O*–4′ structures; A': β–*O*–4′ structures with *p*-coumaroylated γ-OH; PCA: *p*-coumarates; FA: ferulates; H: *p*-hydroxyphenyl units; G: guaiacyl units; S: syringyl units; S′: C_α-oxidized syringyl units; X: xylans; X': C-2 and/or C-3 acetylated xylans; $G_{l(NR)}$: non-reducing end units in cellulose; $G_{l(I)}$: internal units in cellulose; $G_{l(R)}$: reducing end units in cellulose. See Table S3 for signal assignments.

Figure 4. 2D-HSQC-NMR spectra of the lignins extracted from furfural residue upon (A) Treatment 1 – 145 °C, 0.35 M NaOH; (B) Treatment 2 - 100 °C, 0.35 M NaOH; and (C) Treatment 3 - 100 °C, 0.175 M NaOH.

TABLES

Table 1. Chemical Composition of the Original Furfural Residue and Extracted Lignin

sample	Component (%)				
	glucan	xylan	acid insoluble lignin	acid soluble lignin	ash
bagasse	43.0	17.4	16.8	4.7	9.4
furfural residue	35.0	2.0	39.8	1.0	11.7
Treatment 1	2.4	nd[1]	80.2	3.9	
Treatment 2	2.3	nd	79.4	3.5	
Treatment 3	0.7	nd	81.4	3.3	
soda lignin	0.5	1.3	89.7	4.0	0.6

[1] not determined

Table 2. Hydroxyl Content (mM) in Residues from Soda Pretreatment and Furfural Residue Treatments 1, 2, and 3 by ^{31}PNMR

assignment	δ 31P-NMR	soda	furfural residue treatment		
			1	2	3
aliphatic OH	150.0 – 145.5	2.336	0.003	0.001	0.001
condensed phenolic OH (4-O-5)	142.8 – 141.7	0.409	nd[1]	0.001	0.001
condensed phenolic OH (5-5')	141.7 – 140.2	0.110	nd	nd	nd
guaiacyl OH	140.2 – 139.0	0.039	nd	nd	nd
catechol OH	139.0 – 138.2	0.312	nd	0.004	0.0004
p-Hydroxylic phenyl	137.6 – 136.8	0.554	0.0004	0.007	0.0057
carboxylic acid OH	134.5 – 133.7	0.512	0.0025	0.017	0.015
total OH	-	4.272	0.0056	0.03	0.0231

[1] not detected

Table 3. Identification and Relative Molar Abundance (%) of the Compounds Released upon Py-GC/MS of Lignin Samples

Label	Compound	Bagasse	Furfural residue	Lignin treatments		
				1	2	2
1	phenol	1.3	12.8	10.8	11.8	11.1
2	guaiacol	3.2	7.6	9.4	10.4	10.3
3	3-methylphenol	0.3	1.4	1.3	1.5	1.2
4	4-methylphenol	0.8	6.1	6.7	7.9	6.7
5	4-methylguaiacol	2.3	2.3	6.3	7.0	6.4
6	4-ethylphenol	0.8	5.6	5.7	5.7	6.1
7	4-ethylguaiacol	0.7	1.2	2.6	2.6	2.8
8	4-vinylphenol	45.1	31.4	20.2	13.5	17.7
9	4-vinylguaiacol	17.0	5.1	4.8	3.9	4.8
10	3-methoxycatecol	0.0	0.0	0.4	1.7	0.5
11	eugenol	0.6	0.1	0.3	0.3	0.2
12	4-allylphenol	0.1	0.3	0.2	0.2	0.2
13	syringol	6.2	8.5	10.7	10.8	11.3
14	*cis*-propenylphenol	0.0	0.1	0.2	0.1	0.1
15	*cis*-isoeugenol	0.4	0.2	0.2	0.4	0.2
16	*trans*-4-propenylphenol	0.3	0.4	0.6	0.6	0.7
17	*trans*-isoeugenol	1.9	0.3	0.8	0.6	0.7
18	4-methylsyringol	2.5	1.7	5.5	5.3	5.7
19	vanillin	2.7	2.4	1.3	1.5	1.1
20	4-ethylsyringol	0.6	0.7	1.5	1.3	1.6
21	vanillic acid methyl ester	0.1	0.1	0.2	0.2	0.2
22	acetovanillone	0.7	0.7	1.0	1.2	0.9
23	4-hydroxybenzaldehyde	0.2	0.5	0.8	0.8	0.6
24	4-vinylsyringol	3.9	1.7	1.9	1.5	1.9
25	guaiacylacetone	0.3	0.3	0.3	0.5	0.3
26	4-allylsyringol	0.6	0.2	0.2	0.1	0.2
27	4-hydroxyacetophenone	0.0	0.3	0.7	0.9	0.6
28	*cis*-4-propenylsyringol	0.4	0.3	0.2	0.2	0.3
29	*trans*-4-propenylsyringol	2.4	0.9	0.8	0.5	0.9
30	syringaldehyde	1.5	2.3	1.4	1.5	1.2
31	syringic acid methyl ester	0.1	0.0	0.2	0.3	0.2
32	acetosyringone	1.3	0.8	1.7	2.9	1.5
33	syringylacetone	1.1	1.5	0.3	0.8	0.5
34	propiosyringone	0.2	0.2	0.1	0.2	0.3
35	syringyl vinyl ketone	0.3	2.0	0.1	0.5	0.3
36	syringic acid	0.0	0.0	0.6	0.8	0.5
	%H[a]=	48.9	58.9	47.2	43.0	45.0
	%G[a]=	30.0	20.4	27.0	28.5	28.1
	%S[a]=	21.2	20.8	25.3	26.8	26.4
	S/G[a]=	0.7	1.0	0.9	0.9	0.9
	%H[b]=	11.1	44.4	36.9	36.4	36.1
	%G[b]=	38.1	24.7	30.5	30.3	30.8
	%S[b]=	50.8	30.9	32.0	31.2	32.4
	S/G[b]=	1.3	1.3	1.1	1.1	1.1

[a]Calculated by using all the H, G and S-lignin derived products. [b]Estimated by ignoring 4-vinylphenol (peak 8; mostly arising from *p*-coumarates) and 4-vinylguaiacol (peak 9; which also arises from ferulates), and the analogous 4-vinylsyringol (peak 24).

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