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Mechanochemical Treatment Facilitates Two-Step Oxidative Depolymerization of Kraft Lignin

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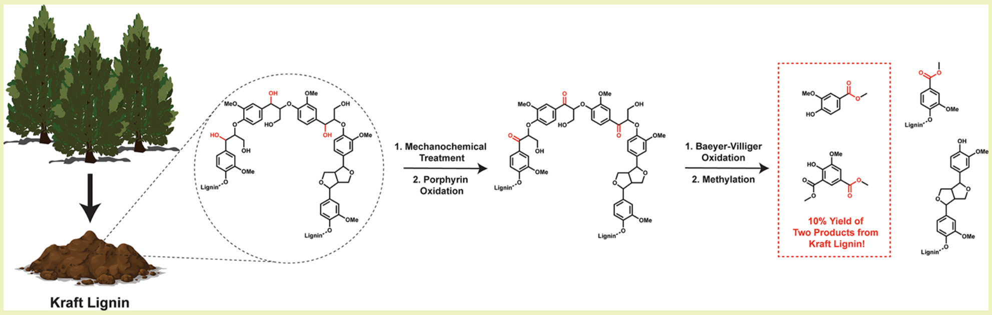
Supporting Information



ABSTRACT: Mechanochemical treatment of Indulin AT kraft lignin by ball-milling with KOH and toluene produces significant carbonyl functionality, among other changes. The chemical reactivity of the lignin is increased, resulting in greater lignin degradation from porphyrin oxidation followed by Baeyer−Villiger oxidation. The mechanochemical treatment produces a level of lignin oxidation that is similar to that produced by porphyrin-catalyzed oxidation. Combining mechanochemical treatment with porphyrin oxidation has a synergistic positive effect on the depolymerization of lignin, as demonstrated by a significantly higher yield of monomers. The methyl ester of vanillic acid was obtained as the main monomeric product (after methylation), along with of methyl 5-carbomethoxyvanillate, in a 10% yield, which is among the highest reported from kraft lignin.

KEYWORDS: *Lignin oxidation, HSQC, Lignin depolymerization, Vanillic acid*

INTRODUCTION

Lignin is a natural organic polymer that is characterized by high chemical stability and structural complexity. Owing to its aromatic nature and high energy content (lignin represents about 40% of lignocellulosic biomass by energy content),[1] it has been targeted for years as a renewable source of aromatic platform chemicals[2] and biofuels.[3−6] However, practical usage and application of lignin requires a cost-effective method for depolymerization of the polymer into small molecules, a goal that has been shared for decades by numerous research groups.[2,7−25] Due to the assortment of linkages within the combinatorial polymer, depolymerization as well as separation of the newly cleaved units presents a significant challenge.

The first step in lignin utilization is isolation of the polymer from lignocellulosic biomass. This typically involves pretreatment,[26−34] usually including milling which pulverizes the raw biomass, reducing the particle size to 0.8 mm or less, and helps maximize the amount of extracted lignin.[35] In general, the more extensive the milling, the more lignin can be extracted from the biomass.[36] However, mechanical treatment of biomass is known to alter the lignin. This includes a decrease in molecular weight[37] and polydispersity,[38] as well as an increase in the carbonyl functionality,[37] and cleavage of aryl ether (β−O−4) linkages,[39−42] with an accompanying increase in phenolic−OH content.[38] When applied in the presence of alkali (e.g., NaOH, KOH), mechanical treatment facilitates depolymerization of lignin into oligomers and monomers,[43] as well as depolymerization of cellulose and hemicelluloses into monomeric carbohydrates.[42] Mechanical treatment also makes cellulose more accessible to enzymatic hydrolysis and fermentation, due to decreased particle size and a loosening of the cellulosic fibrillar structure.[44−47] However, due to the associated cost arising from the high energy requirement in reducing particle size,[44,47] milling is not recommended for use as a stand-alone pretreatment approach, but can be combined with other methods including chemical and physicochemical procedures.[47]

In this work, we apply a mechanochemical method as a strategy to promote lignin depolymerization. Herein we present evidence on how mechanochemical treatment can improve the reactivity of lignin, as manifested in the isolation of aromatic

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compounds. We applied mechanochemical treatment to lignin by ball-milling in the presence of potassium hydroxide and toluene, and monitored changes in the structure of the lignin using IR and HSQC NMR spectroscopies. Finally, we determined how the changes in lignin structure and functionality that are brought about by the mechanochemical treatment affected the success of an oxidative approach to depolymerization. We used a two-step oxidation in which benzylic alcohols present in the lignin structure were oxidized to carbonyls via porphyrin oxidation, and the resulting products were then subjected to Baeyer−Villiger oxidation. The second oxidation step produces esters that can be hydrolyzed, resulting in cleavage of linkages between lignin monomeric units.

RESULTS AND DISCUSSION

Mechanochemical Treatment of Indulin AT Kraft Lignin. Mechanochemistry typically involves solvent-free reactions in the solid state and is often performed in ball-milling equipment, where reactant mixtures can be efficiently mixed and subjected to significant energy input.[42] In this study, Indulin AT kraft lignin (KL) was ball-milled with KOH (lignin/KOH = 2:1 w/w) and toluene (enough to cover the steel ball bearing) at a rate of 25 Hz. It was found that toluene helped with vibratory milling by preventing the material from adhering around the steel ball bearing. After 1−2 days, IR spectra (intensities normalized to a lauronitrile internal standard) showed significant enhancement of the band at 1715 cm^{-1}, indicating an increase in the carbonyl functionality (Figure 1).

Two-Step Oxidative Lignin Depolymerization. Taking advantage of the observed enhancement of the carbonyl functionality upon mechanochemical treatment, we applied a two-step oxidative approach to lignin depolymerization of ball-milled kraft lignin (KLBM) in parallel with the same reactions on untreated KL. The method consists of initial oxidation of the benzylic hydroxyl groups to carbonyl groups using a TPPFeCl/*t*-BuOOH catalyst system. This is followed by Baeyer−Villiger (BV) oxidation of ketones to esters with HCOOH/H_2O_2, followed by *in situ* hydrolysis of the esters to carboxylic acids and phenols (Scheme 1).[48] Oxidation of the primary alcohol group at C_γ was not observed when the TPPFeCl/*t*-BuOOH catalyst system was applied to β-O-4 model compounds.[48]

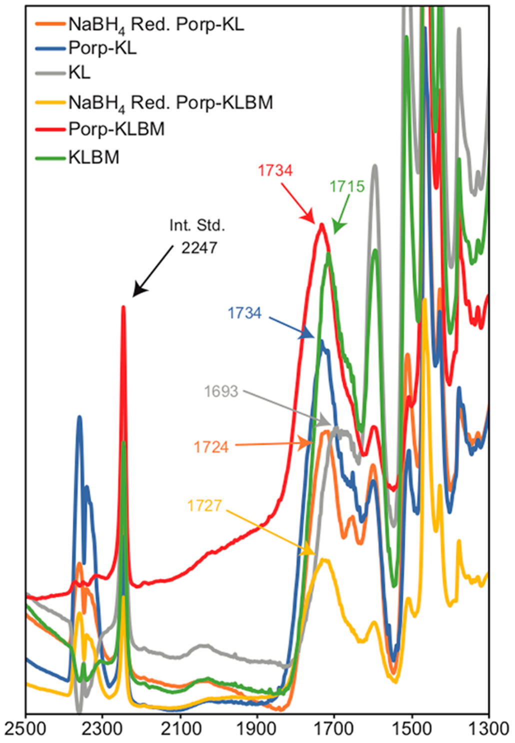
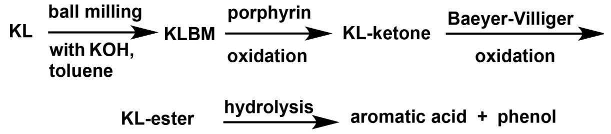


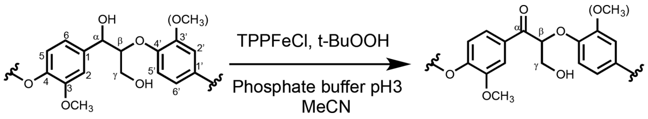
Figure 1. IR spectra of kraft lignin (KL, gray) and ball-milled KL (KLBM, green), porphyrin oxidized KL (Porp-KL, blue) and porphyrin oxidized KLBM (Porp-KLBM, red), $NaBH_4$ reduction products of Porp-KL ($NaBH_4$ Red. Porp-KLBM, orange) and Porp-KLBM ($NaBH_4$ Red. Porp-KLBM, yellow).

Scheme 1. Reaction Sequence for Two-Step Oxidative Lignin Depolymerization



Porphyrin Oxidation of KL and KLBM. In order to determine how the mechanochemical treatment affects the first oxidation step, KLBM (treated for 2 days) was oxidized with the TPPFeCl/*t*-BuOOH catalyst system in parallel with oxidation of KL (untreated). This was done by stirring TPPFeCl, *t*-BuOOH (70% aqueous), phosphate buffer (pH 3), and MeCN for 26 h at room temperature (Scheme 2).[48]

Scheme 2. Porphyrin Oxidation, The First Oxidation Step Toward Lignin Depolymerization, Converts Benzylic (C_α) Hydroxyl Groups to Ketones



We followed the progress of porphyrin oxidation by measuring IR spectra at several time intervals. After 26 h of stirring, we observed that the intensity of the carbonyl spectral band for KL (1693 cm^{-1}) had significantly increased and shifted to 1734 cm^{-1}, whereas that for KLBM (1734 cm^{-1}) increased only slightly (Figure 1). We then performed HSQC NMR experiments to examine changes in the lignin after mechanochemical treatment and after subsequent porphyrin oxidation. The resulting spectra are divided into aliphatic (Figure 2) and aromatic (Figure 3) regions. The aliphatic region is where lignin units with their characteristic interunit linkages are typically profiled, whereas the aromatic region provides insight into possible oxidation of the α-carbon, the effect of which is observed on the aromatic ring.

Frame I in Figure 2 shows the HSQC spectrum for KL, where units characterized by linkages including β−O−4 (**A**), β−5 (**B**), and β−β (**C**) are visible. Similar units are also evident in KLBM (Frame II). After porphyrin oxidation (Frames III and IV), the cross-peaks representing the aforementioned units were of noticeably lower intensity (in most cases completely absent), suggesting that nearly all of the aliphatic alcohols, or at least all of the benzylic ones, present in KL had been oxidized. Indeed, the absence of a cross-peak representing $\mathbf{A}_\beta$ of the β−O−4 unit suggests that the peak at ca. 4.9/71 (δ_H/δ_C, Frames III and IV of Figure 2) does not represent $\mathbf{A}_\alpha$ but rather some

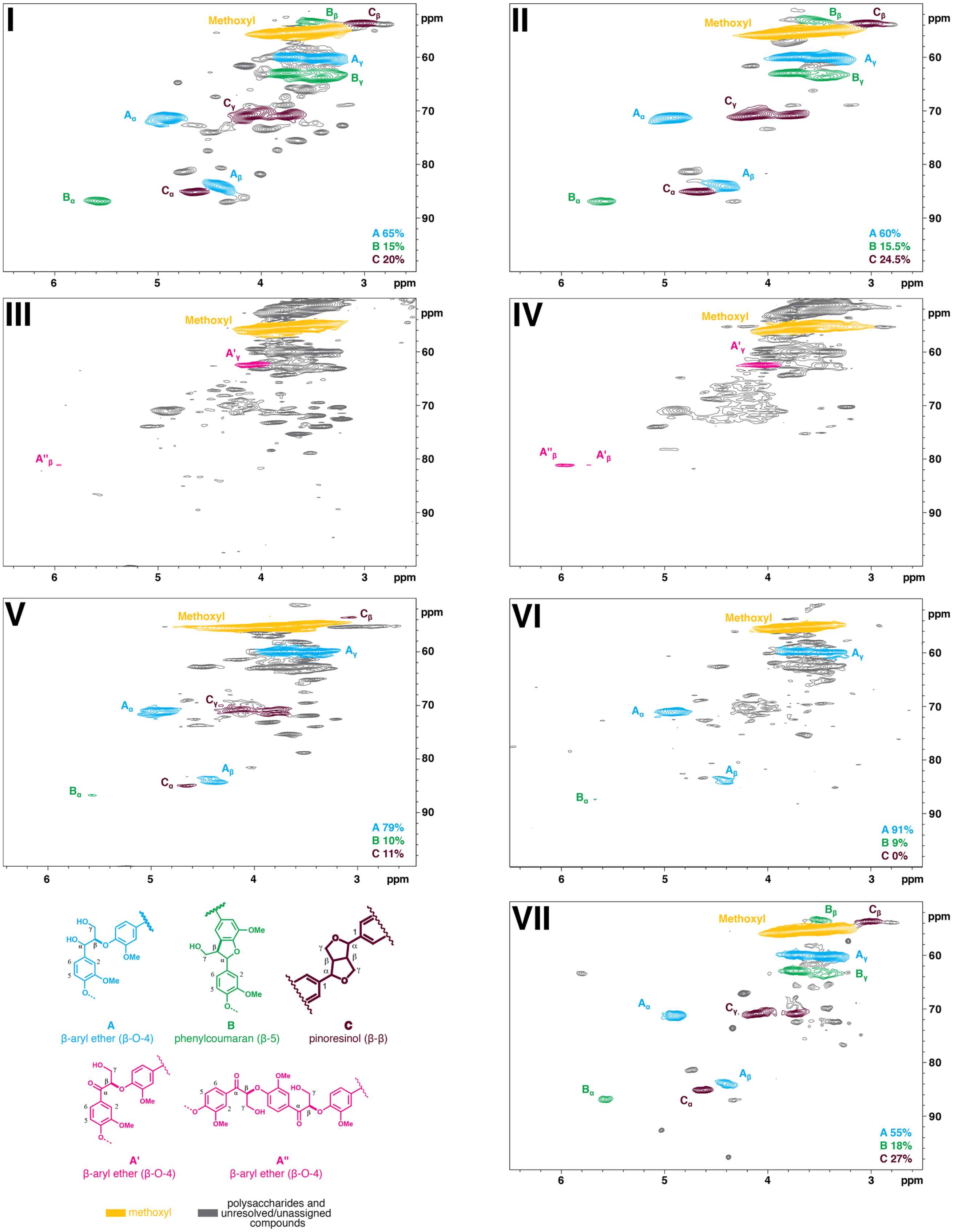


Figure 2. HSQC spectra of lignin samples, aliphatic region. Key: (I) KL, (II) KLBM, (III) porphyrin-oxidized KL, (IV) porphyrin-oxidized KLBM, (V) $NaBH_4$-reduced porphyrin-oxidized KL, (VI) $NaBH_4$-reduced porphyrin-oxidized KLBM, and (VII) $NaBH_4$-reduced KL. Relative percent abundance of units **A**, **B**, and **C** were determined via volume integration of their respective α-C/H correlation peak divided by the sum of the three linkages.

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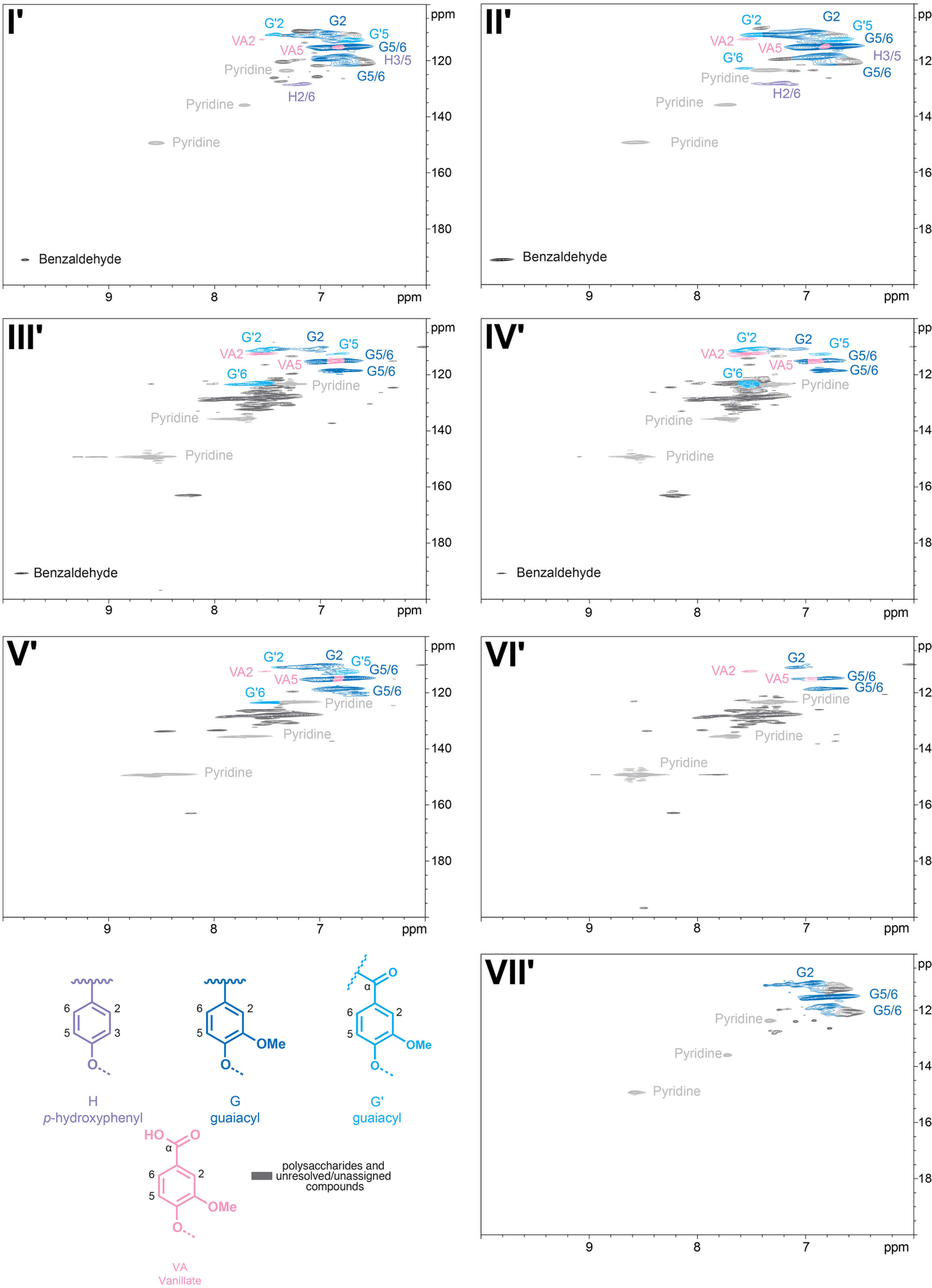


Figure 3. HSQC spectra of lignin samples, aromatic region. Key: (I′) KL, (II′) KLBM, (III′) porphyrin-oxidized KL, (IV′) porphyrin-oxidized KLBM, (V′) NaBH$_4$-reduced porphyrin-oxidized KL, (VI′) NaBH$_4$-reduced porphyrin-oxidized KLBM, and (VII′) NaBH$_4$-reduced KLBM.

unknown structure. Furthermore, COSY of the porphyrin-oxidized sample shows that the peak at 5.9 ppm correlates to the cross-peaks at 4 ppm (Supporting Information), clearly identifying it as a β-C/H correlation. This was further confirmed by HSQC NMR spectroscopy of an authentic lignin model compound (Supporting Information).

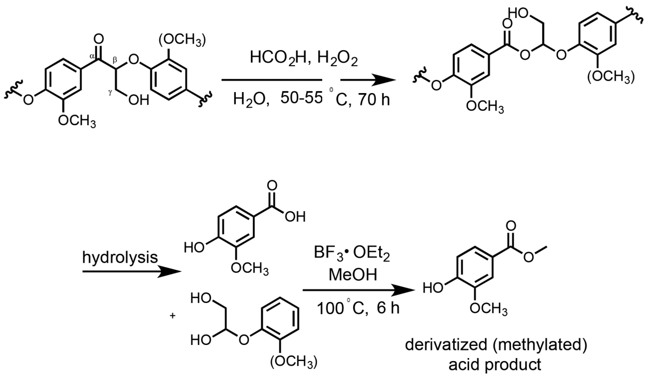
In order to confirm that lignin has indeed been oxidized, we treated both the porphyrin-oxidized KL and KLBM with $NaBH_4$. To our delight, some of the cross-peaks for the normal units reappeared in the HSQC spectra (Figure 2, Frames V and VI). The changes in the cross-peaks in the aliphatic region of the HSQC spectra (before and after porphyrin oxidation, as well as after subsequent treatment with $NaBH_4$), are consistent with oxidation of lignin by the porphyrin catalyst and demonstrates that such oxidation can be reversed by borohydride reduction.

The positions of cross-peaks that appear in the aromatic region of the spectrum (Figure 3) reflect the oxidation state of the α-carbon, and thus give direct evidence of oxidation of that carbon. To illustrate, in Figure 3, cross-peaks associated with the oxidized form of guaiacyl units (G′: G′2, G′5, and especially G′6) are nearly absent in the spectrum of the unmodified KL (Frame I′) but are noticeably present in that of KLBM (Frame II′). This indicates that partial oxidation of KL had occurred during ball-milling. After porphyrin oxidation (Frames III′ and IV′), the cross-peaks corresponding to unoxidized guaiacyl (G) units became smaller whereas those corresponding to oxidized guaiacyl units (G′, especially G′6), became significantly larger, as is consistent with nearly complete oxidation of the α-carbons. Then, after treatment with $NaBH_4$ (Frames V′ and VI′), most of the signals for oxidized guaiacyl (G′) disappeared, indicating that those units had been reduced back to normal guaiacyl (G) units, lending support to the porphyrin oxidation of lignin, and at the same time reaffirming that such oxidation can easily be reversed by borohydride reduction.

The effects of mechanochemical treatment of KL are clearly demonstrated in IR experiments (Figure 1), as well as in the aromatic region of the HSQC NMR spectra, as explained in the preceding paragraph. The HSQC results are also consistent with IR spectra of KL and KLBM after porphyrin oxidation and after sodium borohydride reduction of the resulting porphyrin-oxidized samples (Figure 1). These IR spectra show that the intensity of the carbonyl absorption band at around 1700 cm^{-1} increased significantly after porphyrin oxidation, and then decreased, also significantly, after borohydride reduction.

We then investigated the effect of the initial mechanochemical treatment on the outcome of the Baeyer−Villiger (BV) oxidation step of the two-step lignin oxidation (Scheme 3), to determine whether mechanochemical treatment of the starting lignin is beneficial. As a starting point, we looked at the extent of C_α oxidation of the lignin samples at the beginning of the BV oxidation. As shown in Figure 1, the intensity of the carbonyl absorption band of KL (around 1700 cm^{-1}) is low to start with, those for Porp-KL and Porp-KLBM (1734 cm^{-1}) are much higher. A 50% greater excess of oxidant was used in the oxidation of KL in order to see if the more heavily oxidized KL produced better yields of monomer products than Porp-KLBM, even without mechanochemical treatment.

Scheme 3. Baeyer−Villiger Reaction, the Second Oxidation Step, Further Oxidizes C_α Ketone to an Ester and Cleaves the C_α−C_β Bond Through Hydrolysis of the Resulting Ester



The lignin samples (KL, KLBM, Porp-KL, and Porp-KLBM) were subjected to BV oxidation by heating with formic acid and hydrogen peroxide at 50 °C for 70 h. After oxidation, each reaction mixture was treated with $BF_3 \cdot OEt_2$ and methanol to esterify any carboxylic acids that had been formed, then extracted with water and with ethyl acetate. The water-soluble fractions were back-extracted with ethyl acetate producing four fractions from each BV reaction mixture: material that is only water-soluble, material that is both water and ethyl acetate soluble, material that is only soluble in ethyl acetate, and insoluble material. The amount of material in each fraction is given in Table 1.

Table 1. Fractionation of Baeyer−Villiger Oxidation Mixtures by Solubility

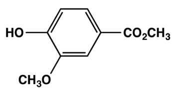
Sample	H_2O (BV-1)	H_2O and EtOAc (BV-2)	EtOAc (BV-3)	Insoluble (BV-4)
KL	31%	20%	0.2%	46%
KLBM	38%	17%	2%	42%
Porp-KL	65%	19%	7%	12%
Porp-KLBM	44%	19%	6%	32%

The amount of the water-soluble fraction (Table 1 column 1, presumably small, polar compounds) obtained from the product mixtures correlates with the degree of C_α oxidation at the start of the BV oxidation. Analysis of the material that was soluble in both water and ethyl acetate (Table 2, column 2) was revealing. From each of these samples we were able to isolate two compounds, one of which was readily identified as methyl vanillate **1**. The other was identified by NMR spectroscopy and X-ray crystallography (see Supporting Information) as methyl 5-carbomethoxyvanillate **2**. Compound **2** is an electrophilic substitution product of **1**. When pure **1** was subjected to Baeyer−Villiger oxidation, a small amount of **2** was formed. The identity of **2** was confirmed by ^{1}H NMR (see Supporting Information) and mass spectroscopy.[49] It is also possible that **2** partly resulted from C5-linked structures in lignin like β−5, or perhaps 5−5. The yields of **1** and **2**, isolated from the reaction mixtures, are shown in Table 2. Due to the solubility of the products in water and ethyl acetate, we presume that the rest of the material in these fractions is likely to be dimeric/trimeric/oligomeric products.[49]

Table 2. Yields of 1 and 2

BV Sample	**1**	**2**
KL	1.5%	0.2%
KLBM	2.2%	0.5%
Porp-KL	2%	0.6%
Porp-KLBM	7.8%	2.2%

Compounds **1** and **2** are methylated derivatives of monomeric products that have previously been identified



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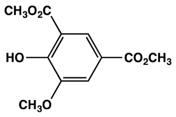

2

from oxidized lignin. Indeed, Villar obtained vanillin and vanillic acid from alkaline oxidation of softwood lignin, as well as syringaldehyde and syringic acid from hardwood lignin.[50] Under acidic oxidation conditions, Werhan detected vanillin and **1** as the major product from an unextracted softwood, along with methyl dehydroabietate and five additional monomers, one of which was believed to be **2**.[49] Assmann[51] adopted Werhan's acidic oxidation procedure for a supercritical extraction study and recovered similar monomer products.[51] Voitl likewise obtained vanillin and **1** from the oxidation of kraft lignin[52,53] whereas Araujo demonstrated that vanillin can be obtained from controlled oxidation of lignin in a batch reactor.[54]

As shown by the results in Tables 1 and 2, all the lignin samples we investigated underwent depolymerization to some extent, including the as-received lignin KL (1.7%). For KLBM and Porp-KL, total yields of **1** and **2** were the same, suggesting that mechanochemical treatment and porphyrin oxidation have more or less the same effect on the efficiency of the two-step lignin depolymerization process. However, the significantly higher total yield of **1** and **2** (10%) obtained from BV oxidation of Porp-KLBM is notable because the yield was nearly quadruple that obtained from BV oxidation Porp-KL and KLBM. These results demonstrate what appears to be a synergistic positive effect of mechanochemical treatment and porphyrin oxidation on the overall lignin depolymerization. In a large and complex molecule like lignin, a high degree of C_α oxidation is not sufficient for the BV reaction to efficiently produce monomeric products. Reduced particle size brought about by mechanochemical treatment, or potentially mechanical shearing of the polymer itself, significantly improved the efficiency of the two-step oxidative lignin depolymerization.

Scalability and Economic Outlook. One of the key challenges with any lignin utilization process is scalability. Addressing the chemical reactions performed in this study, the benzylic oxidation was performed at ambient temperature using a minimal amount of porphyrin catalyst and *t*-BuOOH as the oxidant. Under optimally designed reactor conditions, the solvents and catalyst would be reused, whereas the *t*-BuOOH can be regenerated via dehydration of *t*-BuOH to isobutylene followed by acid-catalyzed oxidation with H_2O_2. Moreover, future endeavors in our lab aim to optimize the amount of *t*-BuOOH used (currently used in excess) and/or replace *t*-BuOOH with a greener oxidant such as hydrogen peroxide or O_2. Baeyer−Villiger oxidation was performed at 50 °C using *in situ* generated performic acid. Consequently, the coproducts of the reaction are water and formic acid, which can be reused. Thus, the terminal oxidant is H_2O_2. Any acid or solvents used during workup can be reused or neutralized to innocuous byproducts (e.g., KCl).

Whereas the chemical processes used here are mild, the mechanical degradation of the lignin is quite energy intensive, due to the extensive ball-milling involved. Though we used a ball-mill due to the scale of the reaction, we envision that future scale-up will involve shatter milling. This should allow for significantly larger batches of biomass to be processed and should considerably decrease the milling time. Although we did not see any obvious toluene oxidation products from our milling process, further scale-up will likely eliminate toluene from the process. Moreover, the KOH used can be neutralized to environmentally benign KCl.

To address the sustainability of the depolymerization process, the *E*-factor (kg waste/kg product)[55] was calculated for each step of the process. It should be noted that the calculation was performed disregarding the water generated and assuming the ability to reuse our catalyst and solvents. Also, because the amount of HCl used to acidify the reaction solution past neutral in the workup steps of milling and porphyrin oxidation was not measured, only the amount of HCl needed to neutralize KOH from milling was used in the calculation. Lastly, the residual/waste lignin was not incorporated in the calculation due to its likely use as fuel for generating process heat/energy. As such, the *E*-factor for the milling process was 0.82, assuming an 81% recovery of lignin. In comparison, the *E*-factor for the porphyrin oxidation process was 8.73, this value being typical of *E*-factors associated with the synthesis of fine chemicals[55] such as vanillic acid. However, the *E*-factor could be substantially decreased by optimizing the amount of *t*-BuOOH used (currently used in excess) and/or by regenerating the *t*-BuOOH as indicated above.

CONCLUSIONS

Mechanochemical treatment in KOH and toluene resulted in an increase in the carbonyl content in Indulin AT kraft lignin, as shown via the absorption band at 1690−1735 cm^{-1} in the IR spectra of KLBM. Treatment of KLBM with $NaBH_4$ caused a decrease in intensity of the carbonyl absorption band, consistent with reduction of the carbonyl functionality, and demonstrating that oxidation of lignin produces structures that can be reduced back to their original forms.

The enhanced carbonyl content, and presumably the reduced particle size of lignin brought about by mechanochemical treatment, or potentially mechanical shearing of the polymer itself, improved the efficiency of two-step oxidative depolymerization, as demonstrated by HSQC NMR experiments and by the isolation of monomeric products. Lignin unit degradation, particularly of β-ether units, was promoted through a first oxidation step using the TPPFeCl/*t*-BuOOH catalyst system, whereas depolymerization to aromatic monomers was achieved via subsequent Baeyer−Villiger oxidation. To the best of our knowledge, a 10% production of two discrete compounds is among the highest reported in the literature on oxidative depolymerization of kraft lignin.[2] Furthermore, the work described here provides a significant starting point for other mechanochemical methods of lignin depolymerization. Further work, involving mechanochemical degradation of less-recalcitrant lignins and characterization of the solubilized lignin fractions via techniques such as GPC, TGA, and ^{31}P NMR, will be subject of future investigations.

EXPERIMENTAL SECTION

Ball-milling was carried out at 25 Hz in a Retsch ball-mill model MM2 equipped with a 1 cm diameter stainless steel ball. Indulin AT kraft lignin was obtained from Ingevity (formerly MeadWestvaco). Indulin AT kraft lignin is a commercial lignin derived from softwood (pine) and has been extensively characterized.[55,56]

FT-IR Spectroscopy. FT-IR spectroscopy was performed on a Nicolet 6700 using KBr pellets. KBr pellets were made with 3.7 ± 0.1 mg of lauronitrile obtained from TCI America, 1.0−2.3 mg of lignin, and ca. 100 mg of KBr. Pellets were formed via bolt press. IR spectra were collected (32 scans, resolution 4 cm^{-1}), background subtracted with a KBr background, and baseline corrected (using the automatic baseline correction feature) with OMNIC 8.2.388 software. Samples were then normalized to the internal standard peak at ca. 2247 cm^{-1}. Finally, because the samples should follow the Beer–Lambert equation, the concentrations of lignin were normalized to the highest concentration (2.3 mg). IR spectra were then plotted with Excel.

Preparation of KLBM for Baeyer–Villiger Oxidation. Kraft lignin was ball-milled with KOH (KL/KOH = 2:1 w/w) and toluene (enough to cover the steel ball) at 25 Hz for 3 days. The ball-milled lignin was dispersed in water, then acidified with 0.2 M HCl until precipitation of lignin took place. The precipitated lignin was recovered by vacuum filtration, washed with deionized water until the washings were at neutral pH, and then dried in a vacuum oven overnight at 80 °C, producing a brown solid in an overall yield of 81%.

Reduction of KLBM. To a solution of KLBM in 1% NaOH was added $NaBH_4$ (KLBM/$NaBH_4$ = 1:2 w/w) and the resulting mixture was stirred overnight at room temperature. The excess $NaBH_4$ was quenched with 0.2 M HCl and the reduced KLBM was filtered, washed with deionized water until the washings were pH neutral, and then dried in a vacuum oven overnight at 80 °C to produce a brown solid in 60% yield.

Porphyrin Oxidation of KL and KLBM.[48,57] A mixture consisting of 26 mg TPPFeCl, 10 mL *t*-BuOOH (70% aq.), phosphate buffer (pH 3, 30 mL), and 10 mL MeCN for every 1 g of lignin was stirred at room temperature for 26 h. The mixture was acidified with 0.2 M HCl until precipitation occurred. The precipitated, oxidized lignin was recovered by vacuum filtration, washed with deionized water until the washings were pH neutral, and the resulting solid was dried in a vacuum oven overnight at 80 °C to produce a brown powder in 62% yield. In the preparation of Porp KL for Baeyer–Villiger oxidation, the same procedure was used except that a greater excess (15 mL) of *t*-BuOOH (70% aq.) was used.

Baeyer–Villiger Oxidation. A mixture consisting of 2 mL HCOOH (95%), 3.4 mL H_2O_2 (30%), and 2.7 mL H_2O for every 1 g of lignin was stirred at 50 °C for 70 h. Deionized water was added until the pH was ∼4. The resulting mixture was filtered by gravity filtration through quantitative filter paper. The water-soluble and water-insoluble fractions were each extracted separately with EtOAc (3 × 30 mL). The residue from each fraction was filtered by gravity filtration through quantitative filter paper, washed with EtOAc, then dried in a vacuum oven overnight giving a 19% yield of EtOAc-soluble fraction (from Porp-KLBM sample).

EtOAc-soluble and water-soluble fractions were derivatized by heating with 200 μL $BF_3{\cdot}OEt_2$ and 20 mL MeOH for every mmol substrate (computed based on theoretical methyl vanillate yield) at 100 °C for 6 h.[8] The reaction mixture was evaporated to dryness, then water was added and the resulting mixture was extracted with EtOAc (3 × 30 mL). The combined EtOAc extracts were evaporated under vacuum and the residue was subjected to column chromatography on silica-gel (EtOH:hexanes 1:10) to give products **1** and **2**: BV-1 (1.5%, 0.2% = 1.7%); BV-2 (2.2%, 0.5% = 2.7%); BV-3 (2%, 0.6% = 2.6%); BV-4 (7.8%, 2.2% = 10%).

HSQC NMR of Lignin. The lignins were collected directly into NMR tubes (ca. 30–40 mg for each sample, except $NaBH_4$ Red. Porp-KLBM which contained 10 mg) and dissolved using DMSO-d_6/pyridine-d_5 (4:1).[58,59] NMR spectra were acquired on a Bruker BioSpin (Billerica, MA) Avance 700 MHz spectrometer equipped with a 5 mm quadruple-resonance $^1H/^{31}P/^{13}C/^{15}N$ QCI gradient cryoprobe with inverse geometry (proton coils closest to the sample). The central DMSO solvent peak was used as an internal reference (δ_C 39.5, δ_H 2.5 ppm). The ^1H–^{13}C correlation experiment was an adiabatic HSQC experiment (Bruker standard pulse sequence 'hsqcetgpsisp2.2'; phase-sensitive gradient-edited-2D HSQC using adiabatic pulses for inversion and refocusing).[60] In the case of KL, KLBM, and $NaBH_4$ Red. KLBM, HSQC experiments were carried out using the following parameters: acquired from +11.5 to −0.5 ppm in F2 (^1H) with 3366 data points (acquisition time 200 ms), +215 to −5 ppm in F1 (^{13}C) with 620 increments (F1 acquisition time 8 ms) of 16 scans with a 1 s interscan delay; the d_{24} delay was set to 0.86 ms (1/8*J*, *J* = 145 Hz). The total acquisition time for a sample was 3 h. For all other samples, HSQC experiments were carried out using the same parameters except for acquiring half the number (1682) of data points (acquisition time 100 ms) in F2, and 112 scans with a 500 ms interscan delay. The total acquisition time was 12 h. In all cases, processing used typical matched Gaussian apodization (GB = 0.001, LB = −0.5) in F2 and squared cosine-bell and one level of linear prediction (32 coefficients) in F1. Volume integration of contours in HSQC plots used Bruker's TopSpin 3.5pl5 (Mac version) software. Integrals were from volume-integration of C/H pairs with similar properties, the α-C/H correlations of **A**, **B**, and **C** units.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.7b04597.

^1H–^1H COSY NMR of KLBM, experimental data and NMR spectra relating to the synthesis of the lignin β-O-4 trimer model compound, NMR spectra of compounds **1** and **2** (PDF)

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S.Y. performed experiments and wrote the paper, J.M. performed model compounds synthesis and NMR experiments as well as edited the paper, J.R. provided NMR insight and edited the paper, M.C. provided experimental guidance and edited the paper, S.P. performed X-ray crystallography measurements, J.S. provided experimental guidance and loaned laboratory equipment, M.M. provided experimental guidance and cowrote the paper.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Zhang, L.; Yan, L.; Wang, Z.; Laskar, D. D.; Swita, M. S.; Cort, J. R.; Yang, B. Characterization of lignin derived from water-only and dilute acid flowthrough pretreatment of poplar wood at elevated temperatures. *Biotechnol. Biofuels* **2015**, *8* (1), 1–14.

(2) Zakzeski, J.; Bruijnincx, P. C. A.; Jongerius, A. L.; Weckhuysen, B. M. The Catalytic Valorization of Lignin for the Production of Renewable Chemicals. *Chem. Rev.* **2010**, *110* (6), 3552–3599.

(3) Bhat, A.; Dasan, Y.; Khan, I. Extraction of Lignin from Biomass for Biodiesel Production. In *Agricultural Biomass Based Potential Materials*; Hakeem, K. R., Jawaid, M., Alothman, O., Eds.; Springer, 2015; pp 155–179.

(4) Kleinert, M.; Barth, T. Towards a lignincellulosic biorefinery: Direct one-step conversion of lignin to hydrogen-enriched biofuel. *Energy Fuels* **2008**, *22*, 1371−1379.

(5) Gellerstedt, G. r.; Li, J.; Eide, I.; Kleinert, M.; Barth, T. Chemical structures present in biofuel obtained from lignin. *Energy Fuels* **2008**, *22* (6), 4240−4244.

(6) Simmons, B. A.; Loque, D.; Ralph, J. Advances in modifying lignin for enhanced biofuel production. *Curr. Opin. Plant Biol.* **2010**, *13*, 312−319.

(7) Rahimi, A.; Ulbrich, A.; Coon, J. J.; Stahl, S. S. Formic-acid-induced depolymerization of oxidized lignin to aromatics. *Nature* **2014**, *515* (7526), 249−252.

(8) Wang, M.; Lu, J.; Zhang, X.; Li, L.; Li, H.; Luo, N.; Wang, F. Two-Step, Catalytic C−C Bond Oxidative Cleavage Process Converts Lignin Models and Extracts to Aromatic Acids. *ACS Catal.* **2016**, *6* (9), 6086−6090.

(9) Li, C.; Zhao, X.; Wang, A.; Huber, G. W.; Zhang, T. Catalytic transformation of lignin for the production of chemicals and fuels. *Chem. Rev.* **2015**, *115* (21), 11559−11624.

(10) Xu, C. P.; Arancon, R. A. D.; Labidi, J.; Luque, R. Lignin depolymerisation strategies: towards valuable chemicals and fuels. *Chem. Soc. Rev.* **2014**, *43*, 7485−7500.

(11) Diop, A.; Jradi, K.; Daneault, C.; Montplaisir, D. Kraft lignin depolymerization in an ionic liquid without a catalyst. *BioResources* **2015**, *10* (3), 4933−4946.

(12) Pandey, M. P.; Kim, C. S. Lignin Depolymerization and Conversion: A Review of Thermochemical Methods. *Chem. Eng. Technol.* **2011**, *34*, 29−41.

(13) Deepa, A. K.; Dhepe, P. L. Lignin depolymerization into aromatic monomers over solid acid catalysts. *ACS Catal.* **2015**, *5* (1), 365−379.

(14) Pineda, A.; Lee, A. F. Heterogeneously catalyzed lignin depolymerization. *Appl. Petrochem. Res.* **2016**, *6*, 243−256.

(15) Wang, H.; Tucker, M.; Ji, Y. Recent development in chemical depolymerization of lignin: a review. *J. Appl. Chem.* **2013**, *2013*, 1−9.

(16) Li, J.; Henriksson, G.; Gellerstedt, G. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresour. Technol.* **2007**, *98*, 3061−3068.

(17) Güvenatam, B. B. *Catalytic pathways for lignin depolymerization*, Technische Universiteit Eindhoven, 2015.

(18) Huang, X. M.; Koranyi, T. I.; Boot, M. D.; Hensen, E. J. M. Catalytic Depolymerization of Lignin in Supercritical Ethanol. *ChemSusChem* **2014**, *7*, 2276−2288.

(19) Lahive, C. W.; Deuss, P. J.; Lancefield, C. S.; Sun, Z.; Cordes, D. B.; Young, C. M.; Tran, F.; Slawin, A. M.; de Vries, J. G.; Kamer, P. C.; et al. Advanced Model Compounds for Understanding Acid-Catalyzed Lignin Depolymerization: Identification of Renewable Aromatics and a Lignin-Derived Solvent. *J. Am. Chem. Soc.* **2016**, *138* (28), 8900−8911.

(20) Roberts, V.; Stein, V.; Reiner, T.; Lemonidou, A.; Li, X.; Lercher, J. A. Towards quantitative catalytic lignin depolymerization. *Chem. - Eur. J.* **2011**, *17* (21), 5939−5948.

(21) Huang, X.; Atay, C.; Korányi, T. I.; Boot, M. D.; Hensen, E. J. Role of Cu−Mg−Al mixed oxide catalysts in lignin depolymerization in supercritical ethanol. *ACS Catal.* **2015**, *5* (12), 7359−7370.

(22) Wu, Y.-R.; He, J. Characterization of anaerobic consortia coupled lignin depolymerization with biomethane generation. *Bioresour. Technol.* **2013**, *139*, 5−12.

(23) Wu, Q.-y.; Ma, L.-l.; Long, J.-x.; Shu, R.-y.; Zhang, Q.; Wang, T.-j.; Xu, Y. *Chin. J. Chem. Phys.* **2016**, *29* (4), 474−480.

(24) Hamidi, N. H. *Enzymatic depolymerization of lignin by laccases*, University of Nottingham, 2013.

(25) Akash, B. A. Thermal Depolymerization of Lignin. *Int. J. Thermal Environ. Eng.* **2016**, *13* (1), 17−22.

(26) Harmsen, P.; Huijgen, W.; Bermudez, L.; Bakker, R. *Literature review of physical and chemical pretreatment processes for lignocellulosic biomass*; 9085857570; Wageningen UR Food & Biobased Research: 2010.

(27) Obst, J. R.; Kirk, T. K. Isolation of lignin. *Methods Enzymol.* **1988**, *161*, 3−12.

(28) Fasching, M.; Schröder, P.; Wollboldt, R. P.; Weber, H. K.; Sixta, H. A new and facile method for isolation of lignin from wood based on complete wood dissolution. *Holzforschung* **2008**, *62* (1), 15−23.

(29) Brodeur, G.; Yau, E.; Badal, K.; Collier, J.; Ramachandran, K.; Ramakrishnan, S. Chemical and physicochemical pretreatment of lignocellulosic biomass: a review. *Enzyme Res.* **2011**, *2011*, 1−17.

(30) Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* **2009**, *48* (8), 3713−3729.

(31) Maurya, D. P.; Singla, A.; Negi, S. An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech* **2015**, *5* (5), 597−609.

(32) Zheng, J. *Mechanical Pretreatment of Corncobs for Bioethanol Production by a Twin-Screw Extruder*, The University of Western Ontario, 2014.

(33) Kumar, A. K.; Sharma, S. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresour. Bioprocess.* **2017**, *4* (1), 7.

(34) Wu, S.; Argyropoulos, D. An improved method for isolating lignin in high yield and purity. *J. Pulp Pap. Sci.* **2003**, *29* (7), 235−240.

(35) Tolbert, A.; Akinosho, H.; Khunsupat, R.; Naskar, A. K.; Ragauskas, A. J. Characterization and analysis of the molecular weight of lignin for biorefining studies. *Biofuels, Bioprod. Biorefin.* **2014**, *8* (6), 836−856.

(36) Fujimoto, A.; Matsumoto, Y.; Chang, H.-M.; Meshitsuka, G. Quantitative evaluation of milling effects on lignin structure during the isolation process of milled wood lignin. *J. Wood Sci.* **2005**, *51* (1), 89−91.

(37) Chang, H.-m.; Cowling, E. B.; Brown, W. Comparative studies on cellulolytic enzyme lignin and milled wood lignin of sweetgum and spruce. *Holzforschung* **1975**, *29* (5), 153−159.

(38) Qu, Y.; Luo, H.; Li, H.; Xu, J. Comparison on structural modification of industrial lignin by wet ball milling and ionic liquid pretreatment. *Biotechnology Reports* **2015**, *6*, 1−7.

(39) Ikeda, T.; Holtman, K.; Kadla, J. F.; Chang, H.-m.; Jameel, H. Studies on the effect of ball milling on lignin structure using a modified DFRC method. *J. Agric. Food Chem.* **2002**, *50* (1), 129−135.

(40) Guerra, A.; Filpponen, I.; Lucia, L. A.; Saquing, C.; Baumberger, S.; Argyropoulos, D. S. Toward a better understanding of the lignin isolation process from wood. *J. Agric. Food Chem.* **2006**, *54* (16), 5939−5947.

(41) Gellerstedt, G.; Northey, R. Analysis of birch wood lignin by oxidative degradation. *Wood Sci. Technol.* **1989**, *23* (1), 75−83.

(42) Kleine, T.; Buendia, J.; Bolm, C. Mechanochemical degradation of lignin and wood by solvent-free grinding in a reactive medium. *Green Chem.* **2013**, *15* (1), 160−166.

(43) Brittain, A. D.; Chrisandina, N. J.; Cooper, R. E.; Buchanan, M.; Cort, J. R.; Olarte, M. V.; Sievers, C. Quenching of reactive intermediates during mechanochemical depolymerization of lignin. *Catal. Today* **2018**, *302*, 180−189.

(44) Lin, Z.; Huang, H.; Zhang, H.; Zhang, L.; Yan, L.; Chen, J. Ball milling pretreatment of corn stover for enhancing the efficiency of enzymatic hydrolysis. *Appl. Biochem. Biotechnol.* **2010**, *162* (7), 1872−1880.

(45) Zakaria, M. R.; Fujimoto, S.; Hirata, S.; Hassan, M. A. Ball milling pretreatment of oil palm biomass for enhancing enzymatic hydrolysis. *Appl. Biochem. Biotechnol.* **2014**, *173* (7), 1778−1789.

(46) Inoue, H.; Yano, S.; Endo, T.; Sakaki, T.; Sawayama, S. Combining hot-compressed water and ball milling pretreatments to improve the efficiency of the enzymatic hydrolysis of eucalyptus. *Biotechnol. Biofuels* **2008**, *1* (2), 1−9.

(47) Barakat, A.; Mayer-Laigle, C.; Solhy, A.; Arancon, R. A.; De Vries, H.; Luque, R. Mechanical pretreatments of lignocellulosic biomass: towards facile and environmentally sound technologies for biofuels production. *RSC Adv.* **2014**, *4* (89), 48109−48127.

(48) Patil, N. D.; Yao, S. G.; Meier, M. S.; Mobley, J. K.; Crocker, M. Selective cleavage of the C[small alpha]-C[small beta] linkage in lignin

model compounds via Baeyer-Villiger oxidation. *Org. Biomol. Chem.* **2015**, *13* (11), 3243−3254.

(49) Werhan, H.; Mir, J. M.; Voitl, T.; Rudolf von Rohr, P. Acidic oxidation of kraft lignin into aromatic monomers catalyzed by transition metal salts. *Holzforschung* **2011**, *65*, 703−709.

(50) Villar, J.; Caperos, A.; Garcia-Ochoa, F. Oxidation of hardwood kraft-lignin to phenolic derivatives with oxygen as oxidant. *Wood Sci. Technol.* **2001**, *35* (3), 245−255.

(51) Assmann, N.; Werhan, H.; Ładosz, A.; von Rohr, P. R. Supercritical extraction of lignin oxidation products in a microfluidic device. *Chem. Eng. Sci.* **2013**, *99*, 177−183.

(52) Voitl, T.; Rudolf von Rohr, P. Oxidation of Lignin Using Aqueous Polyoxometalates in the Presence of Alcohols. *ChemSusChem* **2008**, *1*, 763−769.

(53) Voitl, T.; Rohr, P. R. v. Demonstration of a process for the conversion of kraft lignin into vanillin and methyl vanillate by acidic oxidation in aqueous methanol. *Ind. Eng. Chem. Res.* **2010**, *49* (2), 520−525.

(54) Araújo, J. D.; Grande, C. A.; Rodrigues, A. E. Vanillin production from lignin oxidation in a batch reactor. *Chem. Eng. Res. Des.* **2010**, *88* (8), 1024−1032.

(55) Asikkala, J.; Tamminen, T.; Argyropoulos, D. S. Accurate and Reproducible Determination of Lignin Molar Mass by Acetobromination. *J. Agric. Food Chem.* **2012**, *60* (36), 8968−8973.

(56) Constant, S.; Wienk, H. L. J.; Frissen, A. E.; Peinder, P. d.; Boelens, R.; van Es, D. S.; Grisel, R. J. H.; Weckhuysen, B. M.; Huijgen, W. J. J.; Gosselink, R. J. A.; Bruijnincx, P. C. A. New insights into the structure and composition of technical lignins: a comparative characterisation study. *Green Chem.* **2016**, *18* (9), 2651−2665.

(57) Yao, S. G.; Meier, M. S.; Pace, R. B., III; Crocker, M. A comparison of the oxidation of lignin model compounds in conventional and ionic liquid solvents and application to the oxidation of lignin. *RSC Adv.* **2016**, *6* (106), 104742−104753.

(58) Kim, H.; Ralph, J.; Akiyama, T. Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d6. *BioEnergy Res.* **2008**, *1* (1), 56−66.

(59) Kim, H.; Ralph, J. Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d_6/pyridine-d_5. *Org. Biomol. Chem.* **2010**, *8* (3), 576−591.

(60) Kupče, E.; Freeman, R. Compensated adiabatic inversion pulses: broadband INEPT and HSQC. *J. Magn. Reson.* **2007**, *187* (2), 258−265.

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