Effect of Autohydrolysis Pretreatment Conditions on Sugarcane Bagasse Structures and Product Distribution Resulting from Pyrolysis

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Pyrolysis has been increasingly perceived as a promising technology to produce biofuel precursors (bio-oil) from agricultural residuals; however, there is a significant quality gap between a bio-oil and the fuels used for transportation. In this study, we autohydrolyzed pretreated sugarcane bagasse at three different conditions (180 °C–10 min, 180 °C–40 min, 200 °C–40 min), then we investigated the effect of this pretreatment on a subsequent pyrolysis stage. High-pressure ion-exchange chromatography (HPIC) and the ^{13}C cross-polarization/magic angle spinning (CP/MAS) solid-state nuclear magnetic resonance (NMR) revealed that the autohydrolysis pretreatment significantly disrupted the hemicellulose fractions in the sugarcane bagasse and caused the breakage of lignin ether linkages in the sugarcane bagasse feedstocks. As the ^{31}P NMR results indicated, the autohydrolysis pretreatment removed carboxylic acid groups up to 66.7 %, which could significantly address the corrosion problem of bio-oils. Heteronuclear single quantum correlation (HSQC) analysis suggested that the autohydrolysis pretreatment effectively lowered the presence of the oxygenated aromatic compounds in the bio-oils. Gel permeation chromatography (GPC) analysis of the bio-oils indicated that the oils from severely pretreated sugarcane bagasse pyrolyzed at a low temperature (i.e., 400 °C) contained lower-molecular-weight components similar to those present gasoline products.

Introduction

According to the U.S. Energy Information Administration (EIA), global energy consumption is projected to increase to 858 exajoules in 2040 compared with 549 exajoules in 2012.[1] Though fossil fuels remain the dominant transportation fuel, renewable energy sources are trending upward. The U.S. Department of Energy (DOE) reported that the renewable energy consumption has increased steadily since 2001 and the total renewable energy consumption has expanded by approximately 82 % from 2001 to 2014.[2] Among the renewable energy resources, biomass-based resources play a critical role. Lignocellulosic bioresources are broadly applied to the generation of power, heat, and fuels from home use to industrial production.

Biomass resources include woody feedstocks, energy crops, crop residues, and waste resources. These feedstocks are low-cost and typically avoid direct competition with the use of agriculture land for food crops. Sugarcane bagasse is a typical biomass feedstock from agriculture waste; it is estimated that each ton of sugarcane can yield approximately 0.14 tons of bagasse on a dry basis.[2] The large-volume production of the sugarcane bagasse has attracted significant attention for its use in the biorefinery scenario, especially for its conversion to bioethanol and biopower.[3]

Pyrolysis is one of the promising biomass conversion techniques to produce biofuel precursors. Previous research has been conducted on the pyrolysis of sugarcane bagasse with analysis of the properties of the bio-oils.[4–6] These results showed that the pyrolysis oils from sugarcane bagasse exhib-

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ited low pH values, low heating values, and high water content, which were comparable to the bio-oils generated from other biomass feedstocks.[7]

Pretreatment of biomass is one of the methods currently under investigation to address the unfavorable characteristics of the bio-oils. Researchers have been focused on both thermal pretreatment (i.e., torrefaction) and chemical pretreatment (e.g., leaching/washing, acid pretreatment, autohydrolysis pretreatment) to achieve the optimum feedstock properties prior to pyrolysis.[8] Davidsson et al. conducted water washing and acid leaching on wheat straw, wood waste, and cellulose and pyrolyzed the pretreated feedstocks, which improved the combustion properties of the resulting bio-oils by removing alkali components.[9] Neupane et al. performed both non-catalytic and catalytic pyrolysis on torrefied pine wood; the torrefied pine wood with a reduced content of hemicelluloses achieved a higher yield of aromatic hydrocarbons.[10] Wang et al. compared acid, alkali, and steam explosion pretreatments on the pine wood prior to pyrolysis; here, the results showed that a dilute acid pretreatment resulted in the highest heating value of the bio-oil.[11]

Among pyrolysis pretreatment technologies, an autohydrolysis pretreatment is free of chemical additives, using elevated reaction temperature (140–220 °C) to generate acetic acid from the acetyl groups of hemicellulose, which catalyzes autohydrolysis reactions.[12] In comparison with the pretreatments involving acids or alkali, the autohydrolysis pretreatment process does not require a large amount of chemicals to neutralize the hydrolyzed products. Another benefit of this pretreatment process is that a highly corrosion-resistant reactor is not needed.[13] This pretreatment process has been applied to bioethanol production as a convenient methodology to reduce the recalcitrance of biomass; researchers are now seeking opportunities to combine this technology together with the pyrolysis process to test if the autohydrolysis pretreatment could achieve higher-quality bio-oils. Zheng et al. performed autohydrolysis pretreatment on eucalyptus wood and showed that the pretreatment lowered the amounts of reactive components (i.e., ketones, aldehydes, acids) in the bio-oils.[14] Du et al. conducted autohydrolysis pretreatment on microalgae and concluded that the resulting bio-oil yielded less *N*-containing compounds.[15] However, at the present time, no knowledge exists about the effect of autohydrolysis pretreatment on the resulting pyrolysis oils derived from sugarcane bagasse. In this study, we performed the autohydrolysis pretreatment on sugarcane bagasse using three different conditions: 180 °C–10 min, 180 °C–40 min, 200 °C–40 min. Compositional analysis and ^{13}C CP/MAS NMR were used to investigate the structural changes in the sugarcane bagasse feedstocks. The product distribution and the properties of the bio-oils were characterized by heteronuclear single quantum correlation (HSQC), phosphitylation followed by ^{31}P NMR, and gel permeation chromatography (GPC).

Results and Discussion

Chemical composition analysis

Autohydrolysis pretreatment of sugarcane bagasse was conducted at three reaction conditions: 180 °C–10 min, 180 °C–40 min, and 200 °C–40 min. Figure 1 presents the chemical composition of sugarcane bagasse both before and after the autohydrolysis pretreatment. The untreated sugarcane bagasse was mainly composed of four monosaccharides: glucose, xylose, arabinose, and galactose. The xylose content reached 22.08 % and represented the major hemicellulosic monosaccharide from the untreated feedstock. The autohydrolysis pretreatment significantly removed the hemicellulose fraction in sugarcane bagasse. The pretreatment at 180 °C for 10 min removed 23.96 % xylose, 86.00 % arabinose, and almost 100 % galactose. Prolonging the pretreatment time to 40 min enhanced the removal of xylose up to 73.87 % and led to complete removal of arabinose. Under the most severe pretreatment conditions (200 °C–40 min), it was observed that nearly 100 % of the hemicellulosic components were removed.

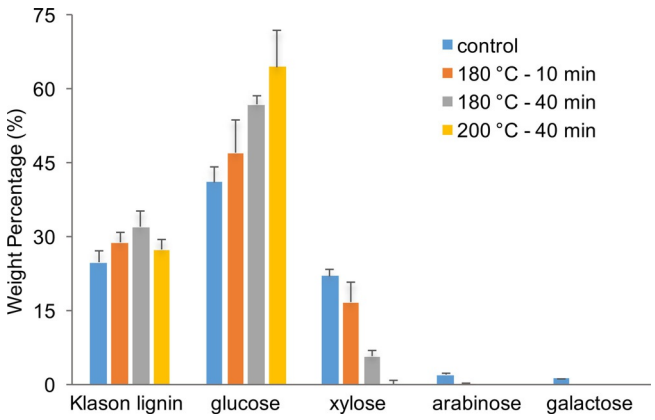

Figure 1. Compositional analysis of the sugarcane bagasse before and after the autohydrolysis pretreatment.

Klason lignin, also known as acid-insoluble lignin, showed an increasing trend after the pretreatment at 180 °C for 10 min and 40 min, and a reduction after the pretreatment at 200 °C for 40 min. The relative increase of the lignin fraction could be attributed to the removal of other components (mainly hemicellulose) as well as "pseudo-lignin" formation.[16] Under the acidic conditions employed, autohydrolysis can lead to hydrolysis of lignin carbohydrate complexes and the depolymerization of lignin by α-*O*-4 and β-*O*-4 bond rupture, and these reactive components can undergo a series of reactions, including dehydration and condensation reactions, resulting in solubilization of lignin fragments and the modification of lignin in the plant cell wall.[17] These competing reaction pathways presumably contribute to the observed changes in the lignin content of the autohydrolyzed products at differing conditions.[18] As for cellulose, the pretreatments

increased the cellulose content, reaching a maximum value of 64.57%. This trend was attributed to the substantial removal of hemicellulose. It has been reported that a longer reaction time did not significantly increase the removal of cellulose due to the high crystallinity of the cellulose.[19]

CP/MAS ^{13}C NMR analysis

The most significant changes in solid-state ^{13}C NMR spectral data of the initial and autohydrolyzed bagasse samples (Figure 2) are observed at approximately 21 and 173 ppm. These two peaks were assigned to the acetyl methyl and carboxyl carbons in hemicellulose, respectively.[20] These two peaks were relatively intense in the untreated sugarcane bagasse; it was clearly observed that the intensity of these two peaks decreased with increasing pretreatment condition severity. After pretreatment at 200 °C for 40 min, the carboxyl and acetyl groups in the hemicellulose were almost eliminated. Another major change can be observed in the lignin regions centered at approximately 148 and 152 ppm, which were assigned to the non-ether linked and ether linked guaiacyl carbons, respectively. The peak intensity of the ether linked guaiacyl carbons decreased after the pretreatment, whereas the peak intensity of the non-ether linked guaiacyl carbons increased. This result indicated the cleavage of the ether linkages, with lignin presumably leading to the formation of free phenolic groups. The chemical shift region of 105–62 ppm was assigned to the cellulose carbons which overlapped with lignin and hemicellulose signals. The peak at 89 ppm contains the C-4 signal for crystalline cellulose, along with other structural components. The peak at 84 ppm was assigned to C-4 of amorphous cellulose and overlapped with signals from lignin and hemicellulose. As the severity of the pretreatment condition increased, the peak intensity ratio of the peaks at 89 and 84 ppm also increased. This result suggested that the autohydrolysis pretreatment enhanced the cellulose crystallinity component in the pretreated sugarcane bagasse.

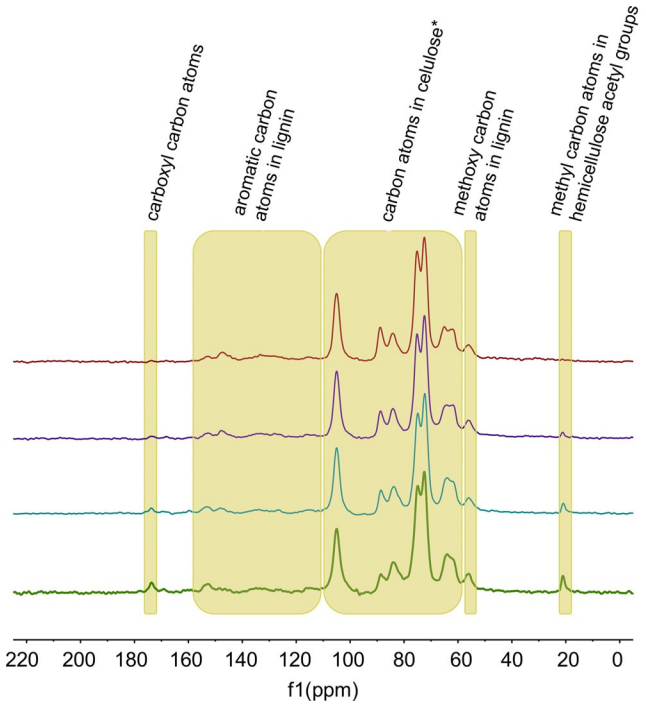

Figure 2. CP/MAS ^{13}C NMR spectra (from bottom to top) of untreated sugarcane bagasse and sugarcane bagasse samples pretreated at 180 °C–10 min, 180 °C–40 min, 200 °C–40 min (*the signals in cellulose region 105–162 ppm overlap with the signals from lignin and hemicellulose).

Yield distribution of the pyrolysis products

Figure 3a,b summarize the product yield distribution from the sugarcane bagasse pretreated at different conditions, based on the masses of pretreated biomass and original biomass, respectively. Figure 3a shows that at a pyrolysis temperature of 400 °C, bio-oils generated from the pretreated sugarcane bagasse were all observed to have higher yields than bio-oil from the control group. However, the most severe pretreatment condition (200 °C–40 min) resulted in decreased bio-oil yields in comparison with the milder pretreatment conditions (180 °C–10 min, 180 °C–40 min), with a char yield increase of approximately 4%. This observation may indicate that, at a lower pyrolysis temperature (i.e., 400 °C), the severe pretreatment condition caused the sugarcane bagasse to undergo more carbonization reactions. At a

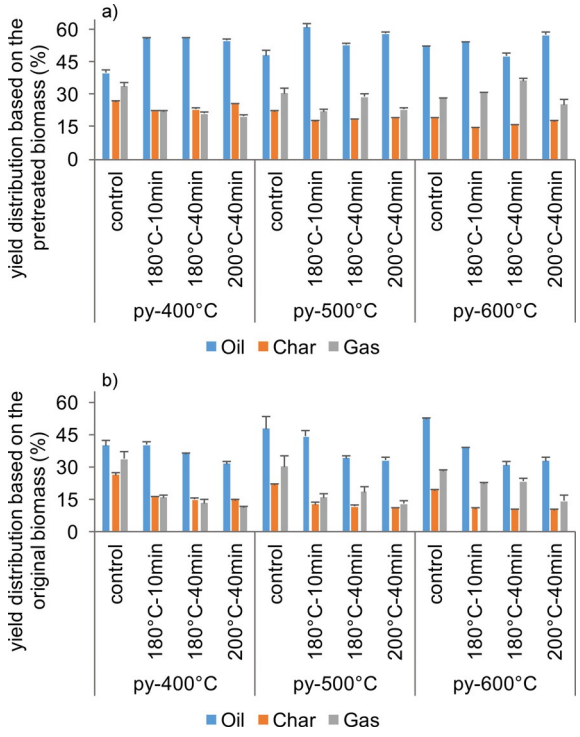

Figure 3. (a) Yield distribution of the bio-oils from the untreated and pretreated sugarcane bagasse pyrolyzed at 400, 500, and 600 °C based on the mass of pretreated biomass. (b) Yield distribution of the bio-oils from untreated and pretreated sugarcane bagasse pyrolyzed at 400, 500, and 600 °C based on the mass of original biomass (mass yields of the three pretreatment conditions 180 °C–10 min, 180 °C–40 min, 200 °C–40 min are 72.50%, 64.40%, and 57.29%, respectively).

pyrolysis temperatures of 500 and 600 °C, the sugarcane bagasse samples that were pretreated at 180 °C–10 min and 200 °C–40 min generated higher bio-oil yields (up to 59.86 %) when compared to the control group, whereas the pretreatment condition 180 °C–40 min showed a negative effect on the oil yields. The lower oil yields from the pretreatment at 180 °C–40 min corresponded to lower char yields and the highest gas yields. This phenomenon suggests that the pyrolysis of the 180 °C–40 min pretreated sugarcane bagasse at higher temperatures (i.e., 500 and 600 °C) leads to secondary decomposition reactions of pyrolysis oils. During secondary decomposition reactions, parts of liquid products were fragmented into gases.[21] Past pyrolysis studies of biomass indicated that carbon monoxide, carbon dioxide, methane, ethane, and ethene are some of the most common components in the gas phase.[22] As shown in Figure 3 b, the autohydrolysis pretreatment lowered the bio-oil yield based on the mass of original biomass due to the mass loss during the pretreatment process. At a pyrolysis temperature of 400 °C, the bio-oils produced from both untreated and 180 °C–10 min pretreated sugarcane bagasse had the similar yields of approximately 40 % based on the mass of original biomass. Considering the energy consumption during the autohydrolysis pretreatment and pyrolysis process, the pretreatment condition of 180 °C–10 min was recommended for achieving higher bio-oil yield.

Molecular weight analysis of the bio-oils

The weight-average molecular weight (M_w), number average molecular weight (M_n), and polydispersity index (PDI) of the bio-oils produced from the control and pretreated sugarcane bagasse are presented in Figure 4. Gasoline-range products have a number molecular weight of 80–120 g mol^{-1}.[23] Usually bio-oils have much higher average molecular weight values, which is not a preferred characteristic for pyrolysis oils. At a pyrolysis temperature of 400 and 500 °C, the bio-oils produced from the 180 °C–40 min pretreated sugarcane bagasse exhibited the highest average molecular weights among the oils. At a pyrolysis temperature of 600 °C, the pretreatments at 180 °C (both 10 min and 40 min) yielded comparable higher average molecular weights (M_n = 136.01 and 138.97 g mol^{-1}). Bio-oil produced from the 200 °C–40 min pretreated and 400 °C pyrolyzed sugarcane bagasse showed the lowest average molecular weight (M_n = 121.88 g mol^{-1}), which was close to the range of gasoline products. The bio-oil produced from this condition also yielded the lowest polydispersity value of 1.42. This could be attributed to the fact that in the bio-oil produced under these conditions had the most favorable thermal fragmentation and secondary reactions.[24]

^{31}P NMR analysis of the bio-oils

Figures 5–8 present the quantitative integration results of ^{31}P NMR analysis of the phosphitylated bio-oils produced from various sugarcane bagasse feedstocks. The chemical shift assignments are based on information from the literature.[25] The aliphatic OH groups were assigned to 150.5–144.5 ppm. Figure 5 shows that at all three pyrolysis temperatures, the bio-oils from the pretreated sugar bagasse exhibited similar trends: pretreatments of moderate conditions (180 °C, 10 or 40 min) increased the aliphatic OH groups compared to the control group. For the 40 min pretreatments, increasing the pretreatment temperature from 180 °C to 200 °C lowered the aliphatic OH content. Most of the aliphatic OH groups came from levoglucosan, which were assigned to signals at 148.68, 147.26, and 147.21 ppm. Figure 6 presents C_5 substituted phenolic OH groups; β-5 and 5-5 phenolic OH groups were generated to a lesser degree in the

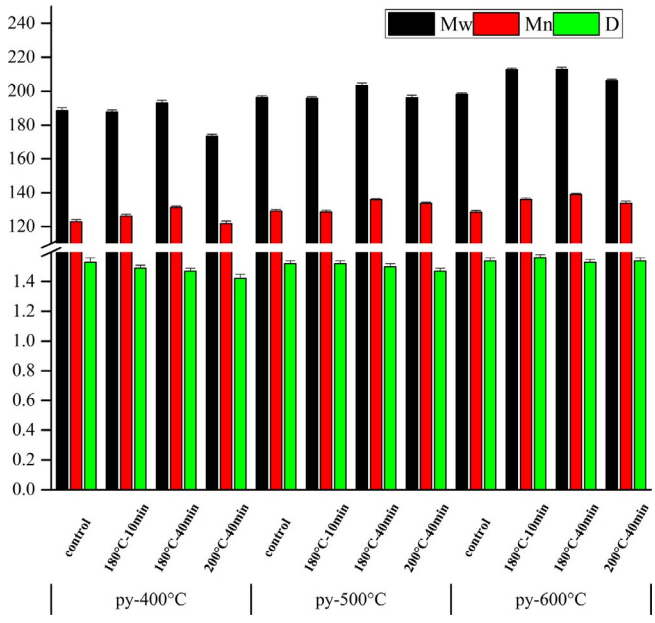

Figure 4. Molecular weight distribution of the bio-oils pyrolyzed from the untreated and pretreated sugarcane bagasse.

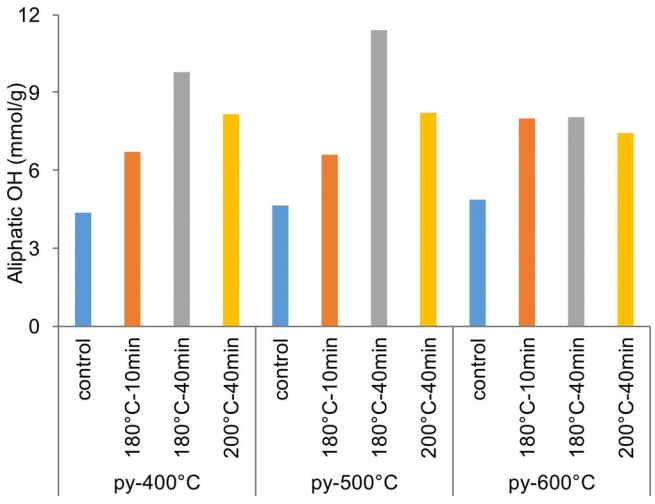

Figure 5. Aliphatic OH content in the bio-oils pyrolyzed from the untreated and pretreated sugarcane bagasse.

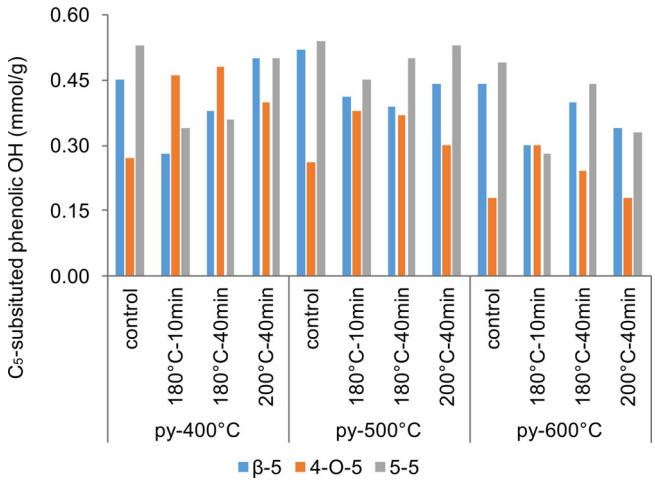

Figure 6. C_5 substituted phenolic OH content in the bio-oils pyrolyzed from the untreated and pretreated sugarcane bagasse.

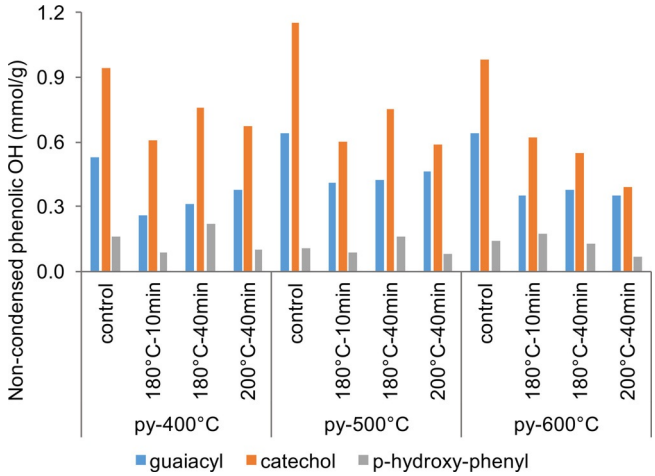

Figure 7. Non-condensed phenolic OH content in the bio-oils pyrolyzed from the untreated and pretreated sugarcane bagasse.

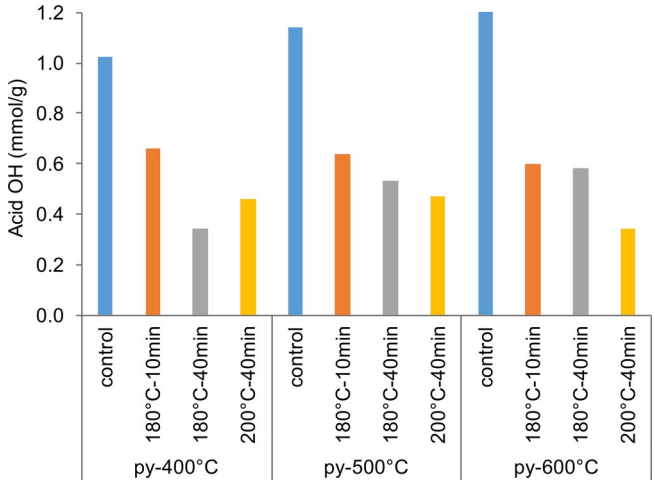

Figure 8. Acid content in the bio-oils pyrolyzed from the untreated and pretreated sugarcane bagasse.

pretreated sugarcane bagasse bio-oils, whereas pretreatment resulted in more 4-*O*-5 phenolic OH groups. Non-condensed phenolic OH groups are shown in Figure 7. It is clear that the pretreatment at all conditions led to a reduced production of guaiacyl and catechol OH groups. Most importantly, as shown in Figure 8, the significant reduction of the acids in bio-oils was presumably due to the pretreatment hydrolysis of acetylated hemicelluloses. At a pyrolysis temperature of 400 °C with bagasse pretreated at 180 °C for 40 min, the resulting bio-oil produced the least amount of acids (0.34 mmol g^{-1}), whereas at a pyrolysis temperature of 500 or 600 °C, the most severe pretreatments led to the least amount of acids (0.47, 0.34 mmol g^{-1}).

HSQC NMR analysis of the bio-oils

Figures 9–12 present the 2D HSQC spectra and assignments of C–H bonds in bio-oils pyrolyzed from untreated and pretreated sugarcane bagasse at 500 °C. The HSQC spectra of bio-oils produced from a pyrolysis temperature of 400 or 600 °C are included in the Supporting Information as Figures S1–S8. The chemical shift assignments are based on information from the published literature.[26] Figure 9 shows the two different methoxy groups in the bio-oils. The C1 type methoxy group does not exist in native sugarcane bagasse lignin and could be rearranged from C2.[26] At all three pyrolysis temperatures, the intensity of both C1 and C2 type of methoxy groups slightly decreased after autohydrolysis. Figure 10 presents the 2D maps of the C–H bonds of levoglucosan in the bio-oils. The intensity of levoglucosan contents increased, especially under the high severity conditions, which are consistent with the ^{31}P NMR results. The aliphatic regions of the HSQC spectra are shown in Figure 11. The content of compound F (5-methylfurfural) increased significantly with increasing pretreatment severity. As with levoglucosan, 5-methylfurfural is a major degradation product from cellulose. The increased percentage of cellulose after the pretreatment resulted in the increased amount of levoglucosan and 5-methylfurfural. The oxygenated compound G (aldehyde type) was slightly removed by the pretreatment whereas the hydrocarbon products D4 (aromatic type) and D5 (aliphatic type) were partially eliminated. Figure 12 presents the aromatic region of the HSQC spectra of the bio-oils. From these data, it was observed that the pretreatments partially reduced the phenol type oxygenated compounds A1 and B1. However, the pretreatment severity does not have a significant effect on the contents of these oxygenated compounds. The contents of the phenol-type oxygenated compounds B3 also decreased after the pretreatment and achieved the minimum quantity following the most severe pretreatment condition.

Conclusions

Three different conditions of autohydrolysis pretreatments (180 °C^{-1}–0 min, 180 °C–40 min, 200 °C–40 min) were conducted on the sugarcane bagasse and the resulting bio-oils

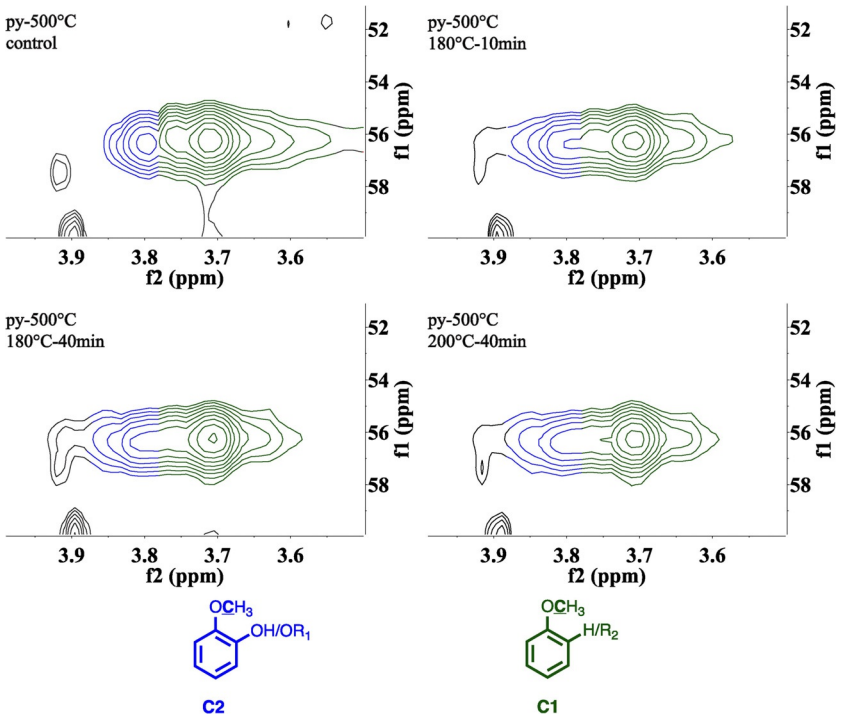

Figure 9. HSQC NMR spectra and assignments of methoxy groups in the sugarcane bagasse bio-oils pyrolyzed at 500 °C (black area: unassigned).

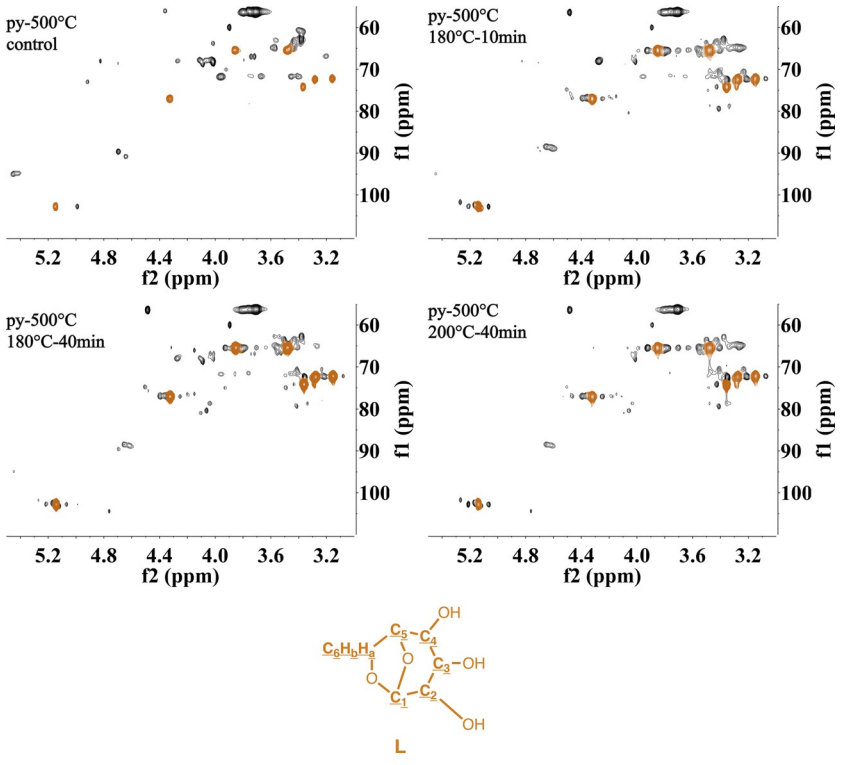

Figure 10. HSQC NMR spectra and assignments of each C–H bond of levoglucosan in the sugarcane bagasse bio-oils pyrolyzed at 500 °C (black area: unassigned).

were analyzed. The autohydrolysis pretreatment led to an optimum bio-oil yield of 59.86 %, and bio-oils of the lowest molecular weight were generated from the sugarcane bagasse pretreated at the most severe conditions, following pyrolysis at 400 °C. The autohydrolysis pretreatment effectively reduced the presence of acids and oxygenated aromatic com-

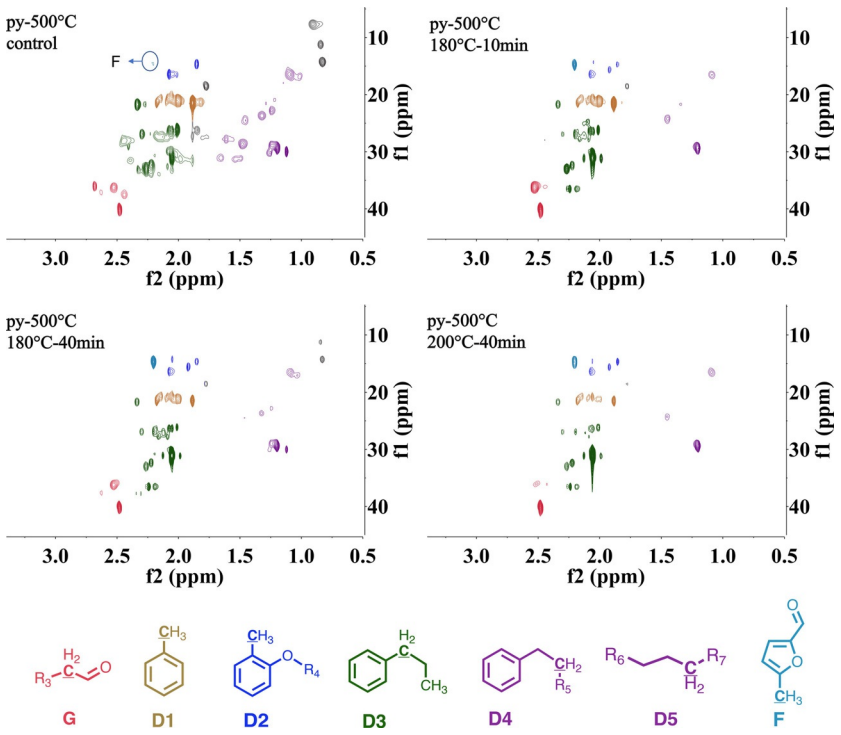

Figure 11. HSQC NMR spectra and assignments of aliphatic C–H bonds in the sugarcane bagasse bio-oils pyrolyzed at 500 °C (black area: unassigned).

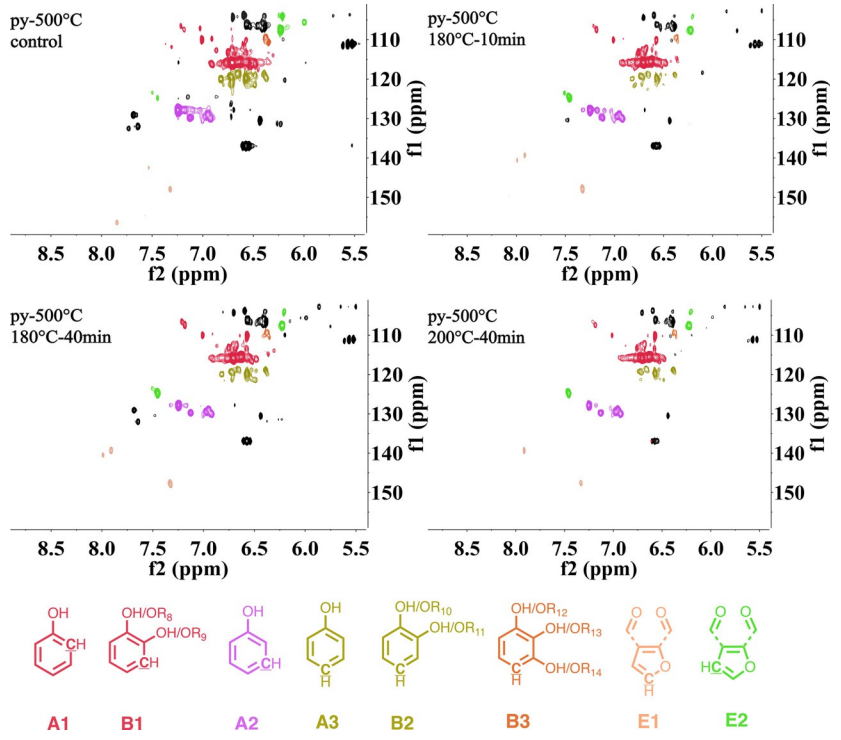

Figure 12. HSQC NMR spectra and assignments of aromatic C–H bonds in the sugarcane bagasse bio-oils pyrolyzed at 500 °C (black area: unassigned.)

pounds in the bio-oils, whereas the yields of undesired levoglucosan and 5-methylfurfural increased. Overall, considering both the reduction in undesired products and lower-temperature pyrolysis conditions, a mild pretreatment condition (180 °C–10 min) is suggested to be applied to enhance the bio-oil properties.

Experimental Section

Materials and sample preparation

Samples of sugarcane bagasse were grown and harvested in Egypt. The samples of sugarcane bagasse were air dried in a fume hood for 24 h. After air drying, the sugarcane bagasse samples were milled through a 2 mm sieve using a Wiley mill. The milled sugarcane bagasse samples were Soxhlet extracted using the mixture of toluene and ethanol ($v/v = 2{:}1$) for 8 h, followed by the acetone for 4 h. After Soxhlet extraction, the sugarcane bagasse samples were air dried in a fume hood. After air drying, the extractive free sugarcane bagasse samples were collected and stored in a refrigerator at 0 °C.

Autohydrolysis pretreatment process

Extractive-free milled sugarcane bagasse samples (≈6.5 g, ≈7.0 % moisture content) were used in the pretreatment. Three different conditions were applied: 180 °C–10 min, 180 °C–40 min, 200 °C–40 min. A mixture of biomass sample and water was loaded into a 200 mL Parr reactor, with a solid-to-liquid ratio of 1:20 (v/v). The Parr reactor was heated to and remained at 180 °C or 200 °C for 10 min or 40 min. The Parr reactor was held at a pressure of 8.27×10^5–9.65×10^5 Pa for the temperature of 180 °C, and 12.41×10^5–13.79×10^5 Pa for the temperature of 200 °C. After the pretreatment reactions, the reactor was cooled to room temperature (RT) using an ice bath. The solid product was filtered and washed with deionized water before collection. The sugarcane bagasse samples pretreated under the same conditions were combined and mixed before storing at 0 °C.

Pyrolysis process

Briefly, the Soxhlet extracted sugarcane bagasse sample was oven dried at 105 °C before pyrolysis. The oven-dried pyrolysis sample (≈3 g) was placed in a quartz sample boat that was positioned in the center of a pyrolysis tube. The pyrolysis tube was connected with two condensers and flushed with nitrogen gas (0.5 L min^{-1}), then inserted into the furnace preheated to 400, 500, or 600 °C. The heating rate was approximately 2.7 °C s^{-1} and was measured by immersing a K-type thermocouple into the sample powders. The condensers were immersed in liquid nitrogen. The pyrolysis outflow passed through the tube and condensers. The pyrolysis process lasted for 30 min. Upon completing the pyrolysis, the tube and the condensers were removed from the furnace and liquid nitrogen, respectively, and were cooled down to RT under constant nitrogen flow. The pyrolysis char and oil were collected for analysis. The liquid products were recovered by acetone wash followed by evaporation under reduced pressure. The char yield was determined gravimetrically, and gas formation was calculated by mass difference.[24]

Klason lignin and carbohydrate analysis

Klason lignin and structural carbohydrates were tested following the laboratory analytical procedure (NREL/TP-510–42618).[27] Approximately 0.15 g extracted biomass sample was hydrolyzed using 72 wt % sulfuric acid for 60 min at 30 °C, and then the mixture was diluted to 3 wt % sulfuric acid and then autoclaved for 60 min at 121 °C. The resulting mixture was cooled down to RT. The precipitate was filtered and dried in an oven at 105 °C overnight. The dried precipitate was referred to as Klason lignin and weighed to determine the Klason quantity. The filtrate was used to determine the carbohydrate composition by high performance anion exchange chromatography using Dionex ICS-3000. NaOH (0.20 M) was used as the eluent and NaOH (0.40 M) was employed as the post-column rinsing effluent. Standard solutions of glucose, xylose, arabinose, mannose, and galactose were used to build a calibration curve. Fucose was employed as the internal standard.

CP/MAS ^{13}C NMR analysis

The untreated and pretreated sugarcane bagasse samples were packed in 4 mm ZrO_2 rotors. The solid-state CP/MAS ^{13}C NMR was performed using a Bruker 400 MHz spectrometer operating at a frequency of 75.48 MHz for ^{13}C. The NMR experiment was performed using a Bruker 4 mm MAS probe. The following parameters were employed in the NMR experiments: 90° proton pulse, 1.5 ms contact pulse, 3072 scans, and 4 s recycle delay.[28]

Molecular weight analysis

The weight average molecular weight (M_w), molar average molecular weight (M_n), and molecular weight polydispersity index (PDI) of the heavy pyrolysis oils were determined by GPC following a procedure from the literature.[29] Before GPC analysis, the heavy oil samples were dissolved in tetrahydrofuran (THF, 1 mg mL^{-1}). The mixture was then filtered through a 0.2 μm filter and injected into the HPLC vials. THF was used as the mobile phase (1.0 mL min^{-1}) with an injection volume of 30 μL. Polystyrene standards (i.e., 1.53×10^3, 1.11×10^3 Da), dioctyl phthalate ($M_w = 390$ g mol^{-1}), 2,2′ -dihydroxy-4,4′ -dimethoxyl-benzophenone ($M_w = 274$ g mol^{-1}), phenol ($M_w = 94$ g mol^{-1}), and acetone ($M_w = 58$ g mol^{-1}) were used as standards to build a calibration curve by fitting a polynomial equation to the retention volumes. The M_w and M_n values were calibrated against the calibration curve. The polynomial order of the standard calibration curve is 3. The R^2 value of the calibration curve was 0.997.

^{31}P NMR analysis

The ^{31}P NMR analysis was acquired using the methods in the published analytical laboratory procedure (NREL/TP-5100-65887).[30] A stock solution of pyridine/$CDCl_3$ ($v/v = 1.6/1$) was prepared first. The chromium acetylacetonate (relaxation reagent) and *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide (internal standard) were then added to the stock solution. Bio-oil (≈25 mg) was dissolved in the solution mixture, and then derivatized using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP). The ^{31}P NMR spectra were acquired using a Varian 500 MHz spectrometer. The following parameters were employed in the NMR experiments: inverse-gated decoupling pulse sequence, 1.2 s acquisition time, 25 s pulse delay, 90° pulse angle, and 64 scans. The data were analyzed using Mestrenova software.

HSQC NMR analysis

The HSQC NMR spectra were acquired using a Varian 500 MHz spectrometer. Heavy oil (≈50 mg) was dissolved in 0.6 mL dimethylsulfoxide (DMSO)-d_6. The following parameters were employed in the HSQC experiments: 1.5 s pulse delay, 0.11 s acquisition time, 24 scans, $^1J_{C\text{-}H}$ of 145 Hz, 716 data points for ^1H, and 256 data points for ^{13}C. The ^1H and ^{13}C widths were 13 ppm and

220 ppm, respectively. The data was analyzed using the software Mestrenova. $\delta_{C/H} = 39.50/2.49$ ppm was used to reference the central solvent peak. The automatic phase and baseline correction were accomplished using the software.[31]

GC–MS analysis

Characterization of bio-oil components was conducted using Agilent 7890A/5795C GC/MS with a HP-5 MS capillary column (30 m × 0.32 mm and 0.25 μm thickness) under helium gas flow. GC samples were prepared by mixing bio-oil with methanol (bio-oil/methanol = 1:10 *w*/*w*). An injection of 0.5 μL with a split ratio of 20:1 and injector temperature of 250 °C was used for each sample. The GC oven was held at 40 °C for 3 min, heated at 5 °C min^{-1} to 260 °C, and then held for 3 min. The molecular mass range (*m*/*z*) of the MS detector was set at 40–400 to avoid the methanol peak in the spectra. Chemical compounds of bio-oil were identified by comparing their spectra with the standard spectra in the National Institute of Standards and Technology (NIST) library and from the previous literature.[32,33] The GC–MS results are included in the Supporting Information as Table S1.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biomass · bio-oil · fuels · NMR spectroscopy · pyrolysis

[1] International Energy Outlook May 2016. Washington, DC: U. S. Energy Information Administration, Office of Energy Statistics, U. S. Department of Energy; **2016** May. Report No.: DOE/EIA-0484(2016).
[2] U. S. Department of Energy. 2016. 2016 Billion-Ton Report: Advancing Domestic Resources for a Thriving Bioeconomy, Volume 1: Economic Availability of Feedstocks. M. H. Langholtz, B. J. Stokes, and L. M. Eaton (Leads), ORNL/TM-**2016**/160. Oak Ridge National Laboratory, Oak Ridge, TN. 448p. https://doi.org/10.2172/1271651.
[3] T. L. Bezerra, A. J. Ragauskas, *Biofuels Bioprod. Biorefin.* **2016**, *10*, 634–647.
[4] W. T. Tsai, M. K. Lee, Y. M. Chang, *J. Anal. Appl. Pyrolysis* **2006**, *76*, 230–237.
[5] M. R. Islam, M. Parveen, H. Haniu, *Bioresour. Technol.* **2010**, *101*, 4162–4168.
[6] M. Carrier, T. Hugo, J. Gorgens, H. Knoetze, *J. Anal. Appl. Pyrolysis* **2011**, *90*, 18–26.
[7] R. H. Venderbosch, *ChemSusChem* **2015**, *8*, 1306–1316.
[8] D. Carpenter, T. L. Westover, S. Czernik, W. Jablonski, *Green Chem.* **2014**, *16*, 384–406.
[9] K. O. Davidsson, J. G. Korsgren, J. B. C. Pettersson, U. Jaglid, *Fuel* **2002**, *81*, 137–142.
[10] S. Neupane, S. Adhikari, Z. Wang, A. J. Ragauskas, Y. Pu, *Green Chem.* **2015**, *17*, 2406–2417.
[11] H. Wang, R. Srinivasan, F. Yu, P. Steele, Q. Li, B. Mitchell, *Energy Fuels* **2011**, *25*, 3758–3764.
[12] P. Alvira, E. Tomas-Pejo, M. Ballesteros, M. J. Negro, *Bioresour. Technol.* **2010**, *101*, 4851–4861.
[13] M. J. Taherzadeh, K. Karimi, *Int. J. Mol. Sci.* **2008**, *9*, 1621–1651.
[14] A. Zheng, L. Jiang, Z. Zhao, S. Chang, Z. Huang, K. Zhao, F. He, H. Li, *Energy Fuels* **2016**, *30*, 3057–3065.
[15] Z. Du, M. Mohr, X. Ma, Y. Cheng, X. Lin, Y. Liu, W. Zhou, P. Chen, R. Ruan, *Bioresour. Technol.* **2012**, *120*, 13–18.
[16] F. Hu, S. Jung, A. Ragauskas, *Bioresour. Technol.* **2012**, *117*, 7–12.
[17] A. Tripathi, A. Ferrer, S. A. Khan, O. J. Rojas, *ACS Sustainable Chem. Eng.* **2017**, *5*, 2483–2492.
[18] Q. Wu, S. Yu, N. Hao, T. Wells, X. Meng, M. Li, Y. Pu, S. Liu, A. J. Ragauskas, *Bioresour. Technol.* **2017**, *244*, 78–83.
[19] H. L. Trajano, N. L. Engle, M. Foston, A. J. Ragauskas, T. J. Tschaplinski, C. E. Wyman, *Biotechnol. Biofuels* **2013**, *6*, 110–125.
[20] C. A. Rezende, M. Aparecida de Lima, P. Maziero, E. Ribeiro de Azevedo, W. Garcia, I. Polikarpov, *Biotechnol. Biofuels* **2011**, *4*, 54.
[21] W.-J. Liu, W.-W. Li, H. Jiang, H.-Q. Yu, *Chem. Rev.* **2017**, *117*, 6367–6398.
[22] H. Yang, R. Yan, H. Chen, D. H. Lee, C. Zheng, *Fuel* **2007**, *86*, 1781–1788.
[23] H. Ben, A. J. Ragauskas, *RSC Adv.* **2012**, *2*, 12892–12898.
[24] H. Ben, A. J. Ragauskas, *Energy Fuels* **2011**, *25*, 2322–2332.
[25] N. Hao, H. Ben, C. G. Yoo, S. Adhikari, A. J. Ragauskas, *Energy Fuels* **2016**, *30*, 6863–6880.
[26] H. Ben, A. J. Ragauskas, *Energy Fuels* **2011**, *25*, 5791–5801.
[27] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, et al. Determination of structural carbohydrates and lignin in biomass. Denver (CO): National Renewable Energy Laboratory; **2012** August. Report No.: NREL/TP-510–42618. Contract No.: DE-AC36-08GO28308. Sponsored by the Department of Energy.
[28] H. Ben, A. J. Ragauskas, *Green Chem.* **2012**, *14*, 72–76.
[29] H. Ben, A. J. Ragauskas, *Energy Fuels* **2011**, *25*, 4662–4668.
[30] M. V. Olarte, S. D. Burton, M. Swita, A. B. Padmaperuma, J. Ferrell, H. Ben, Olarte MV, Burton SD, Swita M, Padmaperuma AB, Ferrell J, Ben H. Determination of Hydroxyl Groups in Pyrolysis Bio-oils using ^{31}P NMR. Denver (CO): National Renewable Energy Laboratoty; **2016** March. Report No.: NREL/TP-5100–65887. Contract No.: DE-AC36-08GO28308. Sponsored by the Department of Energy.
[31] H. Ben, A. J. Ragauskas, *ChemSusChem* **2012**, *5*, 1687–1693.
[32] O. Faix, D. Meier, I. Fortmann, *Holz Roh- Werkst.* **1990**, *48*, 351–354.
[33] O. Faix, I. Fortmann, J. Bremer, D. Meier, *Holz Roh- Werkst.* **1991**, *49*, 213–219.