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Structural and Thermal Characterization of Wheat Straw Pretreated with Aqueous Ammonia Soaking

Allan H. Gao, Mahesh V. Bule, Dhrubojyoti D. Laskar, and Shulin Chen*

Department of Biological Systems Engineering, Washington State University, Pullman, Washington 99164-6120, United States

Supporting Information

ABSTRACT: Production of renewable fuels and chemicals from lignocellulosic feedstocks requires an efficient pretreatment technology to allow ready access of polysaccharides for cellulolytic enzymes during saccharification. The effect of pretreatment on wheat straw through a low-temperature and low-pressure soaking aqueous ammonia (SAA) process was investigated in this study using Fourier transform infrared (FTIR), pyrolysis−gas chromatography/mass spectroscopy (Py-GC/MS), solid and liquid state nuclear magnetic resonance (NMR), and thermogravimetry/differential thermogravimetry (TG/DTG) to demonstrate the changes in lignin, hemicellulose, and cellulose structure. After treatment of 60 mesh wheat straw particles for 60 h with 28−30% ammonium hydroxide (1:10 solid/liquid) at 50 °C, sugar recovery increased from 14% (untreated) to 67% (SAA treated). The FTIR study revealed a substantial decrease in absorbance of lignin peaks. Solid and liquid state NMR showed minimal lignin structural changes with significant compositional changes. Activation energy of control and pretreated wheat straw was calculated according to the Friedman and ASTM methods and found to be decreased for SAA-treated wheat straw, from 259 to 223 kJ/mol. The SAA treatment was shown to remove significant amounts of lignin without strongly affecting lignin functional groups or structure.

KEYWORDS: *soaking aqueous ammonia pretreatment, wheat straw, lignin, structural and thermal characterization*

INTRODUCTION

Lignin is a complex aromatic heteropolymer that in combination with hemicellulose forms a matrix which accounts for 18−40% of the total dry weight of the plant.[1,2] Removal of the lignin matrix is crucial for the accessibility of hydrolytic enzymes, which produce monomeric sugars for further use. Current pretreatment processes, including dilute acid, steam explosion, and ammonia fiber explosion (AFEX), focus on using high temperature and high pressure, which apply heat or chemicals for the partial separation of the lignin fraction from the hemicellulose and cellulose fractions.[3,4] These pretreatments tend to solubilize the hemicellulose, resulting in the hemicellulose fraction being recovered in liquid form and requiring additional steps to purify and use. Hemicellulose comprises 20−50% of the polysaccharides in lignocellulosic material and is therefore a very important biomass fraction for biofuel production in a biorefinery.[5] In addition, high-pressure and -temperature pretreatment methods require a high-pressure vessel design and high energy input, increasing the overall cost of biofuel production. The combined energy cost of the size reduction and pretreatment presents one of the major barriers to making lignocellulosic biofuel an economic and feasible option.

Soaking aqueous ammonia (SAA) is regarded as a valuable pretreatment methodology due to the retention of the hemicellulose fraction and removal of lignin after pretreatment.[6] With current technologies for ethanol production remaining very costly,[4,7] new methods need to be examined for opportunities to lower the cost of pretreatment. SAA is a low-temperature and -pressure treatment that has been shown to be able to remove 74% of the lignin from corn stover while retaining >85% of the xylan and nearly 100% of the glucan.[8] This allows easy downstream utilization of sugars in a single cofermentation process in which net sugar yield was increased due to the presence of hemicellulose.[9]

Previous work on ammonia soaking of cellulose fibers has described a fiber expansion theory that is speculated to be responsible for the increased susceptibility of biomass to enzymatic hydrolysis after ammonium hydroxide pretreatment. The insertion of ammonia molecules is proposed to cause a structural shift, generating cellulose III from cellulose I. This structural separation is believed to cause increased susceptibility to hydrolysis by cellobiohydrolase.[10] Additionally, ammonia is thought to act selectively with lignin bonds, especially C−O−C bonds, as well as ester and ether bonds,[8] which selectively reduces lignin in the lignocellulose structure.

Aqueous ammonia as a chemical has several desirable traits that make it a good candidate for an industrial platform. Flash evaporation is sufficient to remove most of the ammonia from the pretreatment mixture, making the unbound ammonia easily recoverable and recyclable,[11] which is a key feature in the ammonia-based pretreatment scheme. Additionally, ammonia is relatively inexpensive, and minimal harmful byproducts that could inhibit enzymatic hydrolysis are created from the interaction of aqueous ammonia with lignocellulosic biomass.[12]

In comparison to other pretreatment technologies, SAA has both advantages and disadvantages. Most technologies (AFEX, steam explosion, hydrothermal, and dilute acid) require a high operating pressure, usually between 5 and 20 bar.[4,13] Typical

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ammonia freeze explosion and steam explosion treatments require temperatures in the range of 130−200 °C and pressures of 12−41 bar.[14] Sudden release of the high pressure to atmospheric values and turbulent flow defiberizes and fragments the lignocellulosic material, increasing surface area and separating the components of the plant material. Steam explosion, however, does not degrade all of the lignin in the plant cell wall and also requires particle size reduction prior to pretreatment. Furthermore, the inhibitory byproducts produced during such pretreatment processes can later negatively affect enzymatic hydrolysis.[14] In contrast, SAA can be performed effectively at temperatures as low as 40 °C and under atmospheric pressure conditions.[12] The process is significantly longer, though, with a lengthy pretreatment time of 8 h required to obtain good sugar yields. In contrast, steam explosion and AFEX require only 10−20 min.

SAA pretreatment investigation involves optimization of time and temperature for higher yields of sugar from the resulting cellulose, and previous studies have focused primarily on corn stover and barley straw. Studies have shown that SAA at room temperature requires a relatively long period of time to be effective, generally between 10 and 15 days.[8] A further study using temperatures between 40 and 90 °C showed that 24 h of reaction time at 60 °C could result in a net delignification of around 70%.[15] The systematic use of mechanistic analyses will be very beneficial for further understanding of the SAA process. To the best of our knowledge there is a scarcity of literature on the detailed study of lignin modification/structural changes after SAA pretreatment. The main purpose of this study is to investigate structural and compositional changes of wheat straw after SAA pretreatment by different analytical techniques. Pyrolysis−gas chromatography/mass spectroscopy (Py-GC/MS) techniques have been used in the past with great effectiveness for the characterization and identification of lignin deconstruction products >50 kDa[16] and, in combination with Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) (solid and solution state) analysis, will allow the identification of intermediate products as well as lignin components. Thermogravimetric (TG) data will also be used to detect changes in the thermal behavior of pretreated wheat straw and have been widely used in thermal kinetic studies of biomass.[17,18] The results of this study will aid in the evaluation of the process by which SAA works to increase hemicellulose and cellulose digestibility.

MATERIALS AND METHODS

Pretreatment of Wheat Straw by SAA. Wheat straw was pretreated by SAA using 28−30% (w/w) ammonium hydroxide solution (JTB-9721-03 ammonium hydroxide, 28−30%) with a solid to liquid ratio of 1:10. The samples were stirred thoroughly after they had been transferred to 500 mL screw-cap Pyrex solution bottles and kept at 50 °C for 60 h with no agitation. Studies were performed for different time periods of soaking of biomass (6, 12, 24, 36, 48, 60, 72, and 84 h; data not shown), and sugar recovery improved up to 60 h of treatment time. Thus, the time of pretreatment was chosen to be 60 h to obtain the maximum effect of soaking ammonia pretreatment for characterization. After the incubation was complete, the wheat straw was washed twice with deionized (DI) water and vacuum filtered. The first wash consisted of 100 mL of DI water per 3 g of wheat straw, followed by 200 mL of DI water for the second wash; these solutions were mixed together and were used to analyze sugar loss due to SAA treatment. Wheat straw used in the process was obtained courtesy of the Grange Supply Co. in Pullman, WA, and hammer milled at the Wood Materials and Engineering Laboratory, Washington State University. To obtain the maximum possible effect on lignin structure and efficiency, the wheat straw was sieved through a 60 mesh (~0.25 mm) Tyler standard screen scale, and the resultant <60 mesh particles were used for the study.

Carbohydrate Content Analysis of Wheat Straw. Control and pretreated wheat straw samples were dried for 24 h at 105 °C, and then 5 g of material was extracted with ethanol/toluene (2:1 v/v, 8 h) to make it extractive free. Analysis of the carbohydrate content of both wheat straw samples was performed according to NREL's Laboratory Analytical Procedure:[19] 0.3 g of sample was prepared and used in a two-stage acid hydrolysis method. Sugars in the aqueous phase were quantified using ion chromatography (Dionex ICS-3000 with Dionex Pac PA20 column and CarboPac PA20 guard column). Samples were run for 60 min, and the column was flushed between each sample with 100% 200 mM NaOH followed by DI water. Sugar concentration was quantified by measurement against a standard sugar sample, and all of the measurements reported were taken in triplicate.

Acid-Soluble and -Insoluble Lignin Content Analysis and Lignin Extraction. Acid-soluble and -insoluble lignin content was calculated according to NREL's Laboratory Analytical Procedure.[19] Extractive-free cell wall residue was prepared from milled wheat straw (fraction <60 mesh) using ethanol/toluene (50:50) for 24 h, followed by solvation in ethanol (100 mL/g) and water (100 mL/g) for 24 h each. The processed biomass was then freeze-dried and ball-milled in the presence of toluene to avoid any further changes in lignin structure. The ball-milled sample was dried and extracted with 94:6 dioxane/water. The resulting preparation underwent purification for wheat straw lignin extraction as per Björkman's method.[20] The purified lignin was used for liquid state NMR analysis.

Enzymatic Hydrolysis of Wheat Straw Samples. The enzymatic digestibility of ammonia-pretreated wheat straw samples was assessed in triplicate according to the NREL 2008 Laboratory Analytical Procedure (LAP) for Enzymatic Saccharification of Lignocellulosic Biomass. An amount of 0.2 g of sample was treated with 30 FPU/g of cellulase (Novozymes NS 50013) and 30 CBU/g of β-glycosidase (Novozymes NS 50010) and incubated at 50 °C at pH 4.8 in 20 mL of 0.05 M sodium citrate buffer containing 100 μL of 2% sodium azide. The flasks containing the enzymatic hydrolysis preparations were set in an orbital incubator shaker (Gyromax 747) and incubated for 72 h. The total released sugars after 72 h of digestion were used to calculate sugar recovery and enzymatic digestibility. This process was repeated for control wheat straw without SAA pretreatment.

FTIR Analysis. FTIR analysis was performed for analysis of functional groups in control and 60 h SAA pretreated wheat straw samples. FTIR spectra were obtained on an FTIR spectrophotometer (Shimadzu) with 64 scans. Samples were analyzed from 4000 to 800 cm^{-1} at a resolution of 4 cm^{-1}.

Solid State ^{13}C Cross-Polarization/Magic Angle Spinning (CP/MAS) NMR Analysis. The ^{13}C CP/MAS solid state NMR analysis was carried out on both the control and ammonia-pretreated wheat straw biomass without further degradation and/or isolation of its individual components. The finely ball-milled tissues (250 mg) of the control and corresponding SAA-treated wheat straw biomass tissues were individually packed in a 5 mm pencil type rotor, and the spectra were recorded under identical acquisition parameters. The solid state ^{13}C CP/MAS analysis was carried out at 100 MHz on a Bruker Avance 400 spectrometer (NMR Center, Washington State University), equipped with a Chemagnetics double-resonance probe. A contact time of 0.5 ms, proton field of ca. 40 kHz during CP and data acquisition, relaxation delay of 4 s, and spinning speed of 5 kHz were applied to obtain the ^{13}C CP/MAS spectra. All of the corresponding ^{13}C CP/MAS spectra were derived from 18500 scans, with the chemical shifts given in δ (parts per million).

Solution State NMR of Lignin-Enriched Isolates. The solution state 1D NMR spectra of lignin isolates were recorded on a Varian 600 MHz spectrometer operating at 599.69 MHz for proton (^1H) and at 150.8 MHz for carbon (^{13}C), respectively, with the resonance values given in δ. The lignin isolates (~25 mg) from both control and ammonia-pretreated wheat straw samples were individually dissolved in DMSO-d_6 (0.5 cm^3), with the ^1H and ^{13}C NMR spectra individually

recorded. The ^{1}H NMR spectra of both the lignin isolates were recorded individually with a spectral width of 12000 Hz in 256 scans, whereas the ^{13}C NMR spectra were collected with a sweep width of 29304 Hz in 26000 scans. All of the structural analyses were carried out by comparison of chemical resonance values for SAA-pretreated lignin samples to its control.

Py-GC/MS Analysis. Both the control and 60 h SAA pretreated wheat straw samples were analyzed using a CDS Pyroprobe 5000 connected to an Agilent GC/MS. The samples were loaded after a brief incubation at 200 °C to remove residual oxygen before pyrolysis of the sample. Samples were then pyrolyzed at 610 °C for 1 min, with the resulting vapors separated by a 30 mm × 25 mm i.d. (5%-phenyl)-methylpolysiloxane hydrophobic column, with a split ratio of 50:1. The gas flow rate was 1 mL/min, and the oven was retained at 280 °C for 10 min to prevent any residuals from remaining in the chamber. This gas was then analyzed with a mass spectrometer (Inert XL MSD, Agilent Technologies), and CO_2 was used as the internal standard. The abundance of each derived compound was referenced against the area of the internal standard, and compounds were identified through comparison of their mass spectroscopy spectra with a database.

Thermogravimetry/Differential Thermogravimetry (TG/DTG) Analyses. It is important to study the effect of pretreatment on downstream applications. TG and DTG analyses are useful tools to generate information of pretreated biomass to develop thermochemical technologies. Hence, control and SAA-pretreated samples were analyzed using TG and DTG to determine the change in activation energy (E) and pre-exponential factor (A). The analysis was performed by a Mettler Star system (Mettler Toledo TGA/SDTA 851, Switzerland) using five heating rates: 10, 15, 20, 30, and 40 °C/min. All of the experiments were performed in triplicate under a nitrogen atmosphere with a flow rate of 20 mL/min. On the basis of the different heating rates, activation energy and pre-exponential factor were calculated using ASTM[21] and Friedman equations.[22]

Both of these analysis methods use a differential method requiring the assessment of multiple heating rates and were chosen due to the limitations of obtaining biomass kinetic parameters from a single heating rate.[23] The Friedman method is an isothermal analysis process that can be used to calculate the activation energy (E) and pre-exponential factor of the samples (A) and is expressed as

$$\ln\left(\frac{d\alpha}{dt}\right) = \ln f(\alpha) + \ln A - E/RT \quad (1)$$

where $(d\alpha)/dt$ is the instantaneous reaction rate, R is the gas constant, A is the pre-exponential factor, α is the mass fraction remaining at time t, and $f(\alpha)$ is the function regarding the reaction mechanism.

The ASTM method uses an integral method and the assumption that decomposition obeys first-order kinetics to evaluate activation energy and pre-exponential factor, where

$$E = -\left(\frac{R}{b}\right) \times \Delta(\log \beta)/\Delta\left(\frac{1}{T}\right) \quad (2)$$

$$A = \left(-\frac{\beta'}{E_r}\right) \times R \times \ln(1-\alpha) \times 10^{a} \quad (3)$$

where b is the approximate derivative given by the ASTM table of integration constants, β is the heating rate, β' is the heating rate nearest the midpoint of the chosen experimental heating rates, E_r is the refined Arrhenius activation energy, and a is the approximation integral taken from the ASTM table of integration constants.

RESULTS AND DISCUSSION

Compositional and Enzymatic Hydrolysis Analysis. Table 1 describes the sugar and lignin contents before and after the 60 h and 50 °C SAA pretreatment. Approximately 46% (w/w) of the untreated wheat straw is available as convertible carbohydrate, which is comparable to other results.[24,25] It was observed that carbohydrate content was increased to 53% (w/w) after pretreatment, which could be due to removal of lignin compounds and removal of extractives from the sample. The soluble lignin content of pretreated wheat straw was increased by 98% (w/w) as compared to control sample. Earlier SAA pretreatment studies performed on corn stover, barley hull, and straw had observed that the cellulose fraction was retained almost entirely, although a small amount of xylan was lost, typically between 10 and 20% (w/w).[6] The relatively high retention of the hemicellulose fraction allows use of C5 sugars in a cofermentation process, increasing overall sugar yield. Table 1 also describes the results obtained from enzymatic hydrolysis of the control and SAA-treated samples. Sugar recovery from the untreated wheat straw was low, as expected, and the 60 h pretreated samples resulted in a 67% (w/w) sugar recovery. The long reaction time in this study will need to be reduced, either through an increase in the temperature or pressure of the pretreatment conditions or through the addition of a following pretreatment step. The SAA pretreatment (with 15% (w/w) ammonia concentration) performed on barley hull resulted in 83 and 62% glucan and xylan digestibility, respectively, at a temperature of 75 °C and with 48 h of pretreatment.[26]

Table 1. Sugar and Lignin Analysis of Untreated and SAA-Treated Wheat Straw with Enzymatic Hydrolysis Data

component	control wheat straw[a]	SAA-treated wheat straw[a]
sugars		
arabinose	2.09 ± 0.09	2.7 ± 0.08
galactose	0.51 ± 0.07	0.48 ± 0.03
glucose	31.03 ± 1.63	36.83 ± 0.70
xylose	11.96 ± 0.26	12.96 ± 0.60
total sugars[b]	45.6 ± 1.52	52.97 ± 1.20
lignin		
acid insoluble	24.83 ± 1.76	19.55 ± 0.55
acid soluble	4.25 ± 0.24	8.45 ± 0.08
% sugar recovery after enzymatic hydrolysis	14.26 ± 0.02	66.93 ± 1.31

[a]Values in % (w/w). [b]Total sugars in biomass before enzymatic hydrolysis.

FTIR Analysis. FTIR spectroscopy was further used to analyze structural changes of untreated and SAA-treated wheat straw. Figure 1 shows the view of the wavelength region from 800 to 4000 cm^{-1} with bands of interest identified by their

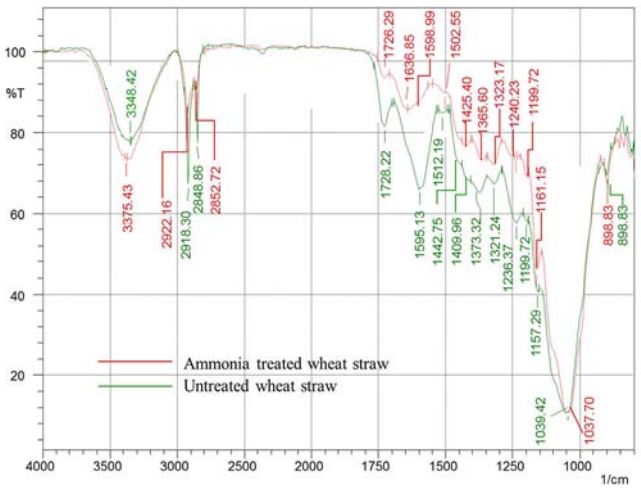

Figure 1. FTIR of untreated versus SAA-treated wheat straw.

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Table 2. Assignment of FTIR Absorption Bands of Untreated and Aqueous Ammonia Treated Wheat Straw[a]

		untreated wheat straw		ammonia-treated wheat straw	
λ (cm^{-1})	attribution and description of FTIR absorption	λ (cm^{-1})	T (%)	λ (cm^{-1})	T (%)
3550−3200	O—H stretching vibrations of polymer	3348.42	77.66	3375.43	73.41
3000−2850	C—H stretching vibration (CH_3, CH_2)	2918.30	67.41	2922.16	84.69
		2848.86	77.52	2852.72	90.84
1740−1720	C═O stretching vibrations	1728.22	81.62	1726.29	93.39
1640−1636	C═O stretching vibrations, characteristic of para-substituted aryl skeleton			1636.85	85.65
1600−1590	C═O stretching and aromatic vibrations; S > G; condensed G > etherified G	1595.13	66.21	1598.99	87.39
1513−1500	aromatic skeletal vibrations; G > S	1512.19	84.78	1502.55	89.81
1450−1400	aromatic skeletal vibrations	1442.75	71.93	1425.40	76.72
		1409.96	68.02		
1375−1360	C—H deformation of cellulose and hemicellulose	1373.32	65.10	1365.60	73.20
1330−1320	C—H vibration in cellulose and C—O vibration of S ring	1321.24	67.35	1323.17	71.98
1241−1236	methoxyl, C—C, and C—O stretching vibrations; C═O stretching vibration	1236.37	57.69	1240.23	74.04
1200−1199	symmetric stretching C—O—C glycoside	1199.72	58.12	1199.72	69.37
1162−1157	C—O—C vibrations in cellulose and hemicellulose	1157.29	40.63	1161.15	46.07
1048−1036	C—O stretch in cellulose	1039.42	10.85	1037.70	10.59
899−887	C—H deformation in cellulose	898.83	69.59	898.83	66.55

[a] λ, wavelength; T, transmittance.

wavenumber. Comparison of the FTIR results (Table 2) of the untreated and SAA-treated wheat straw was performed according to previous literature.[27−29] The untreated wheat straw showed strong hydrogen bond (O−H) stretching at 3348.42 cm^{-1} and C−H stretching vibrations at 2918.30/2848.86 cm^{-1}, whereas the same characteristics were noted at 3375.43 and 2911.16/2852.72 cm^{-1}, respectively, in SAA-treated sample. Figure 1 clearly shows that ester linkage absorbance at 1726.29 cm^{-1} of SAA-treated sample decreased (T%, 90.84) as compared to untreated wheat straw sample absorbance at 1728.22 cm^{-1} (T%, 81.62). A distinct peak was observed at 1636.85 cm^{-1}, which is the characteristic band for carbonyl stretching of a para-substituted ketone or aryl aldehyde,[30] which indicates the presence of an unconjugated carbonyl group. The absorption bands at 1595.13 cm^{-1} (T% 66.21) and 1512.13 cm^{-1} (T% 84.78) of untreated wheat straw, which represents the aromatic skeleton of lignin, have been decreased and can be seen at 1598.99 cm^{-1} (T% 87.39) and 1502.55 cm^{-1} (T% 89.81) for the SAA-treated sample. Furthermore, decreased relative band absorption was observed at 1442.75/1409.96 and 1425.40 cm^{-1} for untreated and SAA-treated wheat straw, respectively; this absorption corresponds to aromatic ring vibrations of lignin.[25] In the SAA-pretreated wheat straw sample a decreased intensity of C−O vibration of S-rings was observed at 1323.17 cm^{-1} (T% 71.98) as compared to untreated sample at 1321.24 cm^{-1} (T% 67.35). In untreated wheat straw the intensity of absorption at 1236.37 cm^{-1} was much stronger than in treated wheat straw, suggesting a high guaiacyl content in the original straw. These results are in agreement with the observation of Lawther et al.,[27] who observed the same trend with sodium hydroxide pretreatment. The characteristic peak of cellulose for C−O stretch/C−H deformation was observed to be identical at 1039.42/898.83 and 1037.70/898.83 cm^{-1}, respectively, for untreated and SAA-treated samples.

Solid State ^{13}C CP/MAS NMR. Figure 2 shows the solid state NMR spectra for untreated and SAA-treated wheat straw. Four regions of the spectra are outlined, showing the lignin, anomeric carbon, methoxyl, and aliphatic resonances. Although the spectra shown do not provide quantitative information,

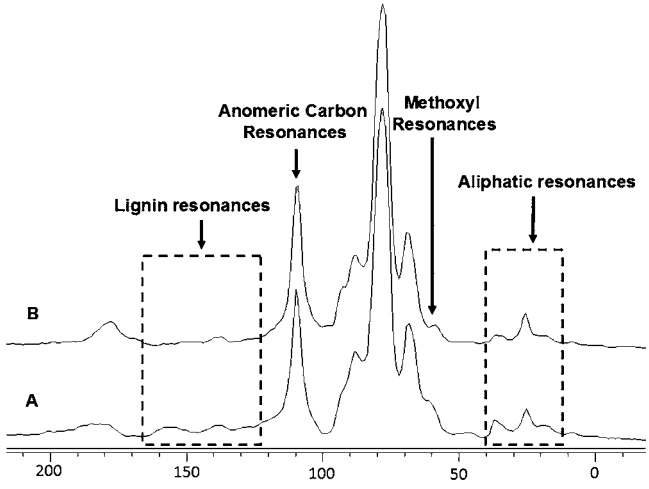

Figure 2. Solid state NMR spectra of control (A) and SAA-pretreated wheat straw (B) samples.

spectra for both show characteristic peaks that correspond to components such as cellulose, hemicellulose, and lignin. Additionally, identical acquisition parameters were used to obtain these spectra and the same amount of sample was used, allowing the comparison of the resultant changes in the spectra. The methoxyl region at 60 ppm in the spectra showed the greatest change, with a decline in the intensity for the SAA-treated samples corresponding to removal of methoxyl resonances. Comparison of the lignin resonance region between 130 and 160 ppm in both the spectra also revealed a lowered intensity in the SAA-pretreated sample (Figure 2). The region at 160 ppm, which is representative of phenolic rings substituted with methoxyl groups, was observed to decrease. This indicated the degradation and/or decrease in lignin structures in the biomass and is supported by the evidence of lignin removal from SAA-treated biomass as shown in the compositional analysis. However, the overall resonance pattern of the spectra was observed to be the same, so the major lignin structure of the biomass likely did not change. The resonances of the carbohydrate and anomeric carbon regions

were observed to be identical for both samples, which showed SAA treatment had less effect on hemicellulose and cellulose structure.

Solution State ^{1}H and ^{13}C NMR Spectroscopy of Lignin Isolates. The ^{1}H NMR spectra (Figure 3) were acquired using identical parameters for lignin isolates of both untreated and SAA samples, respectively. The aromatic regions between 6.5 and 8 ppm (Figure 3) are lignin derived.[31] The pattern in the aromatic region observed with both the control and SAA-treated wheat straw lignin isolates indicated that the chemical resonances were identical. A significant change observed around δ 7.5 could be due to removal of substituents of the lignin aromatic ring. The proton resonance down-shifted to 7.5 ppm is next to methoxyl or other electron-withdrawing groups, and the diminished resonance in this area could be due to removal methoxyl groups from syringl (S) and guaiacyl (G) subunits. Comparison of carbon spectra (Figure 4) showed less significant change in S and G lignin aromatic regions, which again suggested that major lignin structures were not changed significantly after pretreatment with SAA. The non-lignin-derived peaks observed in the spectra (marked by ×) were excluded during comparison. Importantly, the oxygenated aliphatic regions of both spectra look the same, which emphasizes the lack of side-chain modification in the lignin structure.

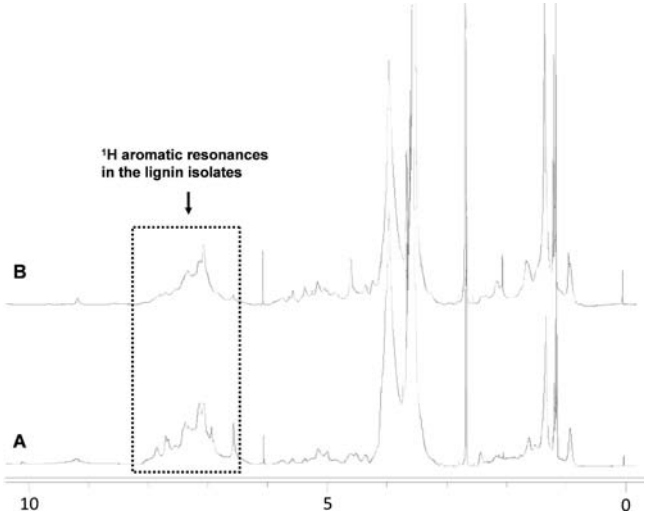

Figure 3. ^{1}H NMR spectra from lignin isolates for control (A) and SAA-pretreated wheat straw (B).

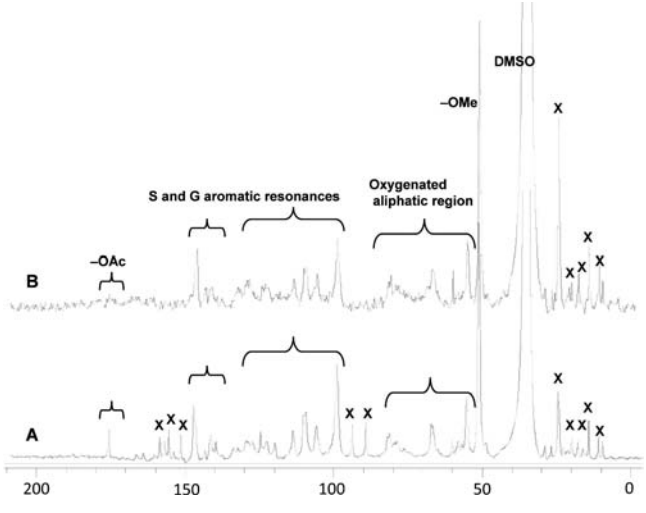

Figure 4. $^{13}CP/MAS$ spectra of control (A) versus SAA-treated (B) samples.

Lignin degradation appeared to occur nonspecifically with minimal qualitative changes. Instead, the overall content of lignin was reduced in SAA-pretreated wheat straw. Figure 4 showed G or S lignin subunits were not changed specifically; this was also evidenced from solid state NMR studies.

Py-GC/MS. Figure 5 depicts the Py-GC/MS chromatograms of the untreated and 60 h SAA treated samples. Low molecular weight monomers produced during pyrolysis of the biomass were identified by their diagnostic mass spectra and indicate the presence of lignin-derived products. The total relative percentage of lignin components was calculated and showed a significant decrease in both G and S units from 31 and 16% in the control to 18 and 7.5% in the SAA-pretreated sample (Supporting Information). The G/S ratio after pretreatment was not affected substantially, changing from 1.96 for the control to 2.43 in the SAA-treated wheat straw. This indicates that lignin was removed nonspecifically. These results were supported by the NMR and FTIR analyses. In the figure, peak 22 shows a dramatic change in content of 2-methoxy-4-vinylphenol, a G-lignin-derived unit, with relative content decreased from 14.1 to 4.8%, for control and pretreated samples, respectively. The middle lamella of plant cell walls is known to be high in guaiacyl subunits,[2] and the reduction in peak area of this compound could be related to removal of much of the lignin from the same section of the wheat straw. Previous studies of the effect of SAA pretreatment on rice straw demonstrated that this method promotes the removal of external fibers.[32]

Additionally, the total relative percentage of *p*-hydroxyphenol (H) units increased from 32 to 42% in pretreated lignin. However, the presence of several H-derived compounds detected in the untreated wheat straw was not found in the SAA-pretreated wheat straw. These results are corroborated by previous studies[33] which demonstrated that treatment of wheat straw with 1.5% NaOH resulted in release of 2−3 times more esterified ferulic acid compared to *p*-coumaric acid.

TG/DTG Analyses. Four typical combustion stages are known to exist during thermal decomposition of wheat straw: A, dewatering period; B, volatilization and burning; C, char burning; and D, burnout.[34] The dewatering period ends at around 390−420 K (117−147 °C), and at this point the wheat straw is completely dry. In this study, TG calculations were performed on the basis that the mass percent at 150 °C is the actual, or dewatered, mass of the wheat straw. Additionally, we considered only the values of E before the 80% conversion ratio of the samples, calculated at 5% conversion intervals. Wheat straw entered the char burning stage around 705−770 K, which correlated to 75−80% conversion, depending on the heating rate. The activation energy during the char burning stage was roughly double that during the volatilization and burning stage; inclusion of these data would lend error to the average activation energy calculation.

Figure 6 presents the TG and DTG curves obtained from the five heating rates used in the experiment for control and SAA-treated wheat straw (panels A and B, respectively). Wheat straw decomposition commenced around 200 °C, and the highest DTG peak was observed at 320 °C for untreated and at 370 °C

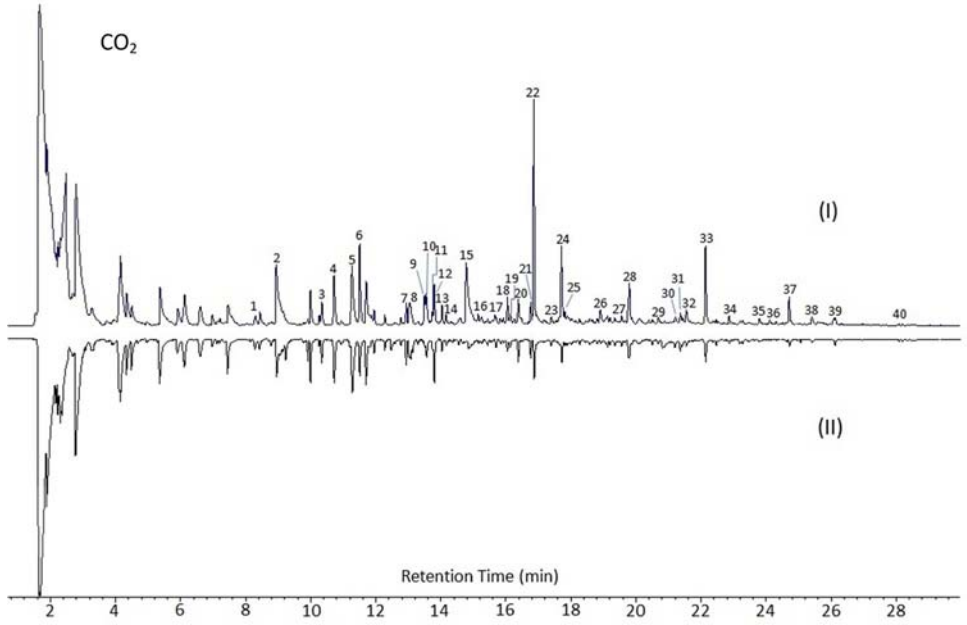

Figure 5. Pyrolysis–gas chromatography traces of control (I) and SAA-treated (II) wheat straw. CO_2 was used as an internal standard. Unlabeled peaks represent non-lignin-derived structures. Nomenclature for all compounds is listed in Supplementary Table 1 (Supporting Information).

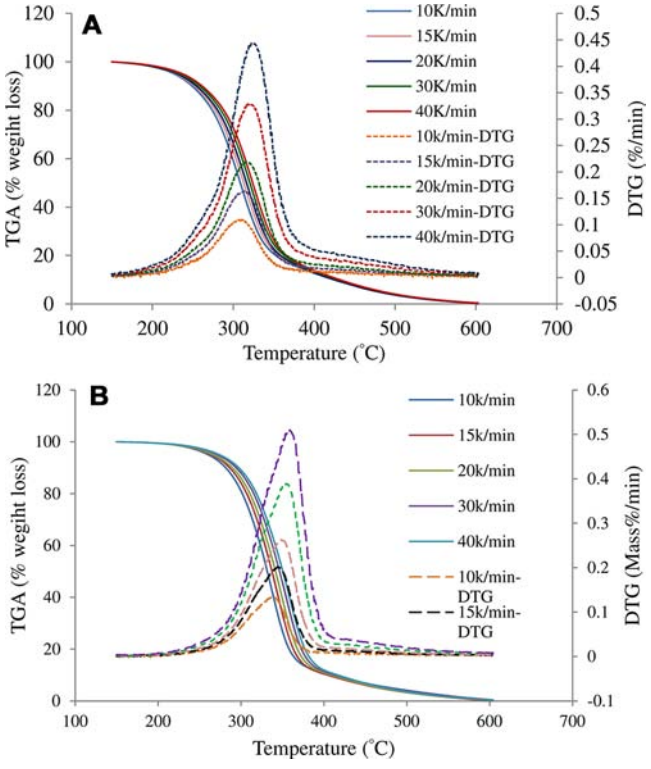

Figure 6. Thermogravimetric analysis of (A) untreated wheat straw and (B) SAA-treated wheat straw with DTG curve.

for SAA-treated wheat straw. The primary peak between 350 and 400 °C corresponds to the decomposition of cellulose,[35] whereas hemicellulose and xylan components decompose between 200 and 300 °C. Decomposition of the SAA-treated wheat straw reached a higher overall rate, and the initial decomposition temperature was observed to shift upward. This could be due to quantitative change in lignin and cellulose, which was supported by Py-GC/MS. Despite an increase in temperature for the reaction rate of the main peak, overall activation energy calculated by both the ASTM and Friedman methods decreased (Table 3). The average activation energies were found to be 260 and 223 kJ/mol for control and SAA-treated wheat straw, respectively. Activation energy calculations differed minimally between the ASTM and Friedman methods. The Friedman method produced a consistently higher activation energy; however, the difference was no more than 10% at any given conversion amount.

Table 3. Average Activation Energy (E) and Pre-exponential Factor (A) for Untreated and SAA-Treated Wheat Straw Calculated by ASTM and Friedman Methods

	ASTM		Friedman	
	untreated	SAA treated	untreated	SAA treated
E (kJ)	259.74	223.14	270.08	226.98
A	2.00×10^{24}	2.46×10^{24}	2.92×10^{23}	7.87×10^{18}
av log(A)	22.86	22.93	23.47	18.90

The activation energy according to stage of decomposition is shown in Figure 7. Prior to 40% conversion, the activation

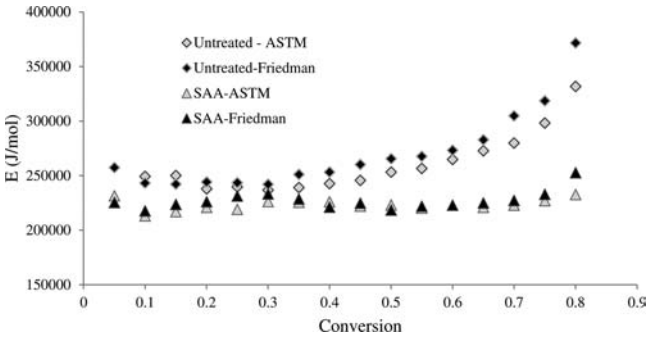

Figure 7. Activation energy of samples compared to conversion amount.

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energy values for both the control and pretreated wheat straws were similar. As the conversion reached 80%, a decreased trend was observed for activation energy with the SAA-treated sample. This difference could be due to the removal of lignin in the SAA process, because lignin contributes the most in the later decomposition stage.[34] The change in activation energy could also be due to the separation of lignin from cellulose in the wheat straw, which is supported by a higher peak in the DTG compared to the control. Additionally, previous papers have indicated that the structure of cellulose III, which results from the ammonia pretreatment, is significantly more amorphous than that of crystalline cellulose I.[10] It is thus possible that cellulose III has a weaker bond structure than cellulose I, resulting in a lower thermal activation energy and resultant change in degradation pattern. The compositional analysis also showed a relative increase in the amount of available sugars after the 60 h SAA pretreatment and a decrease of lignin content with respect to total biomass. The decrease in activation energy could prove useful in further pyrolysis studies of wheat straw and also demonstrates the function of SAA in lowering the energy requirement for decomposition of the pretreated wheat straw.

The effects of SAA pretreatment on wheat straw were studied, and structural and compositional changes as well as sugar yield after pretreatment were determined. A significant amount of wheat straw lignin was removed or solubilized; however, minimal structural changes were detected in the lignin subunits. These results were further verified by solid and solution state NMR, FTIR, and Py-GC/MS analyses. The thermal characterization of the pretreated wheat straw demonstrated a lower activation energy demand required for thermal decomposition. SAA pretreatment appears to have a strong effect on degrading lignin on the surface of the wheat straw to improve cellulose accessibility and sugar yield. However, the length of time required for effective pretreatment at lower temperatures appears to be a major factor for the SAA pretreatment.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1-(509)-335-3743. Fax: +1-(509)-335-2722. E-mail: chens@wsu.edu.

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