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Degradation of Carbohydrates during Dilute Sulfuric Acid Pretreatment Can Interfere with Lignin Measurements in Solid Residues

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ABSTRACT: The lignin content measured after dilute sulfuric acid pretreatment of corn stover indicates more lignin than could be accounted for on the basis of the untreated corn stover lignin content. This phenomenon was investigated using a combination of ^{13}C cross-polarization/magic-angle spinning (CP/MAS) solid-state nuclear magnetic resonance (NMR) spectroscopy and lignin removal using acid chlorite bleaching. Only minimal contamination with carbohydrates and proteins was observed in the pretreated corn stover. Incorporating degradation products from sugars was also investigated using ^{13}C-labeled sugars. The results indicate that sugar degradation products are present in the pretreatment residue and may be intimately associated with the lignin. Studies comparing whole corn stover (CS) to extractives-free corn stover [CS(Ext)] clearly demonstrated that extractives are a key contributor to the high-lignin mass balance closure (MBC). Sugars and other low molecular weight compounds present in plant extractives polymerize and form solids during pretreatment, resulting in apparent Klason lignin measurements that are biased high.

KEYWORDS: *compositional analysis, lignin, mass balance closure, pretreatment*

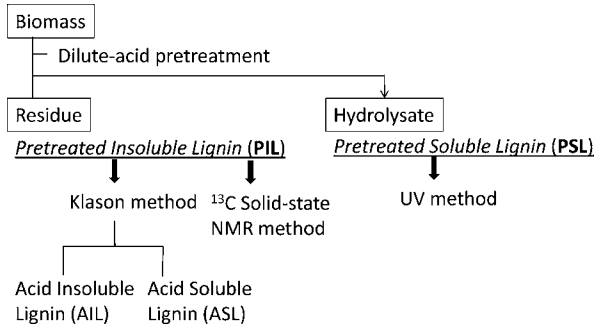
INTRODUCTION

Herbaceous materials such as corn stover are gaining increasing importance as bioenergy feedstocks because they are carbon-neutral, renewable, and not already used in the food supply. The feedstock lignin content is one of the most important factors because lignin acts as a barrier for the enzymatic hydrolysis process, with lower lignin contents in untreated feedstocks leading to higher ethanol yields.[1−4]

Accurate lignin measurements are necessary to complete the mass closure in biomass during a pretreatment process, to identify better herbaceous feedstocks for ethanol production, and to find optimal pretreatment conditions. Several analytical methods have been developed to measure lignin content in raw herbaceous feedstocks and woody materials. The Klason lignin method is frequently used to quantify lignin content, not only in wood but also in herbaceous materials.[5−8] In the Klason method, biomass is hydrolyzed in a two-stage sulfuric acid digestion, and Klason lignin is defined as the solid residue remaining at the end of the procedure. Klason lignin uses a behavior-based definition of lignin and is prone to interferences by components with similar behavior during the acid digestion steps. Total lignin content is calculated as the sum of the Klason lignin and the acid-soluble lignin that is solubilized during the Klason method and is measured by ultraviolet (UV) spectroscopy.[7−10] An alternative method to determine lignin content in biomass materials is the acetyl bromide method, which is also widely used as it is rapid and simple and uses only a small amount of sample.[11−16] In the acetyl bromide method, complete dissolution of lignocellulolytic samples in acetyl bromide is required, and the UV absorbance at 280 nm in the solution is measured. ^{13}C cross-polarization/magic-angle spinning (CP/MAS) solid-state nuclear magnetic resonance (NMR) spectrometry has been used to determine the lignin content in wood and wood products.[17−20] Solid-state NMR has also been used for analyzing lignin chemical structures and for determining syringyl/guaiacyl ratio in hardwoods.[21−23]

During the dilute-acid pretreatment process, part of the biomass is solubilized to provide greater access to the carbohydrates for subsequent processing. A portion of the lignin is released into solution during the pretreatment, and a portion remains behind with the residue (Scheme 1). For purposes of clarity, the lignin that remains in the residue after pretreatment will be called pretreated insoluble lignin (PIL), and the portion that is solubilized during pretreatment will be called pretreated soluble lignin (PSL).

Scheme 1. Nomenclature of Lignin Determinations for Each Fraction Isolated after Pretreatment



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Previously, the lignin content of corn stover residue was determined using the Klason method after dilute-acid pretreatment with a range of severity conditions.[24] It was significantly higher than expected on the basis of the lignin contents measured in the nonpretreated corn stover; that is, the lignin mass balance exceeded 100%. The high lignin content in the pretreated material indicates interfering compounds are being produced during the dilute-acid pretreatment and/or the Klason lignin process. Ellis has suggested that protein in the raw material could be condensed with lignin during the Klason lignin process to yield high lignin contents.[25] Dence has reported that the unexpectedly high lignin content obtained by the Klason method may be caused by carbohydrate degradation products such as furfural and 5-hydroxylmethyl furfural, because those products may be condensed with lignin under acidic conditions.[5] Recently, Sievers et al. reported an overestimation of lignin content in dilute-acid-pretreated loblolly pine.[26]

In this paper, the factors causing the overestimation of lignin content in dilute-acid-pretreated herbaceous material were investigated by combinations of ^{13}C solid-state NMR spectroscopy; removal of lignin using acid chlorite bleaching, removal of extractives, and pretreatment with the addition of ^{13}C-labeled sugars such as fructose, xylose, and glucose.

MATERIALS AND METHODS

Materials. Whole corn stover (Pioneer hybrid 33B51) was knife-milled by a Wiley mill to pass through a 20 mesh screen and then extracted with water and ethanol at 100 °C and 1500 psi by an accelerated solvent extractor (Dionex ASE200, Sunnyvale, CA, USA). The corn stover had water and ethanol extractives of 14.6 and 2.12 wt %, respectively, for a total of 16.7 wt %. The sucrose content in water extractives was measured by a YSI 2700 Select Biochemistry Analyzer to be 4.14 wt %. Fructose content was determined to be 2.07 wt % in the corn stover. Sample moisture content was determined by a moisture analyzer (Mettler Toledo, HR83). The lignin contents of the whole corn stover (CS) and extractives-free corn stover [CS(Ext)] were measured by the composition analysis to be 11.7 and 14.0 wt %, respectively.[27]

Commercial grade sulfuric acid was used. Monosaccharides [D-(+)-fructose, D-(+)-xylose, and D-(+)-glucose] and D-glucose-1-^{13}C were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively. D-Fructose-2-^{13}C and D-xylose-1-^{13}C were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

Dilute-Acid Pretreatment. Ground corn stover (0.3 g) was impregnated in 1.2% (w/w) sulfuric acid (3 mL) overnight in a glass tube. The ratio of solid to solvent was 1:10 (g/g). For pretreatment, the glass tube with corn stover was sealed with a silicone cap and an aluminum cover and was inserted in a stainless steel vessel with water. The vessel was heated at 160−200 °C for 2 or 5 min in a sand bath (Techne, Fluidised Bath SBL-2D). After heating, the vessel was cooled in ice water to room temperature, and the suspension was filtered. The solid residue was washed with deionized water until the filtrate was pH 7.0. The filtrate was adjusted to 100 mL with deionized water and analyzed to determine remaining monosaccharides by high-performance liquid chromatography (HPLC) (Agilent 1100 series; column, Shodex sugar SP0810; column temperature, 85 °C; mobile phase, water; flow rate, 0.6 mL/min).[27] The solid residue was dried under vacuum (22 in Hg) conditions at 40 °C overnight and then analyzed by ^{13}C CP/MAS solid-state NMR spectroscopy. Additionally, a monosaccharide spiking study was carried out to determine the extent to which the sugar degradation products were incorporated in the solid residues. Corn stover was spiked with both ^{13}C-labeled and unlabeled monosaccharides and pretreated as above. The loading amount of monosaccharide was 10 wt % of CS or CS(Ext) for ^{13}C-labeled and unlabeled fructose, 35 wt % for xylose and glucose, and 5.0 wt % for ^{13}C-labeled xylose and glucose.

Delignification with Acid Chlorite. Delignification was conducted according to a modified acid chlorite method.[28] In this technique, the acid chloride opens the aromatic ring and degrades the lignin, removing it from the solids. After Klason digestion, the Klason residue (5 g) was soaked in sodium chlorite (1.5 g)/deionized water (160 mL) solution under vacuum conditions for 0.5 h, and then acetic acid (0.5 g) was added. The suspension was stirred vigorously for 1 h at 70 °C. After the reaction was finished, the mixture was filtered and washed with deionized water to remove acid chlorite. The delignified residue was dried under the same conditions described above.

Lignin Analysis. Pretreated insoluble lignin contents were measured using the Klason method.[27] Samples were first hydrolyzed with 72 w/w % sulfuric acid in a water bath at 30 °C for 1 h with frequent mixing. The slurry was then diluted with water to a concentration of 4 w/w % sulfuric acid and hydrolyzed at 121 °C for 1 h in an autoclave. The resulting slurry was allowed to cool to room temperature before being filtered through a filtering crucible. The insoluble material was defined to be Klason lignin. The solution was analyzed for acid-soluble lignin (ASL) and carbohydrates. The structural carbohydrates were determined according to the HPLC method described above, and the ASL was measured by UV−vis spectrometer (Hewlett-Packard, UV8453, absorbance at 320 nm) according to the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedures (LAP).[27,29]

High-resolution ^{13}C CP/MAS NMR measurements were carried out using a Bruker Avance 200 MHz spectrometer operating at 50.13 MHz at room temperature. The spinning speed was 7000 Hz, variable contact pulse, using a 50% ramp on the proton channel of 2 ms, acquisition time of 32.8 ms, and delay between pulses of 1 s. Lignin content was estimated on the basis of the normalized signal area of both the lignin aromatic carbons (109−165 ppm) and the carbons in carbohydrates (0−109 and 165−240 ppm), according to a previous study.[19] Extractives, including protein and soluble carbohydrates, contribute signal intensity, which can interfere with accurate lignin determinations as demonstrated in the comparison of the lignin measurements of corn stover and extractives-free corn stover shown in Table 1. However, the lignin or lignin-like material estimated using the NMR technique is intended to monitor the increase in the solid degradation products resulting from pretreatment, which will also contribute to the aromatic intensity and be measured as lignin.

Lignin mass balance closure (MBC) was calculated on the basis of the lignin content in the original corn stover using eq 1.

$$\text{lignin MBC (\%)} = \{[\text{lignin content in the residue after pretreatment (wt \\%, based on the residue)} \times \text{residue yield (wt \\%)}]/\text{lignin content in original corn stover (wt \\%)}\} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Lignin Content after Pretreatment. In a previous study, lignin mass balances in pretreated CS under various pilot-scale conditions were determined using the Klason method.[24,30] The high lignin mass balances were confirmed using an alternative method, ^{13}C CP/MAS NMR spectroscopy, as shown in Table 1. When determined by the Klason method, lignin MBCs were between 143 and 181%, with a mean of 165%. The mean value exceeded the theoretical value of 100% and was clearly overestimated. When determined by solid-state NMR, the values for the same samples were between 95 and 141%, with a mean of 124%. A possible explanation for the difference between the NMR and total lignin values determined using the Klason lignin is that the Klason method relies on a measurement of the ASL. The solid-state NMR method does

Table 1. Comparison of Weight Percent Lignin and Mass Balance Closure (%MBC) in the Pretreated Corn Stover under Various Conditions, Determined by the Klason Method and ^{13}C CP/MAS Solid-State NMR Spectroscopy

	pretreatment conditions[d]			Klason method		NMR method	
sample ID	T (°C)	acid[a] (g/g)	solids loading[b] (%)	lignin (%)	% MBC[c]	lignin (%)	% MBC[c]
1	165	0.056	20	27.9	158	21.7	125
2	180	0.03	25	23.5	154	17	117
3	180	0.03	30	29.2	164	15.5	95
4	180	0.045	25	27.4	143	20.6	109
5	190	0.03	25	28.7	181	18.5	125
6	190	0.03	35	28.6	151	21.4	118
7	190	0.045	30	29.3	154	23.7	126
8	190	0.045	30	29.6	172	22.1	131
9	190	0.045	35	32.0	168	22.9	126
10	190	0.06	25	30.0	177	22.5	141
11	190	0.06	30	32.4	164	25.6	134
12	190	0.06	35	34.1	177	23.5	130
13	200	0.045	25	29.2	169	23.4	137
14	200	0.06	25	33.4	161	27.9	135
15	200	0.06	35	37.7	144	30.8	118
corn stover (original)		11.7		12.6			
corn stover (extracted)		14.0		13.3			

[a]Sulfuric acid amount per gram of feedstock material. [b]Weight percentage of solid feedstock in reaction solution. [c]Lignin MBC calculated on the basis of original lignin content. [d]Taken from ref 24.

not require a division into lignin solubilized during the Klason analysis. However, using the UV absorptivity constant to determine ASL is another methodological difference that could introduce error to the Klason method. ASL is measured by UV spectroscopy using a standard absorptivity of 110 L g^{1-} cm^{-1} at 205 nm.[1] The standard absorptivity generated for lignin may not be suitable for pretreated biomass hydrolysates because acid pretreatment generates a significant amount of sugar degradation products that may have different absorption coefficients. The higher mass balances for lignin measured using the Klason determination (41% on average) may be an indication that the current UV absorptivity constants overestimate the amount of ASL in solution.

The high lignin MBCs determined using the two different analytical methods indicate degradation compounds are present along with the residual lignin after pretreatment, causing an overestimation of the lignin values. Protein has been proposed as a possible candidate for interference in lignin measurements. The nitrogen content in the Klason residues after dilute-acid pretreatment, however, showed that 70−80% of the protein was solubilized during the pretreatment procedure (data not shown), suggesting that protein contributes 3.9−5.8% of lignin MBC in CS and 1.9−2.8% in CS(Ext). These results indicate that nitrogenous materials did not significantly contribute to the observed high lignin MBC.

Three other hypotheses were then considered: (1) either carbohydrates are physically protected by the remaining lignin or lignin−carbohydrate linkages are formed during pretreatment, are chemically resistant to the acid hydrolysis, and are not hydrolyzed during the Klason lignin determination; (2) sugar degradation products and/or their condensation products are condensed with the lignin during dilute-acid pretreatment; and (3) the extractable material in CS consisting of monomeric sugars, dimeric sugars, and other low molecular weight compounds forms solid condensation products that precipitate during the dilute-acid pretreatment. The third hypothesis is likely to play a more important role due to the higher percentage of more reactive sugars, for example, fructose, than sugars released from structural carbohydrates by pretreatment.[31]

Acid Chlorite Bleaching Treatment of Klason Residue. The first hypothesis proposes that there may be carbohydrates remaining after pretreatment that are somehow protected from further acid hydrolysis and appear in the acid-soluble residue created during the Klason analysis. If this hypothesis is correct, then the Klason residue from the Klason lignin determination must include, on average, an additional 65% weight from carbohydrates or proteins because the mean value of lignin MBC was about 165%. To measure the remaining sugar and protein content in the Klason residue, we applied acid chlorite bleaching to the residue to remove all lignin and other aromatic compounds for a range of samples (described in Table 1) produced under different severities and having a range of MBCs.

An example of the ^{13}C CP/MAS spectra of (a) Klason residue and (b) the solids that remain after acid chlorite treatment of Klason residue are shown in Figure 1. Peaks

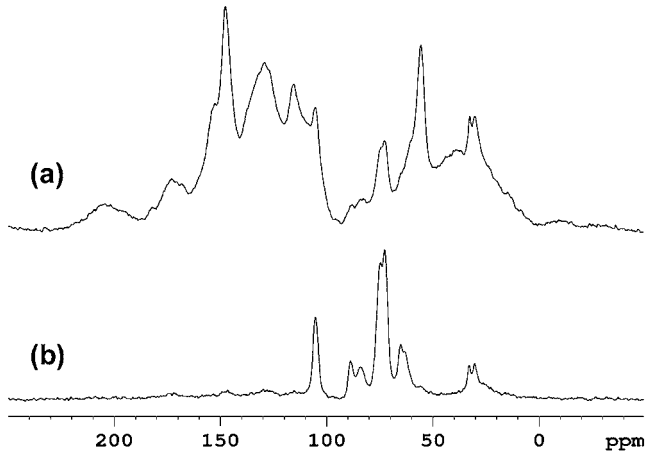

Figure 1. ^{13}C CP/MAS NMR spectra of residues (a) before and (b) after delignification of Klason residue with acid chlorite after Klason procedure from the corn stover residue after dilute-acid pretreatment.

between 10 and 40 ppm in both spectra were assigned to $-CH_2/-CH$ from protein and/or wax. The peaks between 60 and 109 ppm were assigned to carbohydrates and the lignin side chain, and the peaks between 109 and 165 ppm were assigned to lignin aromatic structures. Comparing spectrum a with spectrum b, the intensity assigned to aromatic structures is greatly reduced after acid chlorite treatment. The residual carbohydrate content was determined by integration and was estimated to be 5−10 wt % of the Klason residue of samples selected over the range of different pretreatment severities shown in Table 1. The remaining protein and waxy material content, as indicated by the peak area in Figure 1b, was less than the carbohydrate contamination. These results suggest that unhydrolyzed carbohydrates, protein, and waxy materials in the insoluble residue account for only a small portion of the high MBCs. Therefore, the second and third hypotheses that the sugars and other materials in solution may be degrading and

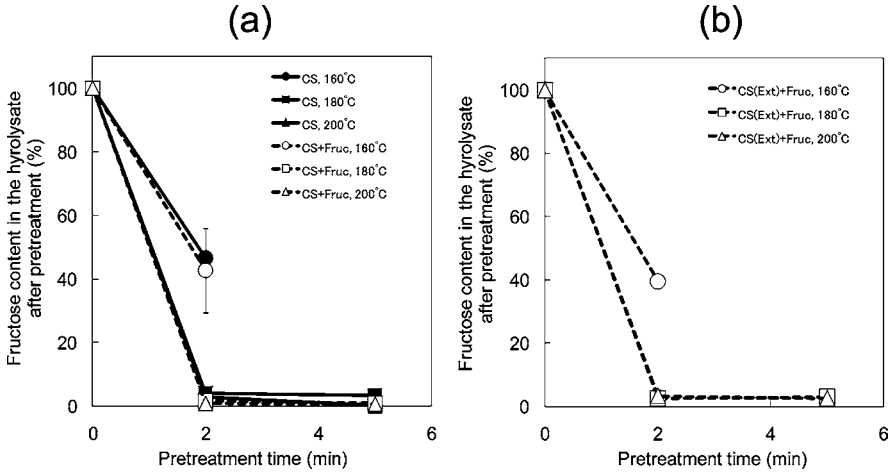

Figure 2. Fructose content in the hydrolysate after dilute-acid pretreatment from (a) whole corn stover (CS) and CS with fructose and (b) extractives-free corn stover [CS(Ext)] with fructose.

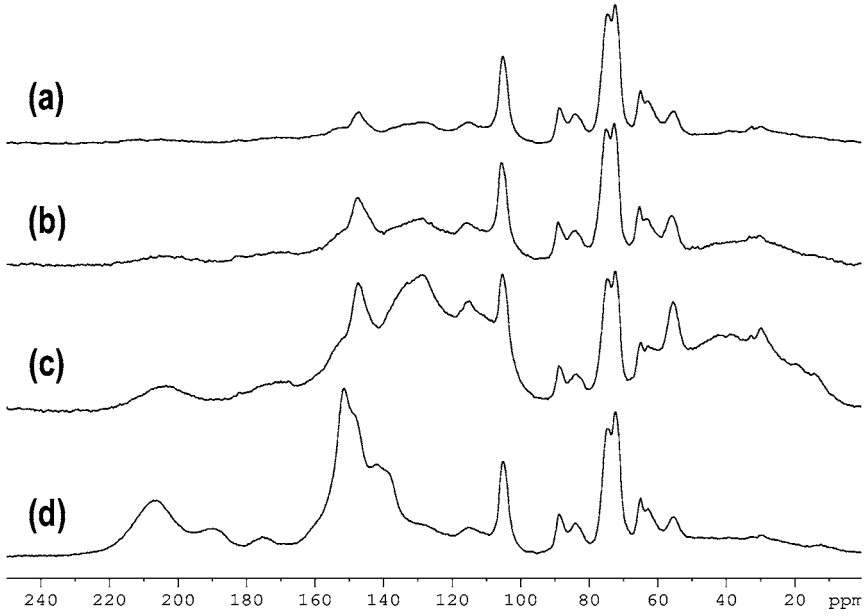

Figure 3. ^{13}C CP/MAS NMR spectra of residue from corn stover with ^{13}C-labeled carbohydrates after dilute-acid pretreatment at 200 °C for 5 min: (a) whole corn stover; (b) corn stover with ^{13}C-labeled glucose; (c) corn stover with ^{13}C-labeled xylose; (d) corn stover with ^{13}C-labeled fructose. All spectra were normalized by the height of the peak at 70−79 ppm.

subsequently condensing with the remaining insoluble lignin after pretreatment were tested.

^{13}C CP/MAS NMR Analyses of Pretreated Corn Stover Spiked with ^{13}C-Labeled Sugar. Glucose and xylose are released during pretreatment as a result of degradation of the cellulose, hemicellulose, and the water-soluble carbohydrates in the extractive fractions of the CS. Under standard pretreatment conditions (180 °C, 5 min, 1.28% of H_2SO_4 aq), 11.8 wt % of the glucose and 67.5 wt % of the xylose were released into solution by hydrolysis of the biomass. Fructose was present in significant quantities (2.07 wt %) in the water-soluble extractives of CS as a moiety of sucrose. Glucose and fructose degrade to 5-hydroxymethylfurfural (HMF), levoglucosan, levulinic acid, and formic acid during dilute-acid pretreatment. Xylose degrades to furfural. The degradation products of glucose, xylose, and fructose are known to undergo complex chemical reactions under the conditions of dilute-acid hydrolysis to form polymeric substances that precipitate and could contribute to an increased bias in lignin amounts determined by both Klason lignin and the solid-state NMR measurements.[32,33] We determined the extent of sugar degradation and polymerization under the pretreatment conditions used in this study by subjecting pure sugar solutions of glucose, xylose, and fructose to dilute-acid pretreatment at 180 °C for 5 min. Under these conditions, solid residues were formed from pure glucose, xylose, and fructose with 2, 11, and 21 wt % yield based on the weight of each sugar in the solution, respectively. After pretreatment, no fructose could be detected in the hydrolysate solution by HPLC (Figure 2), indicating that fructose present in the pretreatment hydrolysate was completely converted to HMF, other small molecular weight degradation products, and solid residues.

^{13}C CP/MAS solid-state NMR can provide chemical information about changes to the solid biomass components in the pretreated CS. Hence, CS was spiked with ^{13}C-labeled sugars (glucose, xylose, and fructose) and pretreated under

normal dilute-acid pretreatment conditions to test the second hypothesis that sugar degradation products could precipitate and contribute to the solids isolated during the Klason procedure. The ^{13}C CP/MAS NMR spectra of the solid residue from CS and from CS spiked with ^{13}C-labeled glucose, xylose, and fructose and pretreated at 200 °C for 5 min were compared (Figure 3). The residues isolated after pretreatment have peaks that can be assigned to chemical moieties of the degradation products derived from the ^{13}C-labeled sugars. Peak intensity assigned to lignin aromatic carbon between 109 and 165 ppm was higher relative to the carbohydrate region (60–109 ppm) in the CS spectrum with labeled fructose (Figure 3d) than the CS spectrum with labeled glucose (Figure 3b) or xylose (Figure 3c). Additionally, carbonyl and carboxyl signals between 165 and 230 ppm increased similar to what was observed in earlier studies of carbohydrate degradation pathways under pretreatment conditions, giving further credence to the hypothesis that the signal intensity from the ^{13}C-labeled sugars is due to polymerized degradation products.[32,33] These results confirm that fructose has a much higher reactivity than both glucose and xylose, and xylose has higher reactivity than glucose under dilute-acid hydrolysis conditions. More importantly, these results confirm that the degradation products observed in the experiments using pure sugar solutions are present in the solid residue from CS after dilute-acid pretreatment.

Contribution of Solid Degradation Products to Overall Lignin Mass Balance. The extractable content in CS consists of a large array of compounds, including many soluble carbohydrates.[31] The results discussed in the previous section using ^{13}C-labeled monosaccharides suggest that carbohydrates either present in the extractives or released as degradation products of cellulose and hemicellulose may play a significant role in the overestimation of lignin. Sucrose, a dimer of fructose and glucose, is one of the principal sugars in the water-soluble fraction. Fructose present in the CS sample accounts for only 2.07 wt % of the original CS, which corresponds to 17.7% of the original lignin content in the CS. Fructose has a higher reactivity than the other common sugars and can be readily converted to HMF during acid pretreatment.[32,33] Thus, to investigate the third hypothesis of solid degradation products derived from the extractable components being counted in the lignin determinations, fructose was used as a model monosaccharide to study the impact of the sugar degradation products. CS and CS(Ext) samples, spiked with labeled and unlabeled fructose, were pretreated under replicate conditions; and the PIL contents in the residues are compared below.

Experiments were performed to investigate the effect of pretreatment conditions, extraction prior to pretreatment, and addition of fructose on the MBC of total lignin, the PIL, and the PSL as defined in eq 1. The mass balances for CS, CS(Ext), CS spiked with fructose (CS+Fruc), and CS(Ext) spiked with fructose [CS(Ext)+Fruc] were determined as a function of pretreatment severity. Figures 4 and 5 show the total lignin MBC under various reaction severities (time and temperature) for samples spiked with fructose and samples with the extractive component removed, respectively. In all cases, the higher severity increased the lignin MBC to values >100%. Secondarily, spiking fructose to the original sample led to higher lignin MBCs than CS in all conditions (dilute acid), showing that degradation products from fructose were counted as pseudolignin and increased the lignin MBC.

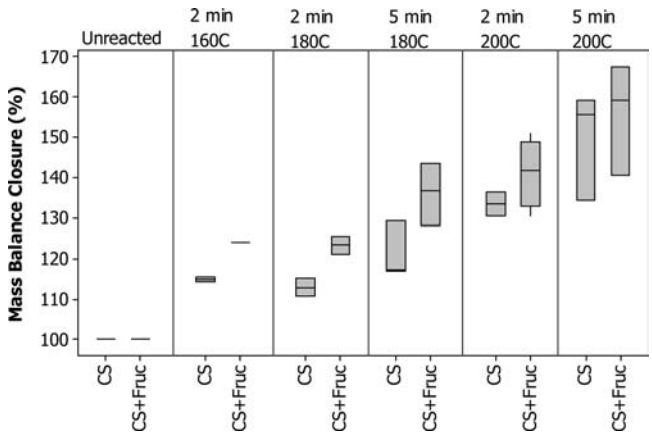

Figure 4. Total lignin mass balance closure (MBC), which is a sum of pretreated soluble lignin (PSL) in the hydrolysate and pretreated insoluble lignin (PIL) in the residue after dilute-acid pretreatment under various conditions from corn stover (CS). CS+Fruc indicates results of samples spiked with additional fructose.

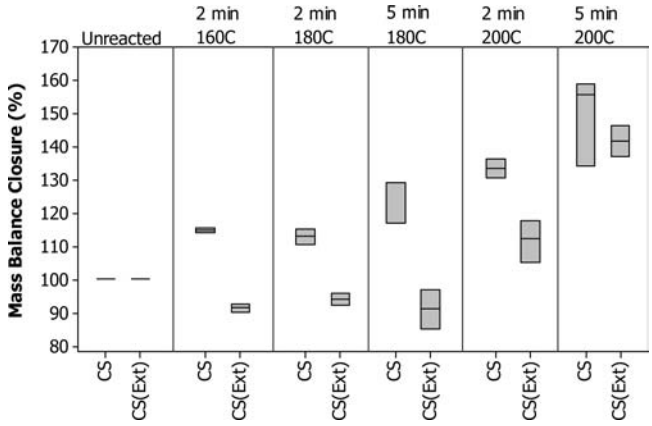

Figure 5. Total lignin MBC from pretreated corn stover (CS) and extractives-free corn stover [CS(Ext)] under various conditions. These show the effect of extractives in corn stover on total lignin MBC.

A direct comparison of the total lignin MBCs for CS and CS(Ext) at various severities (Figure 5) shows that there is a clear increase in the lignin MBC for all samples that contain extractable materials. Lignin MBCs after pretreatment from CS(Ext) remained constant or possibly decreased at temperatures below 200 °C. There was an increase in lignin MBC at the most severe conditions for CS(Ext), suggesting that monomeric and oligomeric sugars released from the structural carbohydrates contributed to the high lignin MBCs at 200 °C. The large contribution of the extractives to the total lignin MBCs is clearly demonstrated by the results of the experiments presented in Figure 5 and shows that extractives materials are a major contributor to the total lignin MBCs measured above 100%.

Figures 6 and 7 show the MBC of PIL obtained under various pretreatment reaction conditions. Addition of fructose to CS(Ext) causes a small increase in the MBCs of 2–11% for PIL (Figure 6), although this slight increase falls within the error of the measurements. Comparing the lignin MBCs for whole CS (Figure 4) and CS(Ext) (Figure 6) shows that adding the fructose affects the total lignin mass closure (Figure 4) to a greater extent and indicates fructose degradation products are predominately measured within the liquid phase.

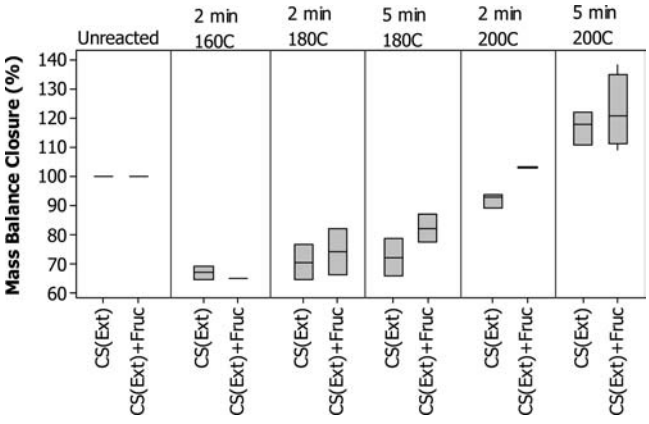

Figure 6. Pretreated insoluble lignin (PIL) MBC from pretreated extractives-free corn stover [CS(Ext)] and CS(Ext) spiked with fructose [CS(Ext)+Fruc] under various conditions, showing the effect of fructose on lignin MBC.

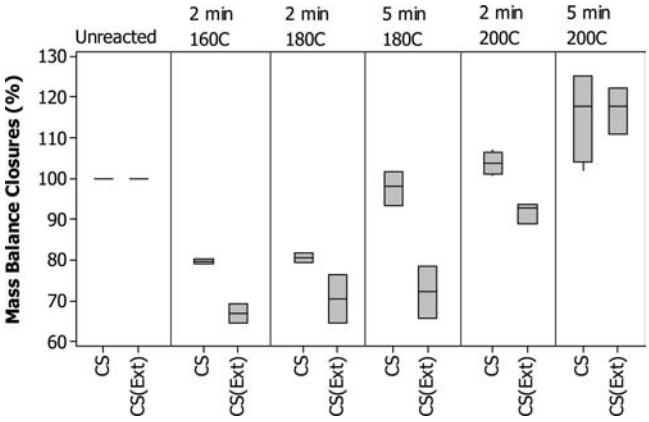

Figure 7. Pretreated insoluble lignin (PIL) MBC from pretreated corn stover (CS) and extractives-free corn stover [CS(Ext)] under various conditions, showing the effect of extractives on corn stover.

Lignin MBCs of PILs at 160 and 180 °C were <100% and then increased at 200 °C, indicating a release of low molecular weight phenolic compounds at lower severities followed by condensation of the low molecular weight compounds and/or sugar degradation products at the higher severities (Figure 7). The results shown in Figure 7 suggest that two kinds of reactions could be occurring during the early stages of acid pretreatment of herbaceous biomass materials: (1) lignin (or other low molecular weight phenolic compounds) in the lignin−hemicellulose complex are solubilized, and (2) sugar oligomers and other lower molecular weight compounds are released. In fact, it has been observed that 84% of xylan in hemicelluloses in CS is quickly dissolved after dilute-acid pretreatment at 180 °C for 0.5−1 min.[34] The results presented in Figures 4−7 indicate that, at lower temperatures and during the first 2 min of pretreatment, lignin, hemicellulose, and extractives are released into the pretreatment solution, followed by degradation and condensation with the insoluble lignin at longer times and higher severities.

The contribution from the PSL to the total MBC for CS ranged from ∼27 to ∼35% under the different pretreatment severities (Figure 8). The increased PSL in whole CS pretreatments performed at the lowest severities with the addition of fructose shows that carbohydrate degradation products can interfere with the PSL measurement. The increase

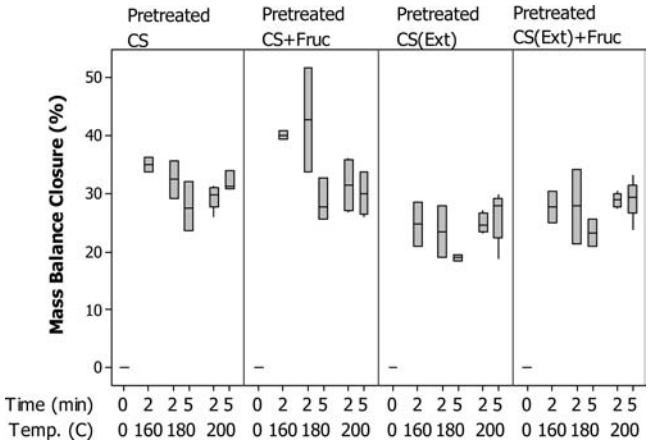

Figure 8. Pretreated soluble lignin (PSL) MBC from pretreated, extractives-free corn stover [CS(Ext)] and corn stover spiked with fructose [CS(Ext)+Fruc] under various conditions, showing the effect of extractives on corn stover.

in PSL was not as dramatic in the CS(Ext) spiked with fructose and was well within the error of the experiments. The contribution to the MBC from the PSL fraction using the CS(Ext) was 19−28%, which was lower than those observed in whole CS. We speculate that the hydrolysate from CS contains degradation products from sucrose and extractives, and these degradation products interfere with the measurement of the PSL. The difference of the MBC of PSL between CS and CS(Ext) decreased under more severe pretreatment conditions (Figure 8), presumably due to lower molecular weight degradation products polymerizing and being accounted for in the solid residues.

Overall, the combination of ^{13}C CP/MAS spectroscopy and ^{13}C-labeled fructose and xylose revealed that fructose and xylose degradation products are present in the PIL fraction after dilute-acid pretreatment and may be incorporated with the lignin. Our studies showed that the extractives present in CS are solubilized during the initial stages of pretreatment and interfere with the PSL measurements, biasing the result high. At higher severities, the extractable components, along with the sugar oligomers and sugar monomers released during pretreatment, were degraded and polymerized to form a solid residue that interferes with the PIL measurement. The results of the experiments presented above clearly show that degradation products interfere with both soluble and solid lignin measurements and bias the resulting MBCs in pretreatment residues high.

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