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Alkaline Pretreatment of Corn Stover: Bench-Scale Fractionation and Stream Characterization

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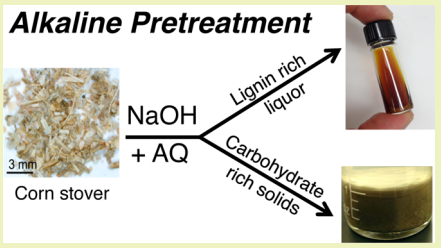
Supporting Information

ABSTRACT: Biomass pretreatment generally aims to increase accessibility to plant cell wall polysaccharides for carbohydrate-active enzymes to produce sugars for biological or catalytic upgrading to ethanol or advanced biofuels. Significant research has been conducted on a suite of pretreatment processes for bioethanol processes. An alternative option, which has received less attention in the biofuels community, is the use of alkaline pretreatment for the partial depolymerization of lignin from intact biomass. A known issue with alkaline pretreatment is the loss of polysaccharides from peeling reactions, but this loss can be mitigated with anthraquinone, as commonly practiced in pulping. Here, we conduct a comprehensive bench-scale evaluation of alkaline pretreatment using corn stover at temperatures of 100, 130, and 160 °C and sodium hydroxide loadings from 35 to 660 mg NaOH/g dry biomass with anthraquinone. Compositional analysis is conducted on the starting material and residual solids after pretreatment, and mass balance is inferred in the liquor by difference. The residual solids after alkaline pretreatment are characterized for crystallinity and imaged by scanning and transmission electron microscopy to reveal the physical changes in the carbohydrate portions of the biomass remaining after pretreatment, which demonstrate dramatic modifications to biomass cell wall architecture with lignin removal but rather insignificant changes in cellulose crystallinity. Our results show that alkaline pretreatment at relatively mild conditions is able to remove substantial amounts of lignin from biomass. Going forward, to be an economically feasibile process, technologies will be required to upgrade the resulting lignin-rich liquor stream.



KEYWORDS: *Lignocellulose, Biomass, Pulping, Biofuels, Lignin, Pretreatment*

INTRODUCTION

The development of processes that utilize renewable lignocellulosic biomass for the production of fuels and chemicals is of paramount importance for improving energy security and mitigating environmental problems associated with the use of fossil fuels. For selective conversion processes to fuels and chemicals, the physical and chemical complexities of plant biomass typically warrants a thermochemical pretreatment step. The aim of pretreatment is generally to make the plant cell wall polysaccharides more amenable to digestion with cellulolytic enzymes to sugars or to catalytic depolymerization.[1−7] Specific pretreatment processes often aim at other key factors, such as hemicellulose depolymerization to monomeric sugars or lignin redistribution and/or partial removal. The residual polysaccharides can be subsequently depolymerized and the resulting sugar streams upgraded either catalytically or biologically to fuels or chemicals.[8−11] Given the importance of pretreatment for subsequent downstream processing and the need for co-design of all steps in biorefineries, significant efforts have been dedicated to developing cost-effective pretreatment technologies.[2,12] In particular, several leading candidates were studied and compared on a uniform basis in a multi-laboratory project, including dilute acid, ammonia fiber expansion (AFEX), steam explosion, lime, and ammonia recycle pretreatments.[3−6] These pretreatment technologies were primarily developed and optimized for bioethanol production, which typically slates the lignin fraction for combustion to provide heat and power, and the yield of sugars for upgrading is the primary objective. The outcome of this large consortium was a side-by-side comparison of the relative merits of leading pretreatment technologies for bioethanol production.

Additional pretreatment technologies that focus on biomass fractionation have begun to receive more attention, as this will enable a more holistic biorefinery concept wherein all fractions of biomass can be upgraded selectively to a broader range of fuels and chemicals. In particular, organosolv pretreatment, which employs organic solvent(s), water, and a mineral acid co-catalyst, has long been studied. One form of organosolv pretreatement, clean fractionation, is quite effective at fractionating woody feedstocks using methyl isobutyl ketone,

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ethanol, water, and sulfuric acid.[13,14] This method has also been shown to be effective on herbaceous feedstocks.[15] Additionally, ionic liquid pretreatment has been widely studied because ionic liquids were first shown to dissolve cellulose.[16] More recently, ionic liquid pretreatment has been compared to dilute acid pretreatment by Li et al.[17] and has also been conducted at high solids loadings.[18] These studies have demonstrated significant potential in the application of ionic liquids as novel solvents for the separation of biomass into its component fractions for subsequent upgrading. In both cases, the recycling of organic solvents or ionic liquid solvents are key cost drivers, which warrant critical consideration from a techno-economic perspective.

Recent work in bioethanol production has shown that very mild alkaline pretreatment of corn stover, prior to an acid pretreatment, fractionates acetate and 11−22% of the lignin into the aqueous phase.[19] This process step, dubbed "deacetylation", was primarily developed due to the significant inhibition of ethanologens such as *Zymomonas mobiliz* by acetate derived from hemicellulose.[20,21] Likely due to the removal of both acetate and some lignin, deacetylation was shown to increase the efficiency of enzymatic depolymerization of the residual solids, which in turn lowers the predicted overall minimum ethanol selling price.[22−24] Using sodium hydroxide alone at much higher loadings, additional bench-scale work has also shown enhanced digestibility and ethanol production from alkaline delignified biomass.[25−27] Significant work has also been done in this vein using alkaline hydrogen peroxide, which has also proven to be quite effective.[28−31] Consequently, the development of a purely alkaline pretreatment strategy, rather than very mild alkaline pretreatment followed by high severity acid pretreatment, may be an attractive approach as enzymatic inhibitors like lignin and acetate are removed from the solid carbohydrate phase. Additionally, the aqueous black liquor phase contains a large fraction of low molecular weight lignin that could provide a valuable stream for upgrading if technologies can be developed to utilize these species. Some technologies currently under investigation include pyrolysis,[32] thermochemical treatment to produce oil products,[33] selective separation of organics,[34] and acid separation using ion exclusion columns[35] or size exclusion chromatography.[36] Additionally, vanillin production via oxidative treatment of black liquor is a well-studied approach to produce a high value chemical.[37] However, the primary drawback to development of this technology is that along with removing lignin and acetate from the solid phase, high severity alkaline pretreatment is known to also solubilize polysaccharides, particularly a significant amount of hemicellulose and minor sugars.[38−40] It is beneficial to mitigate the solubilization of hemicellulose during this process to increase the yield of digestible carbohydrates remaining in the solid phase. This can be difficult in an alkaline process, as large amounts of hemicellulose are removed with increasing treatment severity.[39]

The pulp and paper industry faces a similar challenge, where increased retention of hemicellulose is beneficial, resulting in higher pulp yields with improved tensile strength.[41,42] To accomplish this, several additives for alkaline-based pulping have been developed that boost the retention of hemicellulose. One of the best known is the addition of sodium sulfide to a caustic NaOH solution in the kraft pulping process. Sodium sulfide forms NaSH in solution, which acts as a strong nucleophile increasing the fragmentation of lignin.[38] The chemistry of this reaction is complex, but in short, an OH^- ion removes a hydrogen atom from a phenolic hydroxyl group in the lignin polymer to form a quinone methide. HS^- then adds to the α-carbon on the quinone methide and attacks the β-carbon forming an episulfide that breaks the $\beta-O-4$ linkage.[38,43,44] This reaction increases the rate of lignin removal during the bulk delignification phase and increases the yield of lignin monomers in the liquor. The increased rate of lignin removal enables optimization of conditions for maximum delignification and hemicellulose retention. Unfortunately, the use of a mild kraft pretreatment procedure for the fractionation of biomass in the fuels and chemicals industry is potentially problematic, as sulfur is a known poison for many downstream catalysts and is an inhibitor for organisms in fermentation-based steps. However, the pulp and paper industry also employs the use of an alternative nonsulfur-based additive, anthraquinone (AQ), to maximize hemicellulose retention and delignification. The exact mechanisms of AQ are unknown, but it is hypothesized that AQ works as a redox shuttle catalyst, where AQ is first reduced to anthrahydroquinone (AHQ^-) by oxidizing the aldehyde end groups of carbohydrates to carboxylic acids. This oxidation of carbohydrate end groups mitigates hemicellulose loss via peeling reactions[45] because acid end groups of carbohydrates are more stable in alkali media. AHQ^- then forms AHQ^{2-} in the alkali solution, which is oxidized back to AQ through the reductive cleavage of $\beta-O-4$ linkages in lignin.[38,46,47] This redox cycle simultaneously creates more lignin monomer fragments in the black liquor phase[27] while mitigating the solubilization of hemicellulose and other carbohydrates.

In this work, we investigate alkaline (sodium hydroxide) pretreatment with the additive AQ as a means of fractionating corn stover into a solid carbohydrate-rich fraction, a black liquor fraction containing both high molecular weight lignin and low molecular weight aromatic monomer fragments, carbohydrates, and other components, and an aqueous wash fraction. We examine a wide range of severities, but most of our analysis focuses on the low NaOH loading treatments, as these conditions are likely more economically feasible for a pretreatment process in the biochemical and biofuels industry. In the pulp and paper industry, NaOH concentrations of 1 M or higher at temperatures of ∼160 °C at times greater than 1 h are typical, which is much more severe than the treatments that are the focus of this work. Future work will be published to examine the enzymatic digestibility of the pretreated solids produced from this pretreatment along with techno-economic analysis to determine the applicability of this process at the industrial scale. Additionally, pilot scale pretreatment runs will be reported that investigate methods for enhancing the activity of the AQ additive.

EXPERIMENTAL SECTION

Pretreatment Procedure. Air-dried corn stover from Idaho National Laboratory (Lot #4) was milled to 2 mm or smaller particle sizes and subjected to successive water and ethanol extractions to remove saps and waxes. Compositional analysis of the extracted corn stover showed the material was composed of 40.3% glucan, 26.0% xylan, 18.7% lignin, 6.3% ash, 3.9% arabinan, 2.0% galactan, and 2.9% acetate.

For the pretreatment experiments, the milled corn stover was loaded and sealed into 316 stainless steel vessels at 10 wt % dry solids loading in ultrapure water. Sodium hydroxide (Sigma-Aldrich, Lot MKBP3689 V) loadings were varied between 35 and 660 mg/g dry corn stover. Anthraquinone (Sigma-Aldrich, Lot BCBJ0829 V) loadings were held at 0.2% charge (w/w) to dry corn stover. The

sealed vessels were heated in a large aluminum block heater to 100, 130, or 160 °C with a 30 min heat ramp and held at temperature for 30 min. The temperature was measured with a k-type thermocouple positioned in the slurry with a thermowell. No agitation was employed. After 30 min at temperature, the vessels were removed and submerged in an ice bath to quench the reaction.

The resulting slurry was removed from the reactor and separated into solid, black liquor, and wash fractions. To separate the black liquor, the slurry was first centrifuged for 30 min at 20,000*g* to pelletize the solids, and the caustic black liquor was withdrawn with a Pasteur pipet and stored at 4 °C in a high-density polyethylene (HDPE) container. The solids were then washed through resuspension in 1 L of ultrapure water and centrifuged at 15,000*g* for 30 min. The wash water was decanted from the solid fraction. This process was repeated with an additional 1 L of ultrapure water, and the solids were resuspended but stored in a cold room at 4 °C overnight to increase the removal of the alkalinity retained in the solid fraction. After 12 h, the wash water was decanted off of the settled solids. Finally, to remove the small remaining amount of wash water that could not be decanted off, the solids were vacuum filtered on a 2 μm pore size PTFE filter. The combined 2 L of wash was stored in an HDPE container at 4 °C in a cold room until compositional analysis was performed. Similarly, the washed solids were stored in a cold room at 4 °C in a glass jar with 100 mL of ultrapure water before analysis was conducted.

Compositional Analysis. The mass of the recovered dry solids was measured by drying the solid fraction for several days in a 40 °C vacuum oven until the mass stabilized to a constant value. Compositional analysis of the solids and wash fraction was subsequently performed. For the dried solid fraction, compositional analysis was conducted in accordance with standard NREL laboratory analytical procedures (LAPs).[48,49] Carbohydrate content of the wash fraction was analyzed by high performance liquid chromatography (HPLC), and the lignin content was measured by UV/vis absorbance at 320 nm with an extinction coefficient of 30, in accordance with the NREL LAPs.[48]

The black liquor fraction is a heterogeneous mixture of acids, polysaccharides, monosaccharides, aromatic monomers (derived from lignin), high molecular weight lignin, and acetate.[40] Conventional analytical methods are inadequate, at present, to completely and directly quantify the components present in black liquor.[40] Therefore, for the purposes of this work, we report the composition of the black liquor by difference from the known mass and composition of the dry biomass loaded into the pretreatment vessel and the resulting mass of the retained solids, composition of the retained solids, and composition of the recovered wash. A separate study will be published imminently wherein several new analytical methods alongside established protocols are applied to analyze the black liquor and identify the major chemical components.

Crystallinity Measurements. X-ray diffraction (XRD) was performed to evaluate the crystalline structure of both untreated and alkaline-treated samples by using a Rigaku (Tokyo, Japan) Ultima IV diffractometer with Cu Kα radiation having a wavelength of λ(Kα1) = 0.15406 nm generated at 40 kV and 44 mA. The diffraction intensities of freeze-dried samples placed on a quartz substrate were measured in the range of 8−42° 2θ using a step size of 0.02° at a rate of 2°/min. The crystallinity indexes (CrI) of the cellulose samples were calculated according to the method described by Segal et al.[50] using eq 1

$$\mathrm{CrI} = \frac{I_{200} - I_{\mathrm{Am}}}{I_{200}} \times 100 \tag{1}$$

where I_{200} and I_{Am} are the maximum and minimum intensity of diffraction at approximately 2θ = 22.4−22.5° and 2θ = 18.0−19.0°, respectively.

Stereoscope Imaging. Freeze-dried solids obtained from pretreatment at 130 °C were placed on a white background and imaged using a Nikon SMZ1500 stereomicroscope. Images were captured with a Nikon DS-Fi1 CCD camera operated by a Nikon Digital Sight system.

SEM Imaging. Imaging by scanning electron microscopy (SEM) was performed using a FEI Quanta 400 FEG instrument. The pretreated solids obtained from pretreatment conditions at 130 °C were freeze-dried and then mounted on aluminum stubs using conductive carbon tape. The samples were sputter coated with 10 nm of iridium, and imaging was performed using beam accelerating voltages from ranging from 10 to 25 keV.

TEM Imaging. Biomass samples were processed using microwave EM processing. Samples were fixed in 2.5% gluteraldehyde buffered in 0.1 M sodium cacodylate buffer (EMS, Hatfield, PS). The samples were then dehydrated by treating with increasing concentrations of acetone in a Pelco microwave oven for 1 min for each dilution (i.e., 15%, 30%, 60%, 90%, and 3 × 100% acetone). After dehydration, the samples were infiltrated with Eponate 812 resin (EMS, Hatfield, PA) by incubating at room temperature for several hours to overnight in increasing concentrations of resin (5%, 15%, 45%, 90%, 3 × 100% resin, diluted in acetone). The samples were transferred to capsules, and the resin polymerized by heating to 70 °C overnight. Resin embedded samples were sectioned to ∼60 nm with a Diatome diamond knife (Diatome, Hatfield, PA) on a Leica EM UTC ultramicrotome (Leica, Wetzlar, Germany). Sections were collected on 0.5% Formvar-coated slot grids or 200 mesh copper grids (SPI Supplies, West Chester, PA). Grids were post-stained for 3 min with uranyl acetate and 3 min with 1% $KMnO_4$. Images were acquired with a 4 mega-pixel Gatan UltraScan 1000 camera (Gatan, Pleasanton, CA) on a FEI Tecnai G2 20 Twin 200 kV LaB6 TEM (FEI, Hilsboro, OR).

RESULTS

Alkaline/AQ pretreatment of extractives-free corn stover was performed in sealed vessels for 30 min at temperatures of 100, 130, and 160 °C with an AQ loading of 0.2% charge, which is an intermediate loading relative to that typically employed in the pulp and paper industry (approximately 0.015− 0.4%).[51] Six sodium hydroxide loadings were chosen spaced over a large range (35−660 mg NaOH/g dry stover). The lowest sodium hydroxide loading matches the recent "deacetylation" step employed at the National Renewable Energy Laboratory,[19] and the highest loadings were chosen to examine the structural deformity that occurs in the plant cell wall under extreme NaOH loadings. The results of the percentage of recovered solids are presented below along with the results of w/w compositions. These measured values are used to calculate the yields of each of the compositional components (lignin, glucan, xylan, etc.) of the corn stover in each fraction, as detailed below.

Percentage of Dry Solids Retained after Pretreatment. The percentage of dry solid retained is calculated from the following equation

$$\%\text{Dry Solid Retained} = \frac{m_{\mathrm{s,f}}}{m_{\mathrm{s,i}}} \times 100 \tag{2}$$

where $m_{\mathrm{s,i}}$ is the initial mass of dry corn stover loaded into the reactor, and $m_{\mathrm{s,f}}$ is the final mass of the dry solids recovered after pretreatment. This measurement is warranted as it allows the yield of each fraction (lignin, xylan, glucan, etc.) of the corn stover to be calculated (see below, Yields section). The percentages of dry solids retained after pretreatment at 100, 130, and 160 °C as a function of NaOH loading are shown in Figure 1. At the lowest loading of NaOH (35 mg/g dry stover), the percentage of dry solids retained is essentially the same at 84 ± 3.6% for temperatures at 100, 130, and 160 °C. The error bar represent the 95% confidence interval, which was determined by a set of four replicates at a loading of 70 mg NaOH/g dry stover and pretreatment temperature of 130 °C. We assume that the error on this set of replicates represents the

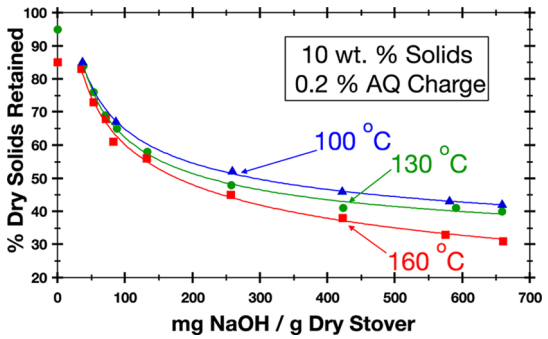

Figure 1. The percentage of the dry solids retained after pretreatment at 100 °C (blue triangles), 130 °C (green circles), and 160 °C (red circles) as a function of sodium hydroxide loading. Here, each point has an error of ±3.6% (see text) and represents pretreatment conditions of 10 wt % dry biomass loading in ultrapure water with 0.2% charge AQ (w/w on dry stover). The solid lines represent the best-fit curve to the data points at each temperature. The equations for the best-fit lines are given in the text with two significant figures and can also be found in Table S1 of the Supporting Information.

error on all points shown in Figure 1 (thus the error bars are not shown explicitly on each point). In Figure 1, it is clear that the traces for the % of dry solid retained for each temperature diverge as the NaOH loading increases. At 100 °C, the percentage of dry solids retained drops to 42 ± 3.6% at the highest NaOH loading of 660 mg NaOH/g dry stover with all of the data points at this temperature being best fit with the power function $y = 19 + 240x^{-0.36}$. However, for pretreatment at higher temperatures, more of the stover is solubilized, and the percentage of dry solids retained decreases. For pretreatment at 130 °C, the percentage of dry solids retained drops to 40 ± 3.6% at the highest NaOH loading, and the data points here are best fit with the power function $y = 23 + 330x^{-0.46}$. At 160 °C, only 31 ± 3.6% of the dry solids are retained at the highest NaOH loading. The data points at 160 °C are also best fit with a power function, $y = -15 + 240x^{-0.25}$. Note that in Figure 1, there are four data points at 0, 53, 70, and 130 mg NaOH/g dry stover at 130 and 160 °C that are not present in the data at 100 °C. Experiments at these points were performed to more thoroughly explore the lower NaOH loading regime at these temperatures because we find the optimal conditions for maximum lignin removal and minimum xylose solubilization are in this region at temperatures above 100 °C (see Discussion section).

Composition of Retained Solids. The measured composition (w/w) of the pretreated solids as a function of NaOH loading and temperature are shown in Figure 2. Note that as a reference, Figure 2 shows the composition of the dry corn stover before pretreatment at the far left. We find that at the lowest severity treatment at 100 °C and 35 mg NaOH/g dry stover, only 0.4 ± 0.1% w/w composition acetate is present in the pretreated solid. Chen et al.[19] found a w/w composition of approximately 1.3% acetate of their alkaline pretreated corn stover. The removal of more acetate at the conditions studied here is likely due to the higher temperatures used here of 100 °C for 30 min, which is slightly more severe than 70 °C for 3 h employed in the deacetylation process.[19] The results in Figure 2 clearly show the influence an increase in temperature has on the composition of the retained solids at a given NaOH loading. As an example, at a loading of 260 mg NaOH/g dry stover, the glucan w/w composition increases from 63 ± 1.6% at 100 °C to 66 ± 1.6% at 130 °C and finally to 68 ± 1.6% at 160 °C. Conversely, the lignin percent composition of the pretreated solid decreases with increasing temperature. As an example, at a loading of 260 mg NaOH/g dry stover, the lignin w/w percent composition decreases from 4.9 ± 1.4% at 100 °C to 3.5 ± 1.4% at 130 °C and finally to 2.9 ± 1.4% at 160 °C. Note the

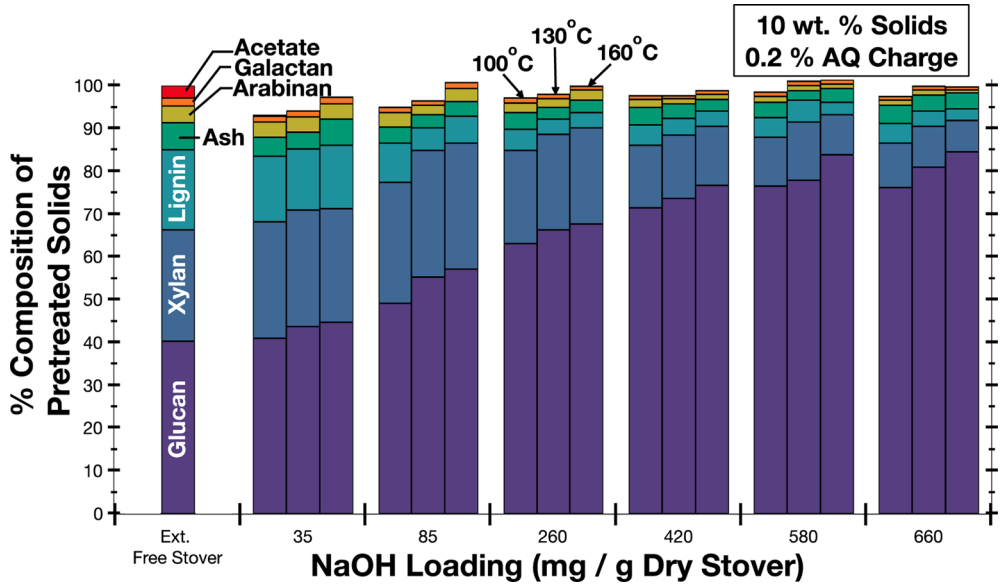

Figure 2. Composition of the pretreated solids at pretreatment conditions of 10 wt % solids and 0.2% AQ charge are shown as a function of NaOH loading and temperature. Here, compositional data for the pretreated solids at temperatures of 100, 130, and 160 °C are binned according to their NaOH loading, with the left-most stack in each bin representing a pretreatment temperature of 100 °C, the middle stack representing a pretreatment temperature of 130 °C, and the right-most stack in each bin representing pretreated solids at 160 °C (as noted in the temperature labels above the stacks in bin 260). The left-most bin labeled "Ext. Free Stover" contains only one stack and represents the composition of dry extractives-free corn stover without any pretreatment, the exact composition of which is given in the Experimental Section. The labels on the stack in the "Ext. Free Stover" bin are consistent for every stack in the figure.

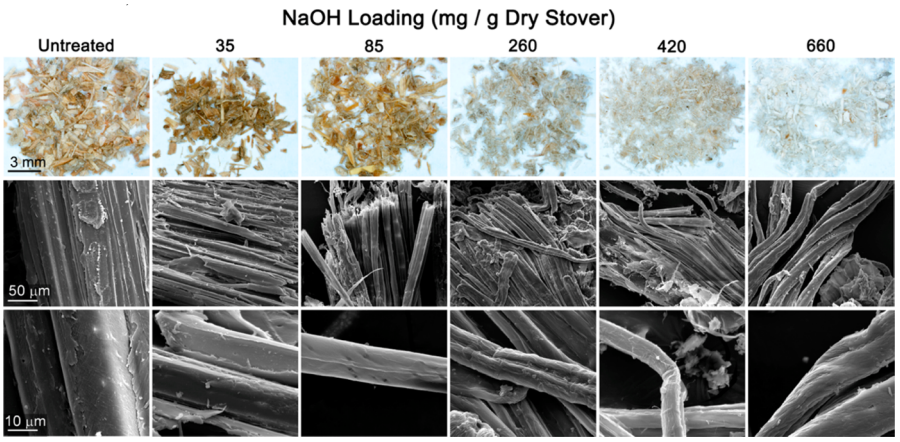

Figure 3. Stereoscope micrographs (top row) and scanning electron microscopy of alkaline/AQ pretreated solids (bottom two rows) from pretreatments at 130 °C and various NaOH loadings. Stereoscope micrographs of pretreated solids show a trend of color change from light brown to white with increasing NaOH loading as more lignin is removed. Moderate magnification SEM images show that higher NaOH loadings result in increased particle fragmentation and separation of individual cells (middle row). Higher magnification SEM images show increased structural deformation of fiber cells as the NaOH loading increases (bottom row).

error bars here represent the 95% confidence interval and were determined by a set of four replicates at 70 mg NaOH/g dry stover at 130 °C; again, we assume that the error on this set of replicates represents the error on all points. The 95% confidence interval for each measured w/w fraction is listed here for reference: ash ±0.26%, lignin ±1.4%, glucan ±1.6%, xylan ±0.12%, galactan ±0.18%, arabinan ±0.08%, and acetate ±0.1%.

While increasing pretreatment temperature has a clear effect on removing lignin, an increase in NaOH loading has an even stronger effect. Taking the results at 130 °C and 35 mg NaOH/g dry stover as an example, the pretreated solid at these conditions exhibits a composition of 14 ± 1.4% lignin and 44 ± 1.6% glucan by weight. Increasing the loading of NaOH to 85 mg NaOH/g dry stover decreases the w/w composition of lignin by ∼9% to 5.2 ± 1.4% and increases the glucan w/w composition by ∼12% to 55 ± 1.6%.

Overall, the data presented in Figure 2 show that the pretreated solid becomes enriched in carbohydrates, particularly glucan, as the severity increases. At the most severe conditions, the pretreated solid is composed of only 2.8 ± 1.4% lignin and 85 ± 1.6% glucan. This enrichment of glucan in solid phase produces a visual trend shown in the top row of Figure 3, where the pretreated solids range from dark brown to white as more lignin is removed. At high NaOH loadings (above 260 mg NaOH/g dry stover), there is a diminishing effect on lignin removal as the NaOH loading is increased.

Crystallinity of Retained Solids. The measured crystallinity of the pretreated solids is presented in Table 1. Here, the changes in crystallinity are found to be relatively insignificant as pretreatment severity is increased. At low NaOH loadings between 35 and 85 mg NaOH/g dry stover, the cellulose crystallinity for treated corn stover is essentially the same as that of untreated corn stover (within the error of the measurement) at all of the pretreatment temperatures. However, at pretreatment temperatures of 100 and 130 °C and between the loadings from 260 to 460 mg NaOH/g dry stover, the cellulose crystallinity increases slightly followed by a moderate decrease in crystallinity at NaOH loadings ≥580 mg/g dry stover. Here the increase in crystallinity is most apparent at a pretreatment temperature of 100 °C. This is likely due to the low removal of glucan (Figure 4, panel A, discussed below)

Table 1. Crystallinity of Pretreated Solids as a Function of NaOH Loading and Temperature[a]

CrI ± 2.9%			
NaOH loading (mg NaOH/g dry stover)	100 °C	130 °C	160 °C
untreated		41%[b]	
35	42%	42%	46%
85	44%	46%	41%
260	50%	48%	40%
420	55%	46%	43%
580	36%	44%	50%
660	38%	42%	44%

[a]The error on this measurement of ±2.9% represents the 95% confidence interval, which was found by measuring a set of four replicates of untreated corn stover. [b]Untreated corn stover milled to particles 2 mm or less in size.

at 100 °C compared to pretreatment at 130 °C where the removal of glucan increases slightly with increasing temperature. This makes the increase in crystallinity less apparent at pretreatment temperatures above 100 °C in this NaOH loading regime. At NaOH loadings ≥580 mg/g dry stover, the crystallinity begins to decrease for pretreatment temperatures at 100 and 130 °C. At a pretreatment temperature of 160 °C, the crystallinity of the residual solids are essentially the same compared to the untreated corn stover (within the error of the measurement), except at a loading of 580 mg NaOH/g dry biomass where a small increase in crystallinity is observed. Because multiple fractions of the corn stover are being removed simultaneously with increasing pretreatment severity and a microstructure change is observed (Figure 3), it is difficult to correlate the measured crystallinity to the removal of any one these fractions because the crystallinity is convoluted by all of these effects.

Microscopy Analysis of Pretreated solids. Stereoscope microscopy images and scanning electron microscopy (SEM) images of the recovered solid at the intermediate pretreatment temperature of 130 °C for increasing NaOH loadings are shown in Figure 3. Stereoscope micrographs of native corn stover and residual solids following pretreatment with various loadings of NaOH are presented in the top row of Figure 3. The stereomicroscopy images show a general trend of particle

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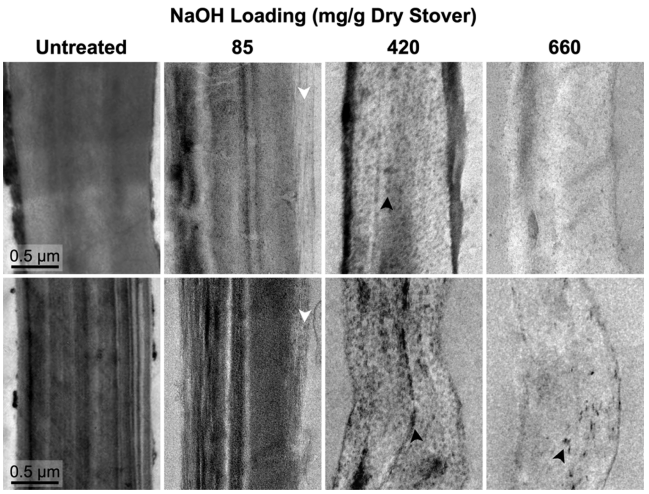

Figure 4. TEM micrographs of alkaline/AQ pretreated solids from pretreatments at 130 °C and increasing NaOH loadings. TEM micrographs of secondary cell walls show a trend of decreasing staining density with increasing pretreatment severity due to less lignin remaining in the cell wall. The pattern of lignin loss causes the walls to appear more striated following the 85 mg/g NaOH pretreatment with an enhanced impact on the wall surface (white arrowheads). The staining density of the remaining lignin in the 420 mg/g NaOH treated walls appears fragmented and discontinuous (black arrowheads). Following the 660 mg/g NaOH pretreatment, so much lignin has been removed that the remaining walls are difficult to discern from the background.

size reduction, with a greater abundance of long, thin, individual fibers observed with the higher NaOH loadings. These images also display a trend of color change from the light brown native material to nearly white for solids treated with a high loading of NaOH, which is an expected result of thermochemical alkaline pretreatments.

In rows 2 and 3 of Figure 3, SEM images of the pretreated solid show dramatic changes in the microstructure of the pretreated solid as the NaOH loading increases. Moderate magnification images of the particle morphology after pretreatment are presented in the middle row. These images show a trend of increased particle fragmentation with increasing NaOH loading. This fragmentation may be attributed to the depolymerization and removal from lignin from the shared compound middle lamella, which facilitates the fragmentation of individual cells from the larger tissue clusters. Higher magnification SEM images of the pretreated solids are presented in the bottom row of Figure 3. The higher magnification SEM micrographs (bottom row) focus on individual fiber cells and show a pattern of increasing deformation with increasing severity. The individual fibers display more frequent bends, kinks, and longitudinal twisting. This can be due to a general weakening of the cell walls and a partial collapse of the fiber lumen.

To investigate the structural changes caused by alkaline pretreatment that take place within the cell walls, we analyzed a subset of the 130 °C pretreated samples by transmission electron microscopy (TEM) (Figure 4). TEM micrographs display a trend of decreasing staining density with increasing pretreatment severity. The staining density is generated by the interaction between $KMnO_4$ and cell wall matrix polymers, so the decrease in staining, not surprisingly, correlates with lignin loss. The staining contrast of the 660 mg/g treated cell walls is so low that the walls are difficult to discern from the background. The TEM analysis also revealed differences in the pattern of distribution of the residual lignin. The 85 mg/g NaOH treated samples were extensively modified at the cell wall surfaces leaving a relatively lignin-free layer (Figure 4, white arrowheads). The rest of the cell wall retained lignin in stratified layers typical of pretreated cell walls. At the higher severities (420 and 660 mg/g), the remaining lignin appears to largely coalesce into discrete structures with the surrounding area devoid of lignin (Figure 4, black arrowheads).

Yields. From the measured values in Figures 1 and 2, the total percentage of each component (lignin, glucan, etc.) remaining in the pretreated solid can be calculated. This is defined as the yield of component *i*, $Y_{i,ps}$, or the percentage of component *i* remaining in the pretreated solid from the original dry corn stover calculated by eq 3

$$Y_{i,\mathrm{ps}} = \frac{\%\text{Dry Solid Retained} \times W_{i,\mathrm{ps}}}{W_{i,\text{dry stover}}} \quad (3)$$

Here, the %Dry Solid Retained is the measured value from Figure 1, $W_{i,\mathrm{ps}}$ is the measured w/w fraction of component *i* in pretreated solid shown in Figure 2, and $W_{i,\text{dry stover}}$ is the measured w/w fraction of component *i* in the untreated ext. free corn stover (Figure 2, left-most stack). In Figure 5, the yields calculated from eq 3 of glucan (Figure 5A), xylan (Figure 5B), lignin (Figure 5C), ash (Figure 5E), and acetate (Figure 5F) are shown for the tested pretreatment conditions. The yields for the minor sugars galactan and arabinan can be found in Figure S4 of the Supporting Information. Note that Figure 5D is a measured value not calculated by eq 3 and is the yield of lignin present in the wash water; *the wash water only contained lignin (discussed below)*. The calculated values in Figure 5 are best fit with power functions of the form $y = A + Bx^C$ over the range of 35 to 660 mg NaOH/g dry stover; the equation for each fit is given in Table S1 of the Supporting Information.

The data in Figure 5 show several trends in the removal of each fraction from the starting corn stover as a function of NaOH loading and pretreatment temperature. For example, the yield of lignin retained in the pretreated solid (Figure 5C) rapidly decreases as the NaOH loading increases. At the lowest severity of 35 mg NaOH/g dry stover and a pretreatment temperature of 100 °C, 70% of the lignin still remains in the pretreated solid phase, and this decreases to only 14% when the NaOH loading is increased to 260 mg NaOH/g dry stover. As mentioned above, increasing the NaOH loading beyond 260 mg NaOH/g dry corn stover only results in slightly more lignin removal, as the percent yield of lignin remaining in the pretreated solid asymptotically approaches 10% (Figure 5C) at 100 °C. Raising the pretreatment temperature has a noticeable effect on increasing the removal of lignin as shown in Figure 5C; here, each trace shifts downward to a slightly lower retention of lignin in the pretreated solid. The ash fraction (which does not include ash from the addition of NaOH) follows a similar trend to that seen for lignin (Figure 5E).

The yields of glucan and xylan in (Figure 5A and B) show significantly different trends with increasing NaOH loading and pretreatment temperature. For glucan at a pretreatment temperature of 100 °C, the amount present in the pretreated solid approaches 80% with increasing NaOH loading. At higher temperatures of 130 and 160 °C, less glucan is present in the pretreated solid, and the dependence on NaOH loading is stronger than at 100 °C. For xylan, Figure 5B, there appears to be no dependency on temperature within the conditions

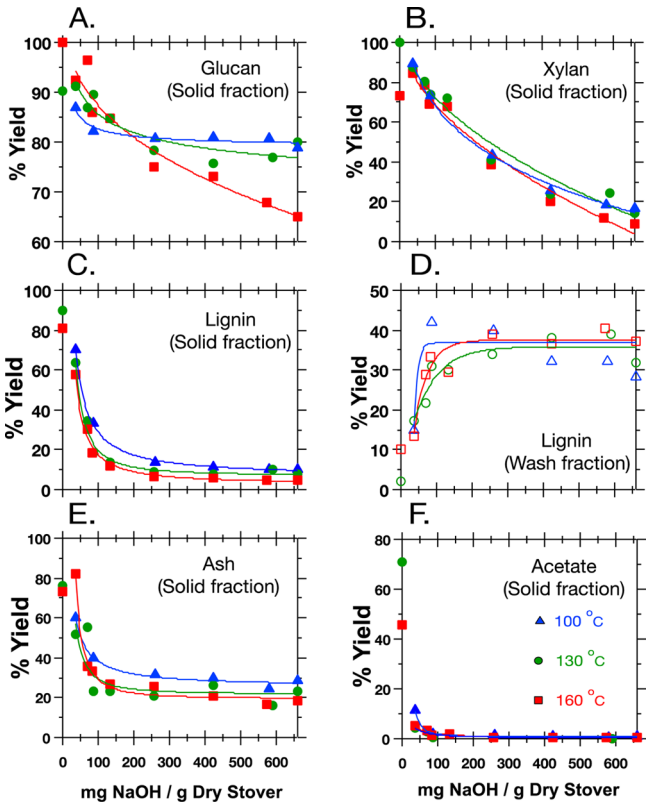

Figure 5. Percent yields of glucan (A), xylan (B), lignin (C), ash (E), and acetate (F) remaining in the pretreated solid phase for pretreatment conditions at 100 °C (blue filled triangles), 130 °C (green filled circles), and 160 °C (red filled squares) as a function of mg NaOH/g dry stover. Yields were calculated from the data in Figures 1 and 2 using eq 3 as described in the text, and the solid traces are the best-fit lines to each data set. For the equations for the best-fit lines, see Table S1 of the Supporting Information. Panel D shows the percent yield of lignin present in the wash phase, which is a measured value; here, data at 100 °C is represented with open triangles, at 130 °C open circles, and 160 °C open squares.

studied here, but a strong dependency on the NaOH loading is apparent. The percent yield of xylan in the pretreated solid decreases from 90% at the lowest NaOH loading to only ~10% at the most severe condition. Lastly, acetate removal is rapid (Figure 5F) with only 11% remaining in the pretreated solid at the lowest severity condition (100 °C 35 mg NaOH/g dry stover), and it quickly drops to 0% in the pretreated solid with a small increase in the NaOH loading.

Wash Fraction. As described in the Experimental Section, compositional analysis of the wash fraction was performed with HPLC for carbohydrate content, and the lignin content was measured with UV/vis. No peak was detected for any sugar (glucose, xylose, or minor sugar) in the HPLC data. The only component detected was lignin through the UV/vis measurement. These measurements are presented in Figure 5D as the yield of lignin present measured in the wash phase (i.e., percent of lignin from the starting dry corn stover that is present in the wash phase). The percent yield of lignin present in the wash phase is best fit with an exponential function of the form $y = A + Be^{Cx}$ (see Table S1, Supporting Information, for the specific power law parameters) and becomes constant at approximately 35 ± 10% for NaOH loadings above 85 mg NaOH/g dry stover. Previous literature has shown that the nature of the lignin removed from pulp by washing is high molecular weight lignin,[52] and it is likely this large heterogeneous lignin distribution present in the wash fraction that is contributing to the noise observed in this measurement.

Black Liquor Composition. The aqueous black liquor phase is a complex mixture of carbohydrates, acids, lignin (both high and low molecular weight), and acetate.[40] The direct analysis of black liquor remains a major analytical challenge due to the heterogeneity of this mixture and its sensitivity to pH change below 7, which causes much of the material to precipitate out into a thick lignin–carbohydrate complex. However, using the data presented in Figure 4, the composition of the stover solubilized into the black liquor phase can be determined by difference from the amount of each fraction (glucan, xylan, etc.) present in the starting dry corn stover and the yields of each fraction present in the pretreated solids. To determine the percent yield of a particular fraction in the black liquor, eq 4 is used

$$Y_{i,\mathrm{bl}} = 100 - Y_{i,\mathrm{ps}} \tag{4}$$

Here, $Y_{i,\mathrm{bl}}$ is the percent yield of component i in the black liquor phase calculated by the difference from the yield of component i in the pretreated solid phase. For the yield of lignin in the black liquor phase, the lignin present in both the pretreated solid phase and the wash phase must be accounted for using eq 5

$$Y_{\mathrm{lignin,bl}} = 100 - Y_{\mathrm{lignin,ps}} - Y_{\mathrm{lignin,w}} \tag{5}$$

In eq 5, the yield of lignin in the wash phase is given by $Y_{\mathrm{lignin,w}}$. Note that the fitted equations for the yields of each component in the pretreated solid and wash phases can be found in Table S1 of the Supporting Information. Equations 4 and 5 give the yield of each component from the starting dry biomass that is present in the black liquor phase. These yields are used to calculate the w/w composition of the black liquor as a function of pretreatment severity using eq 6

$$W_{i,\mathrm{bl}} = \frac{Y_{i,\mathrm{bl}} \times W_{i,\mathrm{dry\ stover}}}{\sum_{N=1}^{i} (Y_{i,\mathrm{bl}} \times W_{i,\mathrm{dry\ stover}})} \tag{6}$$

In eq 6, $W_{i,\mathrm{bl}}$ is the weight fraction of component i in the black liquor. Here, the calculated w/w composition from eqs 4–6 does not include the water content of the black liquor. Also, note this w/w composition does not represent the actual chemical composition of the black liquor as some of the solubilized carbohydrates are cleaved and oxidized to various hydroxy acids, and the lignin polymer is partially fragmented into numerous aromatic compounds including acids, alcohols, and hydrocarbons. Therefore, we stress that the composition reported here is only the w/w composition of the stover material solubilized into the black liquor fraction. Accordingly, Figure 6 displays this w/w composition of the stover solubilized into the black liquor as a function of NaOH loading and temperature calculated from eq 6. Here, we find that at low loadings of NaOH there is roughly an even split of the major components glucan, xylan, lignin, acetate, and ash, each approximately 15% of the w/w composition. An increase in temperature at a given NaOH loading does not have a dramatic effect on increasing the w/w composition of any one component. However, increasing the loading of NaOH does cause a large increase in the amount of xylan solubilized into the black liquor phase, and thus, the w/w composition of xylan increases from approximately 15% at 35 mg NaOH/g dry stover to approximately 40% at 660 mg NaOH/g dry stover.

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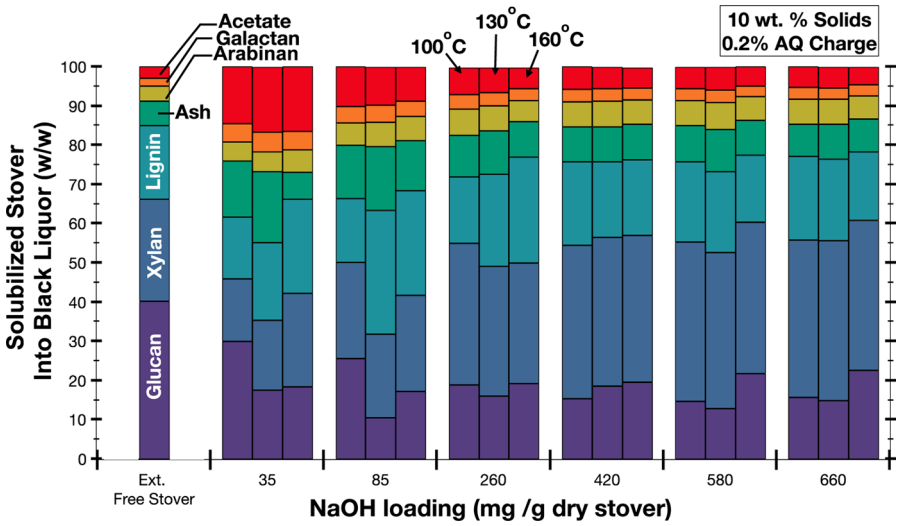

Figure 6. Composition of stover solubilized into the black liquor fraction (w/w, excluding water) shown as a function of increasing NaOH loading. The black liquor composition is calculated by the difference from the measured composition of the pretreated solid and the composition of the original dry corn stover as described in the text. The data presented here are binned the same way as Figure 2 with the composition of the original untreated dry corn stover in the left-most bin labeled "Ext. Free Stover." Compositional data for black liquor at pretreatment temperatures of 100, 130, and 160 °C are binned according to their NaOH loading, with the left-most stack in each bin representing a pretreatment temperature of 100 °C, the middle stack representing a pretreatment temperature of 130 °C, and the right-most stack in each bin representing pretreated solids at 160 °C (as noted in the temperature labels above the stacks in bin 260).

Optimal Pretreatment Conditions. The data presented in this study allow the optimal conditions (NaOH loading and temperature) for alkaline pretreatment of corn stover to be calculated from a comprehensive bench-scale evaluation of severities. Certainly, the most favorable condition for a pretreatment procedure depends on the goals of the process. The attractive feature of alkaline pretreatment is that it creates a soluble stream of lignin that potentially could be upgraded to value-added co-products if appropriate technologies are developed and subsequently enables lower enzyme loadings or catalyst severities in downstream processes due to the partial removal of lignin from whole biomass. Given possible values for the lignin in the black liquor stream and given that sugar yields tend to dominate process economics for fuel and chemical production,[12,53−55] it is likely that the optimal conditions for an alkaline pretreatment process would occur where the maximum amount of lignin is removed into the black liquor phase while minimizing the solubilization of xylan and glucan. On the basis of these requirements, the data collected in this work can be used to calculate the optimal pretreatment temperature and NaOH loading applying eq 7

$$f = \frac{Y_{\text{lignin,bl}}}{Y_{\text{glucan,bl}} + Y_{\text{xylan,bl}}} \quad (7)$$

Here, we create the fractionation optimization function eq 7, where the desired product (lignin yield in the black liquor phase) is divided by the undesired products in the black liquor fraction (glucose and xylose yields). The maximum of this function represents the optimal NaOH loading that yields the most lignin in the black liquor phase while minimizing the loss of glucose and xylose into the black liquor. The fitted equations for the yields of glucan, xylan, and lignin in the black liquor, calculated from eqs 3, 4, and 5 (Table S1, Supporting Information), are used in eq 7, and the maximum of this function is determined. This procedure results in the optimum NaOH loading at 100 °C to be 120 mg NaOH/g dry stover, at 130 °C to be 62 mg NaOH/g dry stover, and at 160 °C to be 47 mg NaOH/g dry stover. To find the optimal pretreatment temperature, the yields of lignin, xylan, and glucan in the liquor phase need to be compared at these optimal NaOH loadings for each temperature. As such, the optimal loading of 120 mg NaOH at 100 °C removes 38% of the lignin, 18% of the glucan, and 35% of the xylan into the liquor phase. While this is the best lignin removal that can be achieved (in ratio to solubilized carbohydrates), there is still a large loss of xylan here (35%) at this pretreatment temperature. At a pretreatment temperature of 130 °C, the removal of lignin is faster so at the optimal NaOH loading, 43% of the lignin, 11% of the glucan, and only 20% of the xylan is removed to the liquor phase. This highlights the fact that an increase in temperature to 130 °C increases the rate of lignin removal significantly (Figure 4C). Increasing the pretreatment temperature further to 160 °C results in only slight improvement in carbohydrate retention compared to the optimal condition at 130 °C. At 160 °C, 38% of the lignin, 7% of the glucan, and 19% of the xylan is removed to the liquor phase.

DISCUSSION

Using the results from this work, comparisons can be made to the recent work of Li et al.[25] wherein four alkaline/AQ pretreatments were performed (70 and 100 mg NaOH/g dry stover with 0.05% AQ charge at 140 and 160 °C). Interpolating from the fitted data in Figure 5, we find nearly identical results to those four conditions reported by Li et al. As an example, at pretreatment conditions of 100 mg NaOH/g dry stover at 160 °C, the interpolated w/w composition of the pretreated solid from this work is 54 ± 1.6% glucan, 29 ± 0.12% xylan, and 7.3 ± 1.4% lignin, nearly identical to that reported by Li et al. of 55% glucan, 22% xylan, and 12% lignin. For comparison between all of the pretreatment conditions of Li et al. and the data presented in this work, see Table S2 of the Supporting Information. Again, at these four conditions, we find very similar values to that reported in their work.

The data collected in this work for such a wide range of alkaline pretreatment severities provides considerably more insight into how each fraction of the biomass is affected by NaOH loading and temperature (Figure 5). Of particular interest are the lignin and xylan fractions. In Figure 5C, lignin is removed rapidly in the NaOH loading regime between 35 and 260 mg NaOH/g stover, but at higher NaOH loadings only a miniscule increase in lignin removal is observed. Additionally, increasing the temperature, specifically to 130 °C and above, results in a noticeable improvement in delignification. Fortuitously, xylan solubilization is found to be independent of pretreatement temperature (Figure 5C) but very strongly correlated with increasing NaOH loading. The strong dependence of xylan removal to NaOH loading is likely due to the ester linkages between the xylan polymer and the lignin polymer. Many of these linkages are formed through coumarates and ferulates and are easily broken in alkaline conditions.[56,57] Thus, xylan becomes more exposed to the alkaline solution where it is more easily dissolved via endwise peeling reactions compared to glucan, which is more resistant to dissolution because it lacks these labile ester linkages. If the goal of the pretreatment process is to retain the hemicellulose in the solid phase while maximizing lignin removal, then these results indicate that the optimal alkaline pretreatment will utilize a low loading of NaOH to mitigate xylan solubilization and use a high pretreatment temperature of 160 °C or possibly even higher to increase the removal of lignin.

The results of this work also demonstrate the importance of a wash step to increase the removal of lignin from the solid phase. At NaOH loadings of 85 mg NaOH/g dry stover and above, approximately 35 ± 10% more lignin can be removed from the solid phase by washing the solids (Figure 5D). It is interesting that the data here show that washing only removes lignin and not any additional carbohydrates. This is likely because solubilized lignin is deposited back onto the pretreated solid as the pretreatment vessel is cooled. We saw evidence for this phenomenon in our TEM analysis (Figure S3C and D, Supporting Information). In pretreatment systems with extensive agitation, this redeposition of lignin onto the solid phase could be mitigated, and a wash step may not be needed. However, the large amount of lignin removed by washing the pretreated solid phase in this work makes this a step worthy of investigation in large-scale alkaline pretreatment processes.

Microscopic analysis presented in Figures 3 and 4 reveal architectural changes in the biomass particles and cell walls as a function of severity. There is a clear pattern of increasing intercellular dislocation with increasing NaOH loadings. This is expected as the increasing severity will more completely degrade the high lignin content of the middle lamella and correlates well with the pattern of particle size reduction due to fragmentation of cellular clusters observed at the light microscopy scale. These images also suggest that increased NaOH loadings result in an apparent mechanical weakening of the secondary cell wall that manifests as bent, deformed, and even twisting and coiling of fiber cells at the highest NaOH loadings. These structural phenomena likely result from the removal of structural polysaccharides and lignin from the cell wall matrix, which reduces the resistance of the secondary wall to mechanical stresses. In addition to these microstructural changes, macrostructural changes are also observed in the pretreated solid. In the NaOH loading regime between 85 and 260 mg NaOH/g dry stover and at all temperatures studied here, a layer of fines is observed (Figure S5, Supporting Information). This layer was analyzed and found to be compositionally identical to the larger particles of pretreated solids that settle below the layer of fines (Figure S5, Supporting Information). At loadings below 85 mg NaOH/g dry stover, this layer is not observed likely because the severity is too low to cause this macrostructure change. At loading above 260 mg NaOH/g dry stover, this layer is also not present; here, the severity is likely so high that any fines become solubilized into the black liquor phase leaving behind only the large particles of pretreated stover. These fine particulates pose challenges in processing as they readily plug filtration membranes and could cause difficulties in large scale processing where pressing and solid transport are common operations.

The feasibility of alkaline pretreatment will undoubtedly depend on the value that can be obtained from upgrading the liquor and solid fractions created during pretreatment. Maximizing the retention of cellulose and hemicellulose in the solid phase is important, as this phase can be enzymatically saccharified and converted to fuels or chemicals by known biological or catalytic processes, but some loss of hemicellulose to the liquor phase may not be detrimental to the applicability of alkaline pretreatment if methods can be developed to recover or convert the chemical components present in the black liquor stream including the xylose and other minor sugars. Certainly utilization of lignin and other minor components in black liquor remains a major challenge in the chemical industry, but lignin valorization is receiving increasing attention as an essential part of a comprehensive biorefinery concept to produce coproducts.[54,58−60]

CONCLUSIONS

Alkaline pretreatment with the additive anthraquinone was performed on corn stover biomass over a wide range of NaOH loadings and at temperatures of 100, 130, and 160 °C. The optimal condition for maximum lignin removal into the black liquor phase and minimum solubilization of xylan and glucan is found at 62 and 47 mg NaOH/g dry biomass at temperatures of 130 and 160 °C, respectively. Pretreatment temperatures of 130 °C and greater are found to notably increase the removal of lignin, and thus, optimal pretreatment temperatures of 130 and 160 °C are reported here rather than at 100 °C where more hemicellulose is lost due to the slower rate of lignin removal. This is because lignin removal is markedly increased as temperatures increase above 100 to 130 °C but only slightly increased at pretreatment temperatures of 160 °C compared to 130 °C treatments, given the same NaOH loading. Xylan is increasingly solubilized as the NaOH loading increases, but increasing temperature does not increase the solubilization of xylan within the error of the measurements reported here. Glucan solubilization is less sensitive to NaOH loading, but at temperatures of 130 °C and above, an increase in solubilization is observed with a minor dependence on NaOH loading. At all conditions studied, washing the pretreated solids with water removed at most 35 ± 10% more lignin from the pretreated solids and may be an important step to investigate in large-scale processes to increase the lignin removal. These results point to optimal pretreatment conditions at temperatures of 160 °C or possibly higher and low NaOH loadings (below 100 mg NaOH/g dry stover).

■ ASSOCIATED CONTENT

Supporting Information

Figures and tables referenced in the above text, expanded data sets for Figures 2, 5, and 6, additional TEM images, and an image of the pretreated solid. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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