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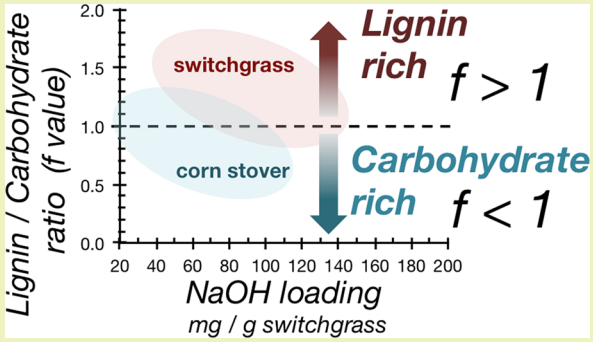
Alkaline Pretreatment of Switchgrass

Eric M. Karp,[†] Michael G. Resch,[†] Bryon S. Donohoe,[‡] Peter N. Ciesielski,[‡] Marykate H. O'Brien,[†] Jennifer E. Nill,[†] Ashutosh Mittal,[‡] Mary J. Biddy,[†] and Gregg T. Beckham*[,†]

[†]National Bioenergy Center and [‡]Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado 80401, United States

Supporting Information

ABSTRACT: Alkaline pretreatment using sodium hydroxide offers a means to extract lignin and acetate from lignocellulosic biomass, in turn enabling higher enzymatic digestibility of the remaining polysaccharides and production of a lignin-enriched stream for potential valorization. Key criteria for alkaline pretreatment processes, which are important for commercial feasibility, include the minimization of carbohydrate loss during pretreatment and the ability to capture carbon lost to the liquor stream, much of which will be feedstock dependent. Here, we present a comprehensive study of alkaline pretreatment of switchgrass over NaOH loadings from 35 to 140 mg NaOH/g dry switchgrass and with a constant charge of 0.2% anthraquinone for pretreatment temperatures between 100 and 160 °C for 30 min. Full compositional analysis of the pretreated solids are reported as a function of pretreatment severity, along with the yields of each biomass component present in the process streams generated during pretreatment (pretreated solid, liquor, and wash fraction). The pretreated solids are further characterized through crystallinity measurements and electron microscopy. Additionally, enzymatic digestions of the residual solids are performed over a range of enzyme loadings for varying pretreatment severities. These results are compared to our recent work with alkaline pretreatment of corn stover using the ratio of lignin fractionation to carbohydrate retention (in the solids after pretreatment), which highlights the greater recalcitrance of switchgrass relative to corn stover. Specifically, compared to corn stover, switchgrass requires approximately twice the NaOH loading to achieve identical delignification and high enzymatic digestibility. From this work, the optimal pretreatment conditions for switchgrass are suggested to be 154 mg NaOH/g dry switchgrass at 130 °C for 30 min at temperature. The results from these bench-scale experiments will serve as a guide to scale up processes for the optimization of lignin removal while minimizing carbohydrate loss during alkaline pretreatment.



KEYWORDS: *Biofuels, Lignin valorization, Enzymatic hydrolysis, Lignocellulose, Pulping*

INTRODUCTION

Thermochemical pretreatment of lignocellulosic biomass is an often critical step in obtaining high yields of cellulose and hemicellulose-derived sugars for the production of renewable biofuels and chemicals.[1−3] Virtually all biomass pretreatment strategies aim to make polysaccharides in the plant cell wall more accessible for subsequent enzymatic, microbial, or catalytic deconstruction to soluble sugars, the development of which was primarily driven by the growth of the lignocellulosic ethanol industry.[2,3] Additionally, depending on the type of pretreatment employed, secondary pretreatment objectives can include removal of either lignin or hemicellulose from the cell wall or physical separation of lignin from the polysaccharides via solubilization and redeposition upon cooling.[4,5]

Acid, steam, and hot water pretreatments typically aim to hydrolyze and remove hemicellulose-derived sugars and redistribute lignin in biomass. These hydrothermal or acid pretreatment approaches are among the primary technologies used by several of the world's first industrial lignocellulosic ethanol plants. Organosolv fractionation pretreatments also employ an acid catalyst, but include at least one added organic solvent to extract lignin from the cell wall into a separate process stream.[6−9] Modification and solubilization of a portion of the lignin from the cell wall is an aim in chemical pulping processes employed in the pulp and paper industry. Similarly, ionic liquid pretreatment has received significant attention given the ability for some ionic liquids to solubilize cellulose, lignin, or whole biomass.[10,11] Base-catalyzed pretreatments are also popular and are being scaled-up for industrial use. The well-studied ammonia fiber expansion (AFEX) approach, which solubilizes a fraction of primary plant cell wall components, is able to render biomass quite digestible by fungal cellulases, likely by increasing cell wall porosity.[4,12,13] Other alkaline approaches include alkaline pretreatment with sodium hydroxide and alkaline peroxide (the latter using a combination of NaOH and H_2O_2), which aim to remove lignin from biomass

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and retain polysaccharides for subsequent downstream depolymerization.[14−20] The economics for cellulosic ethanol production using several leading pretreatment technologies were compared by a large consortium of investigators and were found to be reasonably similar in cost and overall yield (when enzymatic hydrolysis is included) of fermentable sugars.[1,21−24]

As previously mentioned, many of the more industrially relevant pretreatment approaches studied to date have been primarily developed for the bioethanol industry. Going beyond cellulosic ethanol, research is now focusing on drop-in hydrocarbon fuels from lignocellulosic biomass, motivated by several factors including limited market potential for ethanol in the gasoline pool, the ethanol blending wall, and incompatibility of ethanol with the current distribution system for transportation fuel.[25] Given the state of technology for current approaches to produce hydrocarbon fuels from biomass, many of these technologies will still require significant investment in scale-up and process integration to be viable for lignocellulosic biomass conversion to hydrocarbon fuels. A major finding of techno-economic and life-cycle analyses on hydrocarbon biofuel production to date is that lignin valorization will be essential for the long-term economic and environmental sustainability of hydrocarbon biorefineries,[25] a sentiment echoed in several recent reviews of lignin valorization.[26−28]

The need to derive value from lignin in industrial-scale biorefineries will require significant research and development, including in pretreatment technology. This is especially true in the context of maintaining high sugar yields for the production of fuels and chemicals from cellulose and hemicellulose fractions, which include technologies that are typically more mature than those for lignin valorization. Certainly, many technical targets in the biomass value chain can be improved to make lignin valorization a reality, including feedstock engineering,[29−34] fractionation technologies, catalysis or biocatalysis approaches,[26,35−40] and new integrated methods to convert lignin to value-added fuels, chemicals, or materials.[41−43] From a pretreatment standpoint, the evaluation (or re-evaluation) of pretreatment strategies will be required to identify technologies able to produce lignin-enriched streams amenable for upgrading, either during pretreatment or as a residual solid after polysaccharide digestion.

Alkaline catalysis, either employed in pretreatment or post-treatment (i.e., after carbohydrates have been removed from biomass) may be particularly amenable for producing lignin-enriched streams that can be valorized.[41,42] Alkaline pretreatment using sodium hydroxide and anthraquinone (AQ), similar to low severity soda pulping, is able to produce an alkaline pretreated liquor (APL), which depending on the NaOH and AQ loadings and pretreatment temperature, can contain substantial amounts of lignin-enriched species, acetate from hemicellulose, biomass extractives, and some sugar degradation products.[44] This stream is undoubtedly heterogeneous, requiring either an APL fractionation scheme or an upgrading scheme that can deal with its intrinsic heterogeneity. To that end, we have recently reported a biological funneling approach to upgrade APL using aromatic-catabolizing bacteria.[41,42,45] This approach has been employed with wild-type *Pseudomonas putida* KT2440 under nitrogen limiting conditions to produce medium-chain-length polyhydroxyalkanoates.[41] Our group has also conducted metabolic engineering of the same organism to produce muconic acid from lignin-derived species in APL, which can be separated and catalytically upgraded to adipic acid, an important polymer precursor in nylon production,[42] as well as to accumulate either pyruvate or lactate from lignin-derived species.[45] Additional approaches to fractionate and upgrade alkaline liquors, including from flax soda pulping, have found uses as biodispersants and as blending materials.[46,47]

Several of these previous lignin-upgrading experiments were conducted using corn stover as the starting feedstock.[41,42,44] Optimal conditions for lignin removal and sugar retention for corn stover were determined in a previous study from our group as well, wherein we examined a large range of temperatures and sodium hydroxide loadings.[44] In the pulp and paper industry, the choice of specific alkaline treatments is based on their delignification capacity and is feedstock dependent. This, and experience with other pretreatments, suggests the need to develop feedstock-specific, feedstock-agnostic, or mixed-feedstock processing options. While switchgrass and maize are both grasses and share a common order and family, they differ in many ways including their tissue structure and cell wall composition.[48,49] Given the inherent differences in the cell wall architecture in corn stover and switchgrass, namely, that switchgrass exhibits narrow, hollow stems with little pith and corn stover exhibits larger stems with extensive pith and parenchyma tissue, it was hypothesized that switchgrass will perform quite differently than corn stover. To examine this hypothesis, here we perform bench-scale alkaline pretreatment as a function of temperature and NaOH loading, conduct detailed physical and chemical characterization of the residual switchgrass substrate, and conduct enzymatic digestions using a commercial cellulase cocktail. Overall, this study reveals that alkaline pretreatment of switchgrass must be conducted at a much higher severity to achieve optimal lignin removal and optimal polysaccharide digestibility relative to corn stover. This finding has important implications for a mixed-feedstock biorefinery using these two substrates either separately or simultaneously.

EXPERIMENTAL SECTION

Pretreatment Procedure. Air-dried switchgrass from Idaho National Laboratory was milled to 2 mm or smaller particle size and subjected to successive water and ethanol extractions to remove saps and waxes. Compositional analysis of the dry, untreated, extracted switchgrass yielded a w/w composition of 34.8 ± 1.5% glucan, 21.6 ± 0.2% xylan, 23.5 ± 0.6% lignin, 5.3 ± 0.2% ash, 4.8 ± 0.2% arabinan, 2.1 ± 0.1% galactan, and 2.9 ± 0.1% acetate (Figure 2).

Pretreatment experiments were performed by loading the milled and extracted switchgrass into sealed 316 stainless steel vessels at 10 wt % dry solids loading in ultrapure water. Sodium hydroxide (Sigma-Aldrich Lot MKBP3689V) loadings were varied between 35 and 140 mg/g dry switchgrass and the anthraquinone (Sigma-Aldrich Lot BCBJ0829V) loading was held constant at 0.2% charge (w/w) to dry switchgrass. 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 an additional 30 min. A temperature controller automatically adjusted the linear ramp rate to reach the set point in 30 min time. The temperature was measured with a k-type thermocouple positioned in the slurry within a thermo well, and no agitation was employed. At the end of the pretreatment run, 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 three fractions: a pretreated solid, an aqueous alkaline pretreatment liquor (APL), and a wash fraction. The separation was performed by first centrifuging the slurry for 30 min at 20 000 g to pelletize the solids, and the caustic APL 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 000g for 30 min. The wash water was decanted from the solid fraction after centrifugation. This process

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was repeated with an additional 1 L of ultrapure water and the solids were resuspended and stored in a cold room at 4 °C overnight to sufficiently remove any residual alkalinity retained in the solid fraction. After 12 h, the wash water was decanted from the settled solids. Finally, to remove the small remaining amount of wash water that could not be decanted, the solids were vacuum filtered through 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. The recovered washed solids were then 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 pretreated 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 pretreated solids and wash fraction was subsequently performed. For the dried solids, compositional analysis was conducted in accordance with standard NREL laboratory analytical procedures (LAPs).[50,51] 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 to the NREL LAPs.[50]

APL is a complicated mixture of acids (derived from carbohydrate degradation reactions that occur in alkaline conditions), partially depolymerized polysaccharides, monosaccharides, aromatic monomers (derived from lignin), high molecular weight lignin, and acetate.[52] At present, no single method exists to completely identify and quantify all components present in APL. Therefore, for the purposes of this work, we report the composition of the APL 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, the composition of the retained solids, and the composition of the recovered wash. A separate study is underway wherein several new analytical methods alongside established protocols are applied to identify and quantify the compounds present in APL.

Crystallinity Measurements. To examine and compare the crystalline structure of both untreated and alkaline treated samples, X-ray diffraction (XRD) was performed by using a Rigaku (Tokyo, Japan) Ultima IV diffractometer with Cu Kα radiation having wavelength λ(Kα1) = 0.15406 nm generated at 40 kV and 44 mA. Freeze-dried samples were placed on a quartz substrate, and the diffraction intensities were measured in the range of 8−42° 2θ using a step size of 0.02° at a rate of 2°/min. The crystallinity indices (CrI) of the cellulose samples were calculated according to the method described by Segal et al.[53] 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.

SEM Imaging. Imaging by scanning electron microscopy (SEM) was performed using a FEI Quanta 400 FEG instrument (FEI, Hillsboro, OR). Samples were freeze-dried prior to imaging and mounted on aluminum stubs using conductive carbon tape. The stubs were then sputter-coated with ∼10 nm of gold. Imaging was performed with beam accelerating voltage of 10 keV.

TEM Imaging. For transmission electron microscopy (TEM) imaging, biomass samples were dehydrated by treatment with increasing concentrations of ethanol in a Pelco laboratory microwave oven (Ted Pella, Redding, CA) for 1 min for each sample in ethanol solutions of 15, 30, 60, and 100 vol %, followed by two additional dehydration steps using 100% ethanol. After dehydration, the samples were infiltrated with LR White (London Resin Company) by incubating at room temperature for several hours to overnight in similarly increasing resin concentrations. The samples were transferred to capsules and the resin polymerized in an oven at 60 °C overnight in a nitrogen-purged oven. Ultrathin (∼60 nm) sections were positioned on 0.5% polyvinyl formal coated copper slot grids (SPI Supplies, West Chester, PA). Grids were poststained for 1 min with 1% aqueous $KMnO_4$. Images were captured with a 4-megapixel Gatan UltraScan 1000 camera (Gatan, Pleasanton, CA) on a FEI Tecnai G2 20 Twin 200 kV LaB6 TEM (FEI, Hillsboro, OR).

Enzymatic Saccharification of Pretreated Switchgrass. Enzymatic saccharification reactions were carried out according to NREL's Laboratory Analytical Procedure.[54] CTec3 and HTec3 (Novozymes, Franklinton, NC) were applied to an AKTA FPLC (GE, Pittsburgh, PA) using a HiPrep 26/10 Sephadex (GE, Pittsburgh, PA) desalting column to remove stabilizers and other additives that interfere with the bicinchoninic acid (BCA) protein assay and HPLC sugar quantification. Protein concentration was measured by BCA (Pierce, Rockford, Il). Enzymatic hydrolysis reactions with CTec3 and HTec3 in a loading ratio of 10:1 were incubated at 50 °C in 20 mM sodium acetate at pH 5.0. Digestions contained 1% solids (10 mg of biomass per mL) and were conducted in sealed 2 mL HPLC vials with continuous mixing by inversion at 10−12/min intervals. Unless otherwise noted, substrates were loaded as milligram of protein per gram of glucan in 1.4 mL reaction volumes. Slurry samples were withdrawn from well-mixed digestion mixtures at selected time points during the digestions. Released cellobiose, glucose, and xylose in the diluted samples were then measured by HPLC analysis. The sum of the concentrations of *anhydro*-glucose, *anhydro*-cellobiose, and *anhydro*-xylose is equivalent to the weight concentration of the carbohydrate chain that was hydrolyzed to produce the soluble sugars; this value was then divided by the initial weight concentration of cellulose or xylan in the pretreated biomass and multiplied by 100 to yield activity results as percent conversion of cellulose and hemicellulose.

RESULTS

Alkaline pretreatment of the extracted switchgrass was performed in sealed stainless steel vessels, as described above, at 10 wt % solids loading with a 0.2% charge of AQ. The sodium hydroxide loadings were varied between 35 and 140 mg NaOH/g of dry switchgrass and the pretreatment was performed for 30 min at temperature for three different temperatures: 100, 130, and 160 °C. This range of sodium hydroxide loadings was chosen to center on the conditions found optimal in our previous study of alkaline pretreatment of corn stover.[44] Additionally, the AQ charge of 0.2% was chosen because this is an average loading relative to what is typically used in the pulp and paper industry.[55] We also note that the lowest NaOH loading used in these experiments (35 mg NaOH/g) is the same loading used in "deacetylation", commonly applied to corn stover at the National Renewable Energy Laboratory.[56−58] The results of this study are presented with treatment identical to that found in our previous alkaline pretreatment work with corn stover[44] and the same equations used in that study are presented again in this work for clarity.

Percentage of Solids Retained after Pretreatment. After pretreatment, the remaining solids were separated from the slurry, as described above, and the mass of the recovered solids was measured. The percentage of dry solids retained is calculated using this measurement and eq 2:

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

where $m_{\mathrm{s,i}}$ is the initial mass of dry switchgrass loaded into the reactor and $m_{\mathrm{s,f}}$ is the final mass of the dry, pretreated switchgrass recovered after pretreatment. The percentage of dry switchgrass retained after pretreatment is displayed in Figure 1 as a function of sodium hydroxide loading with three traces shown for the different pretreatment temperatures (100, 130, and 160 °C). The error on the values in Figure 1 is estimated to

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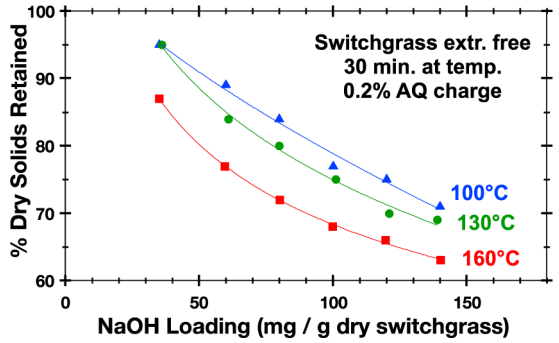

Figure 1. The percentage of dry solids retained after pretreatment at 100 °C (blue triangles), 130 °C (green circles), and 160 °C (red squares) as a function of sodium hydroxide loading. Each point has an error of ±1.0% (see text) and represents pretreatment conditions of 10 wt % dry switchgrass loading in ultrapure water with 0.2% charge AQ (w/w on dry switchgrass). The solid lines represent the best-fit curve for the data points at each temperature. The equations for the best-fit lines are listed in Table S1.

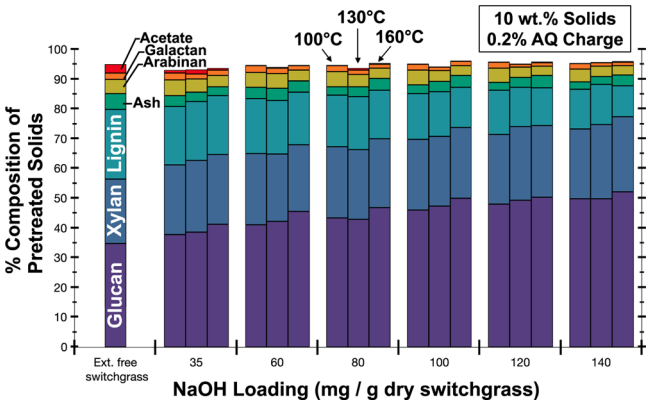

Figure 2. The composition of the pretreated solids at pretreatment conditions of 10 wt % solids and 0.2% AQ charge are shown as a function of NaOH loading and temperature. Compositional data for the pretreated solids at temperatures of 100, 130, and 160 °C are binned according to their NaOH loading, with the leftmost stack in each bin representing a pretreatment temperature of 100 °C, the middle stack representing a pretreatment temperature of 130 °C, and the rightmost stack representing pretreated solids at 160 °C (as noted in the temperature labels above the stacks labeled "80"). The leftmost bin labeled "ext. free switchgrass" contains only one stack and represents the composition of dry extractives of free switchgrass before pretreatment, the exact composition of which is also given in the Experimental Section. The labels on the stack in the "Ext. free switchgrass" bin are consistent for every stack in the figure. The measured compositional closure of the pretreated solids ranged from 93 to 96%.

be ±1.0%. (Note that error reported on a measurement in this work represents the 95% confidence band.) This is based on a set of four replicates at 80 mg NaOH/g dry switchgrass and a pretreatment temperature of 130 °C. The results in Figure 1 display the expected trend in decreasing retention of solids as the sodium hydroxide loading is increased. At 100 °C, increasing the sodium hydroxide loading from 35 to 140 mg NaOH/g dry switchgrass results in decreasing the retained solids from 95 ± 1.0% to 71 ± 1.0%. A strong dependence on biomass solubilization is also found with increasing pretreatment temperature. For example, data at 100 °C are best fit with the line $y = 103 + -0.23x$; however, as the pretreatment temperature is increased, the data exhibit a downward shift and increased curvature. The increase in curvature results in best-fit functions of the form $y = A + Bx^C$ rather than a line that best fits the data at 100 °C (Table S1). Even at the lowest sodium hydroxide loading (35 mg NaOH/g dry switchgrass), increasing the pretreatment temperature to 160 °C decreases the amount of retained solids by ∼9%.

Composition of Pretreated Solids. The weight percent (w/w) composition of the pretreated solids was measured in accordance with NREL's LAPs,[50,51,59] as described in the Experimental Section, and the results are presented in Figure 2. In the leftmost bin of Figure 2, the composition of untreated switchgrass is shown for reference.

There is a clear trend in enrichment of carbohydrates present in the pretreated material as the sodium hydroxide loading is increased. At the lowest sodium hydroxide loading, glucan on average comprises 39 ± 1.5% of the pretreated material, which is 4% greater than in the untreated switchgrass. The glucan fraction in the pretreated material further increases to 50 ± 1.5% by weight at the highest sodium hydroxide loading (140 mg NaOH/g dry switchgrass). The enrichment in carbohydrates is expected as alkaline treatment removes primarily lignin and acetate, a trend clearly seen in these data. For example, lignin comprises ∼20% of the residual material obtained after pretreatment with 35 mg NaOH/g dry switchgrass and decreases to ∼12% at the highest sodium hydroxide loading. Acetate is almost completely removed even at the lowest sodium hydroxide loadings, and at a loading of 60 mg NaOH/g dry switchgrass, no acetate is detectable in the pretreated material. The data in Figure 2 are consistent with the trend seen in Figure 1 showing a dependence on pretreatment temperature. In particular, an increase in glucan and xylan enrichment is seen in Figure 2 at all sodium hydroxide loadings when the pretreatment temperature is increased to 160 °C, due to an increase in lignin removal.

As an example, take the composition of the pretreated switchgrass at 140 mg NaOH/g dry switchgrass (the rightmost bin of Figure 2): the left stack in this bin represents pretreatment at 140 mg NaOH/g dry switchgrass at 100 °C, and the center stack in this bin represents the composition of pretreated material at the same sodium hydroxide loading and 130 °C.

Comparing these two pretreated solids compositions, it is clear that the glucan and xylan compositions are identical, within the error of the measurement, at 49 ± 1.5% and 23 ± 0.6%, respectively, but the rightmost stack for the pretreated solids at 160 °C shows an increase in glucan and xylan enrichment to 53 ± 1.5% and 25 ± 0.6%, respectively. This marked increase in carbohydrate enrichment at the 160 °C pretreatment temperature is evident in all of the rightmost stacks in each bin in Figure 2.

Crystallinity of Pretreated Solids. Table 1 lists the measured crystallinity of the pretreated solids as a function of pretreatment severity. Unlike corn stover,[44] switchgrass displays a trend of increasing crystallinity with increasing sodium hydroxide loading. On average, the crystallinity increases by 1% for every 20 mg increase in sodium hydroxide loading per gram of dry switchgrass (Figure S1) for the conditions examined in this work. Crystallinity of the pretreated solids does not appear to be influenced by pretreatment temperature, and small variations in the slope of the fitted lines for the three pretreatment temperatures are within the experimental error of the measurement (Figure S1). The increase in crystallinity for

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Table 1. Cellulose Crystallinity as a Function of Pretreatment Severity

NaOH loading (mg NaOH/g dry switchgrass)	crystallinity (CrI) ± 1.6%[a] 100 °C	130 °C	160 °C
untreated	43%[b]		
35	48%	46%	48%
60	47%	50%	49%
80	49%	50%	51%
100	51%	50%	51%
120	49%	53%	51%
140	51%	53%	50%

[a]The error on this measurement of ±1.6% represents the 95% confidence band, which was found by measuring a set of four replicates of untreated switchgrass. [b]Untreated switchgrass was milled to particles 2 mm or less in size.

switchgrass is likely due to the high level of carbohydrate retention (Figure 5 below) compared to that of corn stover, which displayed a significant loss of glucan and xylan from the pretreated solid at the same pretreatment conditions.[44]

SEM and TEM Imaging of Pretreated Solids. SEM was used to investigate changes in particle morphology occurring during alkaline pretreatment at a pretreatment temperature of 130 °C, which is the "middle" temperature examined in this work. Untreated switchgrass particles shown in Figure 3a

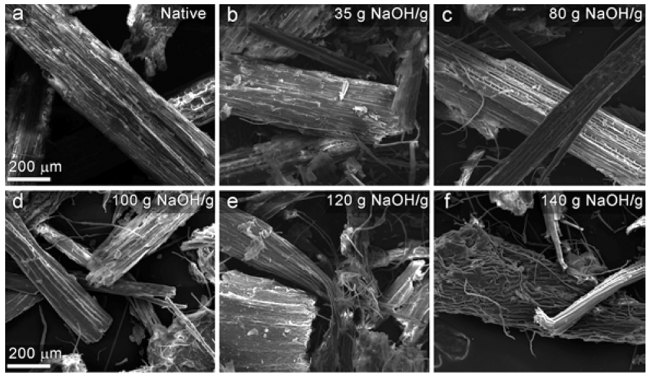

Figure 3. SEM micrographs of alkaline treated switchgrass particles at several different sodium hydroxide loadings (noted in the upper right corner of each panel) and a pretreatment temperature of 130 °C. Alkaline pretreatment facilitates additional fragmentation of particles with respect to untreated particles, shown in panel a. This fragmentation, observed as segregation of fiber bundles and isolation of individual fiber cells in some cases, increases with pretreatment severity as shown in panels b–f. Weakening of the mechanical integrity of particles, evidenced as bending and deformation, is present after the high severity treatments as shown in panels e and f.

appear as high aspect ratio, intact clusters of cells. Micrographs of switchgrass particles treated with increasing loadings of NaOH shown in Figure 3b–f show increased fragmentation whereby smaller clusters of cells, and even individual fiber cells, are loosened and segregated from the larger particles. In addition, high NaOH loadings cause weakening of the mechanical properties of the cell wall, which manifests as particles that are bent, twisted, and structurally deformed relative to untreated particles. These morphological trends are similar to those observed previously for alkaline treated corn stover; however, the extent of cellular disjoining and structural deformation displayed by these samples is not as extensive as that observed for the corn stover feedstock treated at the same severities.[44]

Micro- and nanoscale effects of alkaline pretreatment on the cell wall architecture were observed by TEM. Micrographs of an untreated switchgrass cell wall and cell corner are shown in Figure 4a,b, respectively. Images of cell walls exposed to low

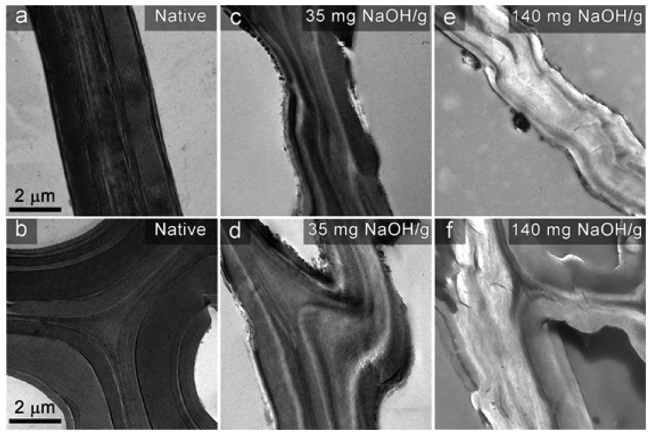

Figure 4. TEM micrographs of native and alkaline pretreated cell walls (top row) and cell corners (bottom row) in cross section. Compared to native switch grass cell walls, shown in a and b, cell walls exposed to low severity alkaline pretreatment, shown in c and d, suggest an increase in intracell wall void space observed as lighter regions within the cell wall, particularly at the interface between layers. High severity treatment produces delamination of cell walls with regions of extremely low density relative to untreated cell walls, as shown in panels e and f. Cell walls exposed to both low and high severity treatments appear generally lighter than the native cell walls, due to lower lignin content, and the extent of this effect trends with the extent of lignin extraction.

severity alkaline treatment, shown in Figure 4c,d, display regions of low density observed as lighter areas within the cell wall. These regions are likely formed by the removal of lignin and hemicellulose from the cell wall interior. Samples exposed to higher severity alkaline pretreatment (140 mg NaOH/g dry switchgrass at 160 °C) show evidence of severe deconstruction by delamination and intracell wall nanofibrillation (Figure 4c,f). The contrast of the cell walls is also significantly reduced compared to the other samples. This observation is consistent with relatively low residual lignin content because the staining agent used in these micrographs ($KMnO_4$) is known to preferentially stain lignin.[60]

Yields. By combining the data from Figures 1 and 2 in eq 3, the yield of the individual biomass components remaining in the pretreated solid can be calculated for each of the pretreatment conditions.

$$Y_{i,\mathrm{ps}} = \frac{\%\ \text{Dry solid retained} \times W_{i,\mathrm{ps}}}{W_{i,\text{dry switchgrass}}} \quad (3)$$

Here, $Y_{i,\mathrm{ps}}$ is the yield of component i (e.g., glucan, lignin, xylan, etc.) remaining in the pretreated solid, "% Dry solids retained" is the measured value from Figure 1, $W_{i,\mathrm{ps}}$ is the measured w/w fraction of each component in the pretreated solid (Figure 2), and $W_{i,\text{dry switchgrass}}$ is the w/w fraction of each biomass component in the untreated extractives free switchgrass material (Figure 2, leftmost stack). The results of eq 3 are presented in Figure 5, and the minor sugar yields (galactan and arabinan) are presented in Figure S2. Note that in Figure 5,

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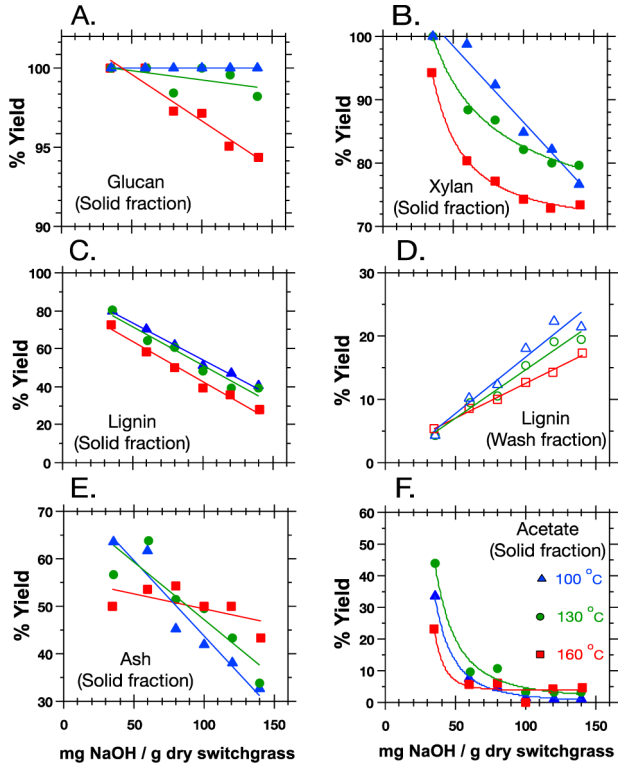

Figure 5. Percent yields of glucan (A), xylan (B), lignin (C), ash (E), and acetate (F) remaining in the pretreated solid phase for pretreatment conditions at 100 °C (blue filled triangles), 130 °C (green filled circles), and 160 °C (red filled squares) as a function of mg NaOH/g dry switchgrass. Panel D shows the percent yield of lignin present in the wash phase, which is a measured value; here, data at 100 °C are represented with open triangles, data at 130 °C are represented with open circles, and data at 160 °C are represented with open squares. 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 for each data set. Table S1 contains the equations of the best-fit lines.

panel D is a measured value not calculated by eq 3, which is the measured amount of lignin present in the wash water. As with our previous work with corn stover,[44] the wash water was found to only contain lignin, and any sugars or sugar degradation products were below detection limits. The data shown in Figure 5 present several trends regarding how each biomass fraction is removed as a function of pretreatment conditions. For example, take the glucan fraction shown in Figure 5A. Here it is clear that at pretreatment temperatures of 100 and 130 °C, glucan is essentially completely retained in the solid fraction; however, at a pretreatment temperature of 160 °C, it is removed linearly with increasing sodium hydroxide loading, up to 6% at the highest NaOH loading. Xylan is the other major carbohydrate fraction of switchgrass, and its removal with increasing pretreatment severity is shown in Figure 5B. Here, xylan is removed linearly with increasing sodium hydroxide loading at a pretreatment temperature of 100 °C, but at higher pretreatment temperatures of 130 and 160 °C, the removal of xylan is much more pronounced and nonlinear, with the best-fit function for these data taking the form of a power function (Table S1). Under the most severe conditions (160 °C and 140 mg NaOH/g dry switchgrass), 28% of the xylan is solubilized into the black liquor fraction.

Higher retention of carbohydrates (glucan and xylan) in the solid phase is expected as alkaline pretreatment is more selective for lignin removal via saponification of ester linkages and through cleavage of C−O linkages in the lignin polymer.[61] This is clearly seen in Figure 5C. Here, lignin is removed at much higher levels than the carbohydrate fractions, and its removal is linearly dependent on the loading of sodium hydroxide (within the loadings examined in this work). Also, it is noteworthy that a marked increase in lignin removal is seen when the pretreatment temperature is increased to 160 °C. This increase in lignin removal was also found with corn stover; however, corn stover displayed this marked increase in lignin removal when the pretreatment temperature was increased from 100 to 130 °C, a temperature lower than that found here for switchgrass.

During alkaline pretreatment, high molecular weight lignin that is solubilized into the APL has a tendency to redeposit back onto the solid carbohydrate enriched fraction during cooling of the pretreatment vessel.[62] A mild wash of the residual solids allows removal of the redeposited lignin. Figure 5D displays the measured yield of lignin present in this wash water. Here, the amount of lignin removed to the wash water is found to increase linearly with increasing sodium hydroxide loading. This is not surprising as an increase in sodium hydroxide during pretreatment removes a greater amount of lignin from the plant cell wall; therefore, more lignin can be removed during the wash. However, as the pretreatment temperature is increased, the amount of lignin washed out of the pretreated solids decreases slightly. This is likely the result of greater deconstruction of the high molecular weight lignin during pretreatment at higher temperatures; thus, a lower amount of high molecular weight lignin is available in the APL to redeposit onto the solid phase.

Other major biomass fractions examined in this work are acetate and ash. Ash (Figure 5E) does not display a distinctive trend with increasing pretreatment temperature or sodium hydroxide loading. The lack of a trend in ash removal is due to sodium ions collecting in the solid phase from the APL. This obscures any trend in removal of the native ash originally present in switchgrass. However, the total amount of ash remaining in the solid phase after pretreatment is important information because any downstream catalytic process that uses this carbohydrate enriched solid as a feedstock would be affected by the ash content. Acetate (Figure 5F) is essentially completely removed at all pretreatment temperatures and at sodium hydroxide loadings greater than 60 mg NaOH/g dry switchgrass.

Alkaline Pretreatment Liquor. APL is a caustic mixture of high molecular weight lignin, low molecular weight aromatics derived from lignin, short chain acids, diacids, and hydroxy acids derived from sugar degradation reactions that occur during pretreatment in the alkaline media as well as high molecular weight partially depolymerized carbohydrates.[52]

In addition to the chemical complexity of these streams, APLs are particularly sensitive to changes in pH, where lowering the pH below 7 causes much of the solubilized components to fall out of solution, forming a thick lignocellulosic precipitate. The chemical complexity of the liquor and its sensitivity to pH changes present major challenges in analytical chemistry, and currently no single technique is capable of directly and completely quantifying or characterizing the solution. The requirement of multiple techniques and complex protocols for sample workup are

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beyond the scope of this work and will instead be presented in a future publication where the liquors are analyzed directly through a wide variety of approaches. However in this work, the data collected in Figures 1, 2, and 5 can be used to determine the w/w composition of the switchgrass material that is solubilized into the liquor phase using eq 4.

$$W_{i,\mathrm{APL}} = \frac{Y_{i,\mathrm{APL}} \times W_{i,\mathrm{dry\ switchgrass}}}{\sum_{N=1}^{i} (Y_{i,\mathrm{APL}} \times W_{i,\mathrm{dry\ switchgrass}})} \quad (4)$$

Here, $Y_{i,\mathrm{APL}}$ is the percent yield of component i in the APL by difference from the yield of component i in the pretreated solid phase (i.e., 1 − the data presented in Figure 5). For the yield of lignin in the APL, the lignin present in both the pretreated solid phase and the wash phase is accounted for by subtracting both the yield of lignin in the pretreated solid (Figure 5C) and the lignin measured in the wash phase (Figure 5D) from unity. $W_{i,\mathrm{dry\ switchgrass}}$ is the measured w/w composition of component i present in the untreated switchgrass (Figure 2, leftmost stack). The calculated compositions of APL from eq 4 are displayed in Figure 6. In Figure 6, several trends are apparent in the

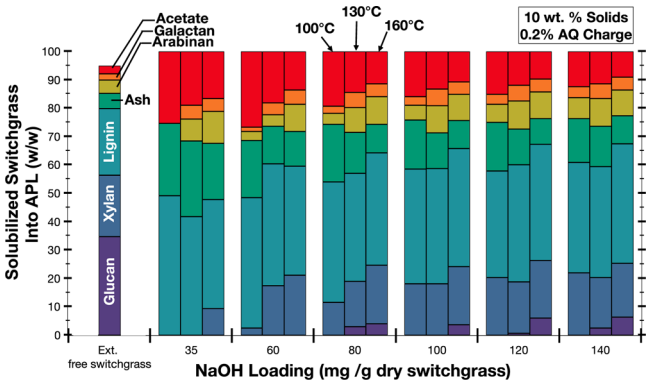

Figure 6. Composition of switchgrass solubilized into the APL fraction (w/w, excluding water) shown as a function of increasing NaOH loading. The APL composition is calculated by difference from the measured composition of the pretreated solid and the composition of the original dry switchgrass, as described in the text. The data presented here are binned in the same fashion as in Figure 2 with the composition of the original untreated dry switchgrass in the leftmost bin labeled "Ext. free switchgrass". Compositional data for black liquor at pretreatment temperatures of 100, 130, and 160 °C are binned according to their NaOH loading, with the leftmost stack in each bin representing a pretreatment temperature of 100 °C, the middle stack representing a pretreatment temperature of 130 °C, and the rightmost stack in each bin representing pretreatment at 160 °C (as noted in the temperature labels above the stacks in bin "80").

composition of the solubilized switchgrass. At low severities, the components in the liquor are derived only from lignin (50%), acetate (25%), and ash (25%). As the NaOH loading is increased, carbohydrates (mostly xylan and minor sugars) begin to comprise a larger fraction of the solubilized switchgrass, up to a maximum of ∼20%. We stress that the w/w compositions in Figure 6 do not represent the chemical composition of the black liquor. As stated above, black liquor is a complex mixture of numerous compounds including monomers, oligomers, and higher molecular weight species. Instead, this result displays the w/w composition of the switchgrass material that has been solubilized to the liquor phase. These values are important for preliminary examination of the liquors without applying advanced analytical tools. For example, using the data in Figure 6, one can adjust pretreatment conditions to maximize the w/w composition of lignin present in the black liquor. This analysis can be beneficial for selecting optimal pretreatment conditions to generate feed liquors for biotechnologies aimed at lignin valorization currently under development.[41,42]

Enzymatic Hydrolysis of Pretreated Solids. To determine the impact that alkaline pretreatment has on enzymatic digestibility of the residual polysaccharide-enriched solids, a commercial fungal enzyme cocktail was used. As expected, we observe an increase in glucose and xylose release as the pretreatment severity is increased. Under the pretreatment conditions tested, the total conversions group into three levels. At the highest pretreatment severity, 160 °C and 140 mg NaOH/g dry switchgrass, 10 mg/g of CTec3 and HTec3 loaded in a 10:1 ratio were able to convert ∼80% of the glucan and xylan within 48 h (Figure 7). We did not observe a

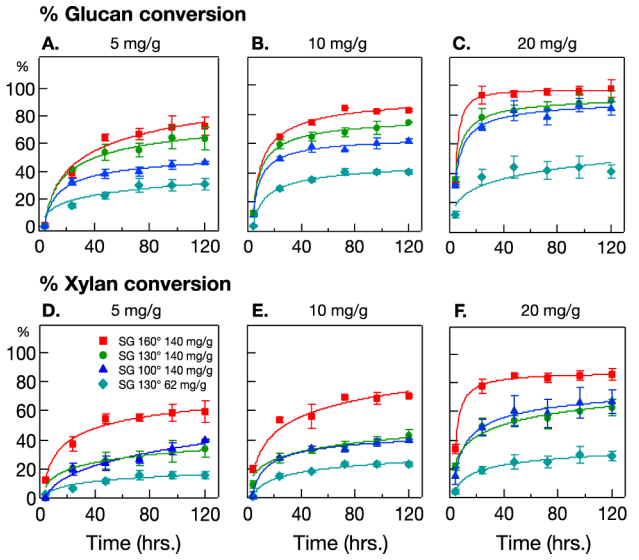

Figure 7. Glucan and xylan conversion of alkaline pretreated switchgrass as a function of incubation time. Switchgrass samples treated with 62 or 140 mg of NaOH/g dry switchgrass at 100, 130, or 160 °C (see legend in panel D) were incubated with CTec3 and HTec3 in a 10:1 enzyme loading ratio at 5 mg (A and D), 10 mg (B and E), or 20 mg (C and F) of protein per gram of glucan to assay for the extent of enzyme saccharification. Enzymatic reactions contained 30 mM NaAc at pH 5.0 and were incubated at 50 °C. Digestions of pretreated biomass contained 1% (w/v) solid slurries in 1.4 mL reaction vials and were mixed by continuous rotation at 10−12 rpm. Conversion of glucan (A−C) and xylan (D−F) was measured by quantifying the amount of glucose, cellobiose, and xylan released using HPLC. The solid lines represent the best-fit power function for the data points for each sample set. The equations for these lines can be found in Tables S2 and S3.

significant difference in the xylan conversion between material treated at 130 and 100 °C with 140 mg NaOH/g dry switchgrass, but at a pretreatment temperature of 160 °C, the saccharification was significantly enhanced by ∼15% after 72 h. This difference may be a reflection of the higher content of xylan and/or lignin remaining in the biomass after pretreatment at lower pretreatment temperatures (Figure 5) or physiological differences such as enzyme accessibility to the substrate. The lowest level of saccharification was observed for switchgrass pretreated at conditions that were found to be optimal for corn stover in our previous work: specifically, a sodium hydroxide

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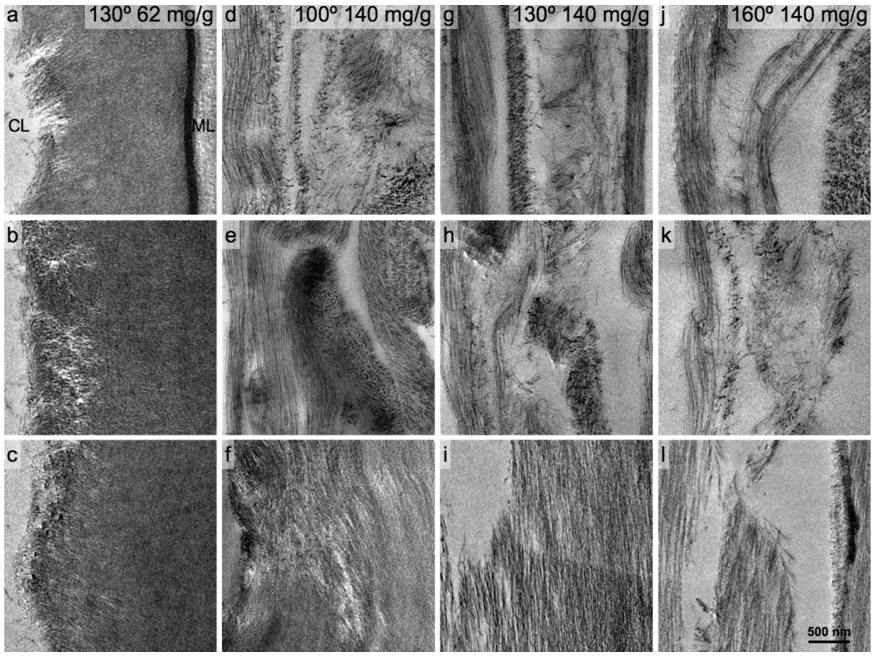

Figure 8. TEM micrographs of solid switchgrass biomass remaining after ~50% conversion of the alkaline pretreated solids by enzymatic saccharification. Switchgrass samples treated with 62 mg (a–c) or 140 mg (d–l) of NaOH per gram of dry switchgrass at 100 °C (d–f), 130 °C (a–c and g–i), or 160 °C (j–l) were incubated with CTec3 and HTec3 (Figure 7). The remaining solids at 50% conversion display evidence for a range of deconstruction and some variability within each treatment. The digested samples that had been pretreated with 62 mg NaOH/g showed surface limited deconstruction. All of the 140 mg NaOH/g dry switchgrass showed evidence of enzymatic deconstruction through the depth of the cell walls, and a subtle gradient of the extent of delamination and fibrillation that correlates with the range of temperatures used during pretreatment. CL, cell lumen; ML, middle lamella; scale bar = 500 nm.

loading of 62 mg NaOH/g dry biomass and a pretreatment temperature of 130 °C.[44]

The observed conversions suggest that switchgrass is more recalcitrant than corn stover in the context of alkaline pretreatment and that pretreatment conditions for switchgrass require a sodium hydroxide loading of at least 140 mg NaOH/g at 160 °C to enzymatically hydrolyze 80% of the glucan using an enzyme loading of 10 mg NaOH/g. Increasing pretreatment time to longer than 30 min would likely yield readily digestible material with decreased NaOH loadings. However, the drawbacks of longer pretreatment time are discussed in the Discussion below in the context of a biological lignin upgrading process.

TEM Imaging of Digested Solids. To investigate structural changes and visualize the increased accessibility in pretreated and partially saccharified material, samples were analyzed by TEM. The four pretreatment severities used in this digestion study were imaged to investigate the morphological differences in the cell walls, which may aid in enzyme accessibility and explain the differences in conversion observed in Figure 7. Partially digested, pretreated switchgrass samples were retrieved at a 50% glucan conversion level for preservation for imaging. The TEM images are shown in Figure 8 and highlight the differences in the cell wall architecture. The left panel is the lowest pretreatment severity at 62 mg of NaOH/g dry switchgrass pretreated at 130 °C (Figure 8a–c). The far right-hand column (Figure 8j–l) shows images of the most severely pretreated material, 140 mg of NaOH/g dry switchgrass and 160 °C.

Digested cell walls from the lowest severity pretreatments retained structural integrity and show evidence of mild enzymatic deconstruction largely limited to the cell wall surface (Figure 8a–c). The higher density and darker staining of the cell walls in the leftmost column is due to their higher lignin content. Also, the cellulose structure in the left most column (Figure 8a–c) appears to be well organized compared to the rightmost column (Figure 8j–l). The middle two columns (Figure 8d–i) appear to have very similar morphology, showing evidence of cell wall delamination and easily discernible cellulose microfibrils. At enzyme loadings of 10 and 20 mg/g, the glucan and xylan conversion levels are also very similar between these two substrates. Figure 8j–l shows cell walls with extensive delamination and disruption of the cellulose microfibrils. All of the samples pretreated with the higher NaOH loading exhibited similar morphology at 50% glucan conversion. The most dramatic difference in morphology was observed between 62 mg NaOH/g dry switchgrass (Figure 8a–c) and 140 mg NaOH/g dry switchgrass (Figure 8d–l).

DISCUSSION

New biotechnologies are being developed that are capable of converting lignin within APL to valuable chemicals and fuels.[41,42,45] For the purposes of lignin valorization, APL generated from this pretreatment will likely be best suited for upgrading if it contains a maximum ratio of lignin to xylan and glucan in the liquor. To meet these requirements and examine differences in the APL produced from both corn stover and switchgrass feedstocks, the fractionation optimization function (f) developed in our previous work is used (eq 5).[44]

$$f = \frac{Y_{\text{lignin,APL}}}{Y_{\text{glucan,APL}} + Y_{\text{xylan,APL}}} \quad (5)$$

Here, the yield of lignin in the APL ($Y_{\text{lignin,APL}}$) is divided by the undesirable yields of glucan and xylan ($Y_{\text{glucan, APL}}$ and

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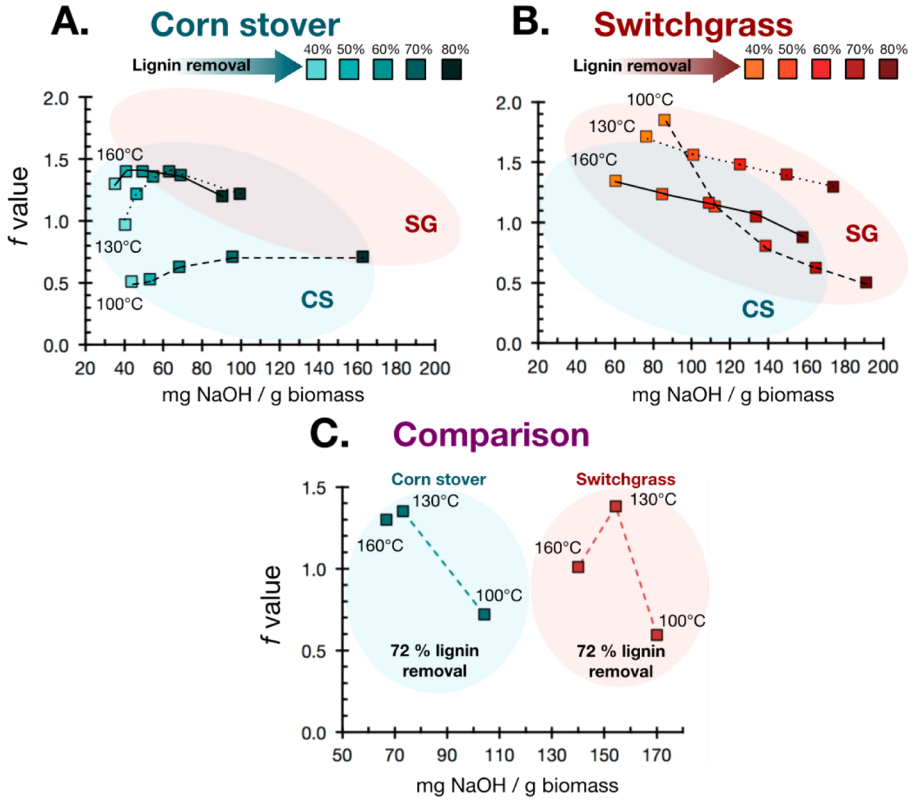

Figure 9. The values of the fractionation optimization function, *f*, for corn stover (A) and switchgrass (B) are plotted as a function of the sodium hydroxide loading. In panels A and B, the data are shown for three different pretreatment temperatures. As the trace for a given pretreatment temperature is followed from left to right, the total amount of lignin removal from the substrate is denoted by the color gradient of the data points. For ease of comparison, shaded regions for corn stover and switchgrass are overlaid on both A and B. Panel C shows the *f* values for pretreatment conditions for both corn stover and switchgrass that remove 72% of the lignin from the substrate. As discussed in the text, 130 °C appears to be the pretreatment temperature that is most selective for lignin removal, showing the highest *f* value for both switchgrass and corn stover substrates.

$Y_{xylan,APL}$ respectively) solubilized in the liquor, which measures the selectivity of lignin to the APL and retention of carbohydrates in the residual solids after pretreatment. To track how the fractionation optimization function, or the lignin richness of the liquor, changes with pretreatment conditions, the *f* values for corn stover and switchgrass pretreated at 100, 130, and 160 °C are plotted as a function of the sodium hydroxide loading in Figure 9A,B. As each trace for a given pretreatment temperature is followed from left to right with increasing sodium hydroxide loading, the total amount of lignin removal from the substrate is also shown, denoted through the color of the data point indicated in the color key at the top of panels A and B in Figure 9. Plotting the data in this fashion clearly shows how the lignin richness of the APL is affected by pretreatment conditions and highlights the substantial differences in the behavior of corn stover and switchgrass during alkaline pretreatment. From Figure 9A,B, it is quickly apparent that switchgrass is more recalcitrant than corn stover, requiring higher sodium hydroxide loadings to achieve lignin removal identical to that of corn stover. However, switchgrass does allow for higher *f* values to be achieved for the produced APL, particularly at lower sodium hydroxide loadings, where APL from switchgrass is roughly three times more enriched in lignin than xylan and glucan compared to corn stover APL. This is the result of the greater retention of glucose in the switchgrass substrate compared to corn stover where a substantial amount of glucose is solubilized into the APL even at low pretreatment severities. We note that xylan retention is moderately better for switchgrass compared to corn stover. The retention of more glucan in switchgrass during alkaline pretreatment is likely why a trend in increasing crystallinity is found (Figure S1 and Table 1) in the pretreated solids for increasing pretreatment severity, but such a trend is not evident with pretreated corn stover, due to increased glucan loss to the APL. However, even though the liquors produced from switchgrass at low severities are more enriched in lignin than those produced from corn stover, the total yield of lignin removed from the substrate at these conditions is still low at ~30%.

The results shown in Figure 9A,B can be further used to select optimal pretreatment conditions in a biorefinery where lignin utilization is a major objective by following these series of steps: (1) Determine the amount of lignin removal from the substrate required by the process. (2) Use the data in Figure 9A or 9B (depending on the feedstock) to select pretreatment conditions at sodium hydroxide loadings for different pretreatment temperatures that achieve the level of lignin removal specified in step 1. (3) Choose the pretreatment condition from those identified in step 2 that has the highest *f* value.

This analysis allows selection of pretreatment conditions that meet two simultaneous goals of achieving a desired lignin removal from the substrate and maximizing the richness of the APL for downstream lignin upgrading technologies.

As an example of this analysis, let us take the data in this work and predict optimal pretreatment conditions for a hypothetical biorefinery using switchgrass as a feedstock. For step 1, we determine the target amount of lignin removal. For

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this value, we refer to the enzymatic hydrolysis results of Figure 7. These results find a pretreatment condition of 140 mg NaOH/g dry switchgrass at pretreatment of 160 °C produced pretreated solids that were rapidly digestible. At these pretreatment conditions 72% of the lignin was removed from the switchgrass substrate, suggesting that a threshold of 72% removal of lignin from the substrate is needed to achieve rapid saccharification. Thus, we select a criterion that 72% of the lignin must be removed from the substrate. Next, we select from Figure 9B the pretreatment conditions that achieve 72% lignin removal. These selected pretreatment conditions and their respective *f* values are plotted in Figure 9C. Here we find that at a pretreatment temperature of 100 °C, 72% lignin removal can be achieved at a sodium hydroxide loading of 170 mg NaOH/g dry switchgrass, but the *f* value for the produced liquor is relatively low at 0.59. At a pretreatment temperature of 130 °C, 72% of the lignin can be removed at a lower sodium hydroxide loading of 154 mg NaOH/g dry switchgrass, and the *f* value of the APL is significantly higher at 1.38. At a pretreatment temperature of 160 °C, the sodium hydroxide loading needed to achieve 72% lignin removal can be lowered further to 140 mg NaOH/g dry switchgrass, but the *f* value for the APL decreases to 1.0, mostly due to an increase in hemicellulose removal at the increased temperature. Thus, from this analysis the optimal pretreatment conditions are identified as 154 mg NaOH/g dry switchgrass and a pretreatment temperature of 130 °C. We note that this sodium hydroxide loading is slightly outside of the data collected in this work and is found through extrapolation of the fits in Figure 5. For comparison, the same analysis is applied to corn stover to achieve 72% lignin removal, and the results are also plotted in Figure 9C, finding optimal conditions for corn stover of 130 °C and 73 mg NaOH/g dry stover.

In Figure 9C, two key results are illustrated. First, 130 °C seems to be the most selective temperature for lignin removal during alkaline pretreatment for both switchgrass and corn stover. At 100 °C, the rate of lignin removal may be too slow to produce lignin rich APL streams; at 130 °C, the rate of lignin removal increases dramatically, yielding the most lignin rich APL. At 160 °C, the rate of lignin removal is still high, but the rate of sugar solubilization also begins to increase, yielding slightly less lignin rich APL. The second observation from Figure 9C is the extent to which switchgrass is more recalcitrant than corn stover. For 72% lignin removal at 100, 130, or 160 °C, switchgrass requires a much higher NaOH loading; for example, compared to corn stover at 130 °C, switchgrass requires roughly twice the NaOH loading to achieve 72% lignin removal. The explanation for the greater recalcitrance of switchgrass could arise from differences in the chemical composition and molecular connectivity between the lignin in corn stover and switchgrass. Indeed, 2D-NMR studies have reported differences in the chemical makeup of lignin in these feedstocks. Corn stover lignin from stem and cob residue was found to have an average S/G ratio of 1.4,[61] and the S/G ratio for switchgrass was found to be 0.8.[63,64] Higher S/G ratios have been correlated with increased ease of pulping, due to an increase in the relative amount of β-O-4 linkages that are more labile under alkaline environments.[29,65−67] Furthermore, a study by Wang et al.[68] demonstrated that genetically modified switchgrass containing a higher S/G ratio than native switchgrass delignified to a greater extent than native switchgrass under identical alkaline pretreatment conditions and yielded more sugars during enzymatic hydrolysis.

Secondary to chemical linkages, differences in microscale architectures could also contribute to increased recalcitrance of switchgrass compared to corn stover. Comparing SEM images of switchgrass in this work (Figure 3) to SEM images of corn stover (Figure 3 in ref 44) from our previous work, it appears that cellulose microfibrils in switchgrass are more condensed than those in corn stover. This condensed architecture displayed by switchgrass could provide increased resistance to transport of the catalyst into and resulting lignin-derived oligomers out of the switchgrass substrate, thus overall requiring higher pretreatment severity for lignin removal.

Finally, the results of this work can be compared to other reports examining the behavior of switchgrass during alkaline pretreatment. Previous literature has shown that the release of lignin-derived monomers during alkaline pretreatment is time dependent and maximum release occurs around 30−40 min.[62,69−71] Therefore, in this work, we chose to investigate pretreatment conditions at high temperatures (≥100 °C) and short residence times of 30 min at temperature to maximize the concentration of monomeric lignin-derived species in the APL. However, alkaline pretreatment of switchgrass at temperatures lower than and sodium hydroxide loadings similar to those reported here have been found to remove up to 85.8% of the lignin present but only with much longer residence times (>10 h).[16−20] These longer pretreatments allow sufficient time for additional reactions to occur within the APL that increase its average molecular weight, which may not be optimal for a feed stream for downstream biological upgrading.[62] However, one could envision extending the analysis in Figure 9 along an additional dimension of pretreatment time given a complete data set of pretreatment time on the resulting composition of the recovered solids.

Other studies have investigated additives in switchgrass alkaline preatreatment such as $Ca(OH)_2$,[17] which has been found to achieve similar delignification rates and reduce the amount of NaOH needed. Moreover, H_2O_2 has also been identified as a promising additive as it diminishes the amount of dissolved sugars during alkaline pretreatment by preventing endwise peeling reactions of carbohydrates.[72,73]

CONCLUSIONS

Alkaline pretreatment of switchgrass has been investigated at pretreatment temperatures between 100 and 160 °C, NaOH loadings between 35 to 140 mg NaOH/g dry switchgrass, and at a pretreatment time of 30 min. The results and analysis of this work find that pretreatment at 130 °C is beneficial as a marked increase in lignin removal is observed at this temperature. This increased delignification at 130 °C as opposed to 100 °C is seen for both corn stover and switchgrass, but switchgrass is found to be significantly more recalcitrant than corn stover, requiring approximately twice the sodium hydroxide loading to achieve delignification identical to that of corn stover. Coupling the pretreatment results with the enzymatic hydrolysis results of the pretreated solid and applying the analysis developed in Figure 9, the optimal pretreatment conditions are suggested to be 130 °C and 154 mg NaOH/g dry switchgrass to achieve pretreated solids that are rapidly digestible and produce the most lignin rich APL for downstream lignin conversion through biotechnological approaches. These conditions are approximately twice the amount of NaOH loading needed compared to the optimal pretreatment conditions found for corn stover.

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■ ASSOCIATED CONTENT

Supporting Information

Contains additional figures and tables referenced in the above text. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.5b00201.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: gregg.beckham@nrel.gov.

Present Address

J.E.N: Department of Chemical Engineering and Materials Science, University of California Davis, Davis, California 95616-5294, United States.

Notes

The authors declare no competing financial interest.

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