Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production

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Abstract
The performance of two pretreatment methods, sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) and dilute acid (DA), was compared in pretreating softwood (spruce) for fuel ethanol production at 180 °C for 30 min with a sulfuric acid loading of 5% on oven-dry wood and a 5:1 liquor-to-wood ratio. SPORL was supplemented with 9% sodium sulfite (w/w of wood). The recoveries of total saccharides (hexoses and pentoses) were 87.9% (SPORL) and 56.7% (DA), while those of cellulose were 92.5% (SPORL) and 77.7% (DA). The total of known inhibitors (furfural, 5-hydroxymethylfurfural, and formic, acetic and levulinic acids) formed in SPORL were only 35% of those formed in DA pretreatment. SPORL pretreatment dissolved approximately 32% of the lignin as lignosulfonate, which is a potential high-value co-product. With an enzyme loading of 15 FPU (filter paper units) per gram of cellulase, the cellulose-to-glucose conversion yields were 91% at 24 h for the SPORL substrate and 55% at 48 h for the DA substrate, respectively.

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1. Introduction
Second-generation bioethanol, produced from lignocellulosic materials, is a promising alternative to fossil fuel for vehicular transportation (Ragauskas et al., 2006; Carroll and Somerville, 2009). The benefits of cellulosic ethanol include, but are not limited to, reduced greenhouse gases emission, value-added utilization of agricultural and forest residues, enhancement of the rural economy, and improved national energy independence and security (Farrell et al., 2006; Scharlemann and Laurance, 2008). A typical process for cellulosic ethanol production consists of three steps: feedstock pretreatment to enhance cellulases accessibility to cellulose, enzymatic saccharification of the cellulose-to-glucose, followed by fermentation of the glucose to ethanol. One of the barriers to the commercialization of cellulosic ethanol is the lack of economical and effective technologies for the feedstock pre-treatment. Pretreatment is a necessary operation required to achieve optimal bioconversion for all forms and types of lignocellulosic feedstocks to ethanol, but is particularly important for the more recalcitrant softwoods. The significance and importance of the pretreatment step cannot be overemphasized, as the effectiveness of the pretreatment affects the upstream selection of biomass, the yield of fermentable sugars, and the chemical and morphological characteristics of the pretreated substrate which in turn govern downstream hydrolysis/saccharification (Wyman et al., 2005).

An effective pretreatment method should be economical (in terms of both capital and operating costs) and effective for a variety of lignocellulosic biomass. Specifically, it should require minimal feedstock preparation and preprocessing prior to pretreatment, maximally recover all lignocellulosic components in usable forms with minimal formation of fermentation inhibitors, and produce a readily digestible cellulosic substrate that can be easily hydrolyzed with a low loading of enzymes. In the last several decades, research and development efforts have made significant progress in pretreatment technologies for lignocellulosic feedstocks (Lynd et al., 2002; Mosier et al., 2005; Wyman et al., 2005; Chandra et al., 2007; Hendriks and Zeeman, 2009). Many pretreatment technologies, such as lime, dilute acid, hot water, ammonia, steam explosion, and organosolv pretreatments (Nguyen et al., 2000; Pan et al., 2005a; Chandra et al., 2007; Sendich et al., 2008; Wingren et al., 2008; Gupta and Lee, 2009; Kim et al,
2. Experimental

2.1. Materials

Fresh spruce chips were generously provided by the Wisconsin Rapids Mill of Stora Enso North America (now New Page Corporation, Maminbush, OH). After being air-dried, the chips were ground to pass a 40-mesh (0.42 mm opening) screen using a Wiley mill. Chemical composition of the spruce wood is presented in Table 1. Commercial enzymes, Celluclast and β-glucosidase produced by Novozymes, were purchased from Sigma–Aldrich (St. Louis, MO) and used as received. All the chemical reagents used in this study were purchased from Fisher Scientific (Pittsburgh, PA) and used as received.

2.2. Pretreatments

Chemical pretreatments were conducted in batch mode using a lab-scale rotatable reactor, as described previously (Wang et al., 2009; Zhu et al., 2009a). Both SPORL and dilute-acid pretreatment were carried out in triplicate; the average results of the three runs were reported. In general, ground spruce (100 g oven-dry material) was loaded into a 1-L stainless steel vessel. Prepared pretreatment solution (500 mL 1% H2SO4 for DA pretreatment or 500 mL 1% H2SO4 + 9 g Na2SO3 for SPORL pretreatment) was then poured into the vessel. Three sealed 1-L vessels were mounted inside a 23 L stainless steel reactor. The system was heated via the external steam jacket and rotated at a speed of 2 rpm to provide mixing during pretreatments. The temperature was raised to 180 °C in about 7 min and maintained for an additional 30 min. At the end of the pretreatment, the pretreatment spent liquor was separated from the solid (pretreated substrate) by filtration and stored for fermentation study and chemicals analysis. The solid substrate was collected in a Buchner funnel on filter paper and washed thoroughly with water. Substrate yield was determined from the measured wet weight and moisture content of the washed solid substrate.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated substrates and original ground spruce was conducted as described previously (Pan et al., 2008). Briefly, the hydrolysis was carried out at 50 °C on a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 150 rev/min. Substrate equivalent to 2 g glucan was loaded into a 250 mL Erlenmeyer flask with 100 mL of 0.05 M sodium acetate buffer (pH 4.8). Approximately 4 mg of tetracycline chloride was used to control the growth of microorganisms and prevent consumption of liberated sugars. Cellulase (5 or 15 FPU, Filter Paper Units, per gram glucan) and β-glucosidase (10 or 30 IU, International Units, per gram glucan) were loaded into the flask. Hydrolysates were sampled periodically and subjected to glucose analysis.
The hydrolysis was conducted in duplicate for each substrate; the average is reported here.

2.4. Analytical methods

Acid-insoluble lignin of spruce and pretreated SPORL and DA substrates was determined according to National Renewable Energy Laboratory (NERL) Analytical Procedure (http://www.nrel.gov/biomass/analytical_procedures.html) with modifications. Acid-soluble lignin was determined by UV at 205 nm using an extinction coefficient of 110 L g\(^{-1}\) cm\(^{-1}\) (Dence, 1992).

Saccharide analysis was conducted using a Dionex HPLC system (ICS-3000) equipped with an integrated amperometric detector and Carbopac™ PA1 guard and analytical columns at 20°C. Eluent was provided at a rate of 0.7 mL/min, according to the following gradient: 0-25 min, 100% water; 25.1-35 min, 30% water and 70% 0.1 M NaOH; 35.1-40 min, 100% water. To provide a stable baseline and detector sensitivity, 0.5 M NaOH at a rate of 0.3 mL/min was used as post-column eluent.

Fermentation inhibitors generated in pretreatment including acetic acid, formic acid, furfural, levulinic acid and 5-hydroxymethylfurfural (HMF) were analyzed using the Dionex ICS-3000 equipped with a Supelcogel C-610H column at temperature 30°C and UV detector at 210 nm. Eluent was 0.1% phosphoric acid at a rate of 0.7 mL/min.

Cellulase activity was determined using the filter paper assay recommended by the International Union of Pure and Applied Chemists (Ghose, 1987) and is expressed in terms of filter paper units (FPUs). β-Glucosidase activity was determined using p-nitrophenyl-β-D-glucoside as the substrate (Wood and Bhat, 1988) and is expressed in terms of International Units (IUs).

2.5. Whole cell-wall NMR of original spruce and pretreated substrates

Whole cell-wall NMR of pretreated substrate and original spruce was conducted according to Lu and Ralph (2003). In brief, 1.5 g of extractive-free spruce powder or pretreated substrate was loaded into a 50-mL ZrO\(_2\) jar and ball-milled on a Retsch PM-100 ball mill for 10 h (20 min on and 10 min off). The ball-milled sample (600 mg) was dissolved in 10 mL dimethyl sulfoxide (DMSO) and 5 mL N-methylimidazole (NMI). A clear solution was formed in approximately 3 h, depending on the sample. Excess acetic anhydride (3 mL) was added to the solution, and the mixture was stirred for 1.5 h. The resulting clear brown solution was added dropwise to 2 L of water by a glass pipette, and the mixture was allowed to stand overnight. The precipitate was recovered by filtration through a nylon membrane (0.2 lµm). The product was washed with water (250 mL) and freeze-dried. About 60 mg dried powder was dissolved in CDCl\(_3\) for NMR spectrometry. NMR spectra were acquired on a Bruker DRX-360 instrument fitted with a 5 mm 1H/broadband gradient probe with inverse geometry.

2.6. Degree of polymerization of cellulose

The degree of polymerization of cellulose was indirectly determined by a viscometry method. The viscosity of cellulose in cupriethylenediamine solution was measured using a kV 3000 kinematic viscosity bath (Koehler Instrument Company) with a
Cannon Ubbelohde capillary viscometer, according to Technical Association of Pulp and Paper Industry (TAPPI) Standard Method T230-om-99. Cellulose samples for viscosity measurement were prepared from original spruce and SPORL and DA substrates by careful delignification using sodium chlorite according to the PPTAC (Pulp and Paper Technical Association of Canada) Useful Method G.10U.

2.7. Fermentability of SPORL and DA pretreatment liquors

Fermentability of the pretreatment spent liquors was evaluated according to an in vitro ruminal fermentation assay (Weimer et al., 2005). In vitro ruminal experiments were conducted at 39 °C using a single replicate of each sample, and replication was achieved through a second in vitro run. Triplicates of a standard ryegrass were included in each run. Incubations were conducted in nominal 60-mL serum bottles (volume-calibrated to 0.01 mL) and that contained the equivalent of 100 mg (weighed to 0.1 mg) of biomass material, 6.7 mL of Goering and Van Soest buffer, 0.3 mL of cysteine-sulfide reducing agent (6.25 g/L each of cysteine HCl and Na2S-9H2O) and a CO2 gas phase. Gas pressure readings were made at 24 and 96 h using a SenSym digital pressure gauge modified to accept a 20 gauge hypodermic needle.

3. Results and discussion

3.1. Changes in cell-wall components during SPORL and DA pretreatments

As described in Section 2, ground spruce wood (-40-mesh) rather than wood chips was used as the feedstock, and mechanical size reduction was not included in either SPORL or DA pretreatment. The consideration of doing so is that a refining step adds difficulties to mass balance evaluations. In addition, only physical size changes and no (significant) chemical reactions are expected during the mechanical size reduction. Both SPORL and DA chemical pretreatments were carried out at the same temperature (180 °C), pretreatment time (7 min to attain the desired temperature and 30 min at that temperature), ratio of liquor to wood (5:1), and acid loading (5% on a dry wood basis). The only difference is that 9% (w/w of wood) sodium sulfite was added in SPORL pretreatment.

Chemical composition of the spruce wood is presented in Table 1. It had a typical softwood composition with 29.0% acid-insoluble lignin and 46.7% glucose (or 42.1% glucan). The majority of the hemicellulosic sugars were hexoses (10.8% mannose and 2.6% galactose), accompanied by 5.5% xylose and 1.2% arabinose. The chemical compositions of SPORL and DA pretreated substrates are compared in Table 1. The DA pretreatment apparently dissolved all hemicellulose from the feedstock spruce, leaving only cellulose and lignin in the DA substrate. Essentially no delignification (but lignin condensation) occurred during the DA pretreatment, so the lignin was enriched in the substrate (48.5% acid-insoluble lignin) after the hemicelluloses and part of the cellulose were dissolved. In contrast, SPORL pretreatment retained more cellulose (SPORL 86.3% vs. DA 71.3%, Fig. 2) and a low level of mannan, but less lignin (32.9% acid-insoluble lignin), in the substrate. The extent of delignification (percentage of original wood lignin dissolved) during the SPORL pretreatment was approximately 32%, calculated from the total lignin contents of spruce wood and the SPORL substrate (Table 1) and the substrate yield (Fig. 2). The differences between DA and SPORL substrates were caused by the addition of the sulfite. First, sodium sulfite is alkaline, buffering the pH value from 1.2 in the DA pretreatment liquor to 2.7 in the SPORL liquor, which protected the cellulose and hemicelluloses from extensive hydrolysis and further degradation to inhibitors at elevated temperature (Tables 1 and 3) and prevented lignin from extensive condensation. Second, the sulfite introduced sulfonic groups at the lignin benzylic carbons, which may (1) partially depolymerize and dissolve the lignin and (2) increase the hydrophilicity of the residual lignin that is retained in the pretreated substrate. These two effects are important to the enzymatic digestibility of the SPORL substrate, as discussed below. The partial removal of lignin in SPORL pretreatment produced a substrate with lower lignin content than the DA substrate.

The concentrations of sugars and lignin in the SPORL and DA liquors, listed in Table 1, provided insight into the effects of sulfite on the reactions of carbohydrates and lignin, discussed above. The SPORL liquor contained more sugars, except for glucose, than the DA liquor. The reason is that the higher pH value of the SPORL liquor limited sugar degradation at high temperature. This is supported by the level of inhibitors derived from the sugars (Table 3) in the spent liquors. The slightly higher content of glucose in the DA liquor was due to the enhanced acidic hydrolysis of cellulose in the DA pretreatment, which has been reflected by the low cellulose content in the DA substrate. The SPORL liquor contained significantly more soluble lignin detected by the UV method than did the DA liquor. The lignin in the SPORL liquor was in the form of lignosulfonate with a yield of 8.3% (on dry wood, calculated from the amount of the dissolved lignin in the SPORL liquor estimated by an UV method). Lignosulfonate has been the most successful lignin product in the market. It has been widely used as dispersants for carbon black, pesticides, dyestuffs and pigments; emulsifiers for soils, asphalt, waxes and oil in water; additives for drilling mud and concrete; and adhesives and binders for animal-feed pellets, minerals, and laminates (Fengel and Wegener, 1984). The SPORL pretreatment therefore has potential for high-value lignin co-products development.

To investigate the behavior of cellulose during the SPORL and DA pretreatments, viscometry was used to provide an indication of cellulose depolymerization (Table 2). The viscosity of the cellulose solution from the substrates was only approximately one tenth of that from original spruce wood, implying that the cellulose was significantly depolymerized (hydrolyzed) during both pretreatments. This is one of the reasons why the pretreated substrate had better enzymatic digestibility than the untreated wood (Fig. 4). No significant difference was found between SPORL and DA substrates in cellulose viscosity. DA cellulose solution showed a slightly lower viscosity because the lower pH value of DA pretreatment enhanced hydrolysis of cellulose.

To further understand the changes of cell-wall components, in particular lignin, during the pretreatments, whole cell-wall solution-state two-dimensional HSQC NMR methods (Lu and Ralph, 2003) were used to compare the SPORL and DA pretreated substrates with the original spruce. As shown in Fig. 2, the cell-wall HSQC NMR spectrum from untreated spruce is typical for a softwood, showing the dominant C–H correlations (colored in green) from cellulose and hemicelluloses (colored in black) along with correlations from major substructures (β-aryl ethers A and phenylcoumarans B) of lignin. The C–H correlations in the aromatic region of the spectrum also show typical softwood lignin correlations from (almost) solely guaiacyl (G) units. After pretreatment with SPRL and DA, the spruce residues contain mainly cellulose and lignin-derived material as shown by their HSQC NMR spectra, which are consistent with the chemical analysis results (Table 1). For the residual lignin, although the methoxyl signal is readily seen, the absence of recognizable lignin subunits A and B suggests that considerable degradation has occurred. Although no detectable monomeric sugars are in the DA pretreated sample, HSQC NMR shows some correlations from non-cellulosic carbohydrates (colored in black). These may be condensed with the residual lignin preventing their release by acid hydrolysis (Yasuda and Murase,
and thus would be unavailable for further conversion to ethanol. Meanwhile lignin itself can undergo condensation reactions under acidic conditions. The most common condensation reactions occur between benzylic (i.e., α) positions of lignin sidechains and 5-positions of other aromatic rings, producing diarylmethane structures (Gierer, 1985). The C–H correlations (colored in pink) in the aromatic regions of these spectra from pretreated spruce suggest that such condensation reactions indeed occurred during both pretreatment processes. During SPORL treatment lignin was partially sulfonated producing lignin sulfonates soluble in water so that wood substrates were partially delignified. That explains why SPORL treated substrate has less lignin than the DA treated material. Sulfonated lignin structures in the SPORL treated spruce residues were not detected by NMR, although model work is needed to confirm this. The reason for such observations may be either that such structures in these residues were too minor to be detected by NMR or, more likely, that the lignin remaining in such residues contains few sulfonated units because the sulfonated lignin components were removed in the liquid phase.

3.2. Mass balance of sugars during SPORL and DA pretreatments

An ideal pretreatment is not only able to produce a readily digestible substrate, but also to maximally recover all available

<table>
<thead>
<tr>
<th>Material</th>
<th>Viscosity mPa s at 25 °C</th>
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<tr>
<td>Untreated spruce</td>
<td>25.6 ± 0.3</td>
</tr>
<tr>
<td>SPORL substrate</td>
<td>2.7 ± 0.0</td>
</tr>
<tr>
<td>DA substrate</td>
<td>2.4 ± 0.0</td>
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![Fig. 2. HSQC NMR spectra of untreated and pretreated spruce cell-walls.](image-url)
sugars in feedstock in fermentable form with limited formation of inhibitors. As shown in Fig. 3, the feedstock spruce wood was separated into two fractions after the pretreatments, solid substrate and a liquid stream (spent pretreatment liquor) containing dissolved sugars, lignin and sugar degradation products. The SPORL substrate appeared lighter in color than the DA substrate because of its lower lignin content and less-condensed lignin. SPORL liquor was darker than DA liquor, probably because the former contained more soluble lignin (Table 1) than did the latter. To compare the performance of SPORL and DA in sugar recovery during the pretreatment, a mass balance of sugars during the two pretreatments was performed (Fig. 3). From 100 g of oven-dry spruce wood (containing 46.7 g glucose, 2.6 g galactose, 10.8 g mannose, 1.2 g arabinose, and 5.5 g xylose), 64.1 g (containing 33.3 g glucose) and 60.5 g (containing 40.3 g glucose and 7.1 g mannose) substrates were obtained from DA and SPORL pretreatments, respectively. High substrate yield from DA pretreatment was due in part to the retention of almost all of the original lignin. Total detected sugars in the pretreatment liquors were 4.6 g (3.0 g glucose, 0.4 g galactose, 0.9 g mannose, 0.1 g arabinose, and 0.2 g xylose) for DA pretreatment and 11.3 g (2.9 g glucose, 1.3 g galactose, 4.5 g mannose, 0.4 g arabinose, and 2.2 g xylose) for SPORL, respectively. The calculations from these data indicated that total sugar recovery was 56.7% (DA) and 87.9% (SPORL), and glucose recovery was 77.7% (DA) and 92.5% (SPORL). Compared to the recovery of pentoses, that of hexoses was much higher, 62.8% vs. 4.5% for DA and 93.7% vs. 38.8% for SPORL, respectively, indicating the pentoses were subject to greater degradation at high temperature and low pH than were the hexoses. The results above clearly indicate that under the same acid loading, temperature and reaction time, SPORL is superior to DA for the recovery of both hexoses and pentoses. As discussed above, this is likely because of the higher pH value of the SPORL pretreatment liquor formed by the addition of sulfite. High sugar recovery in SPORL process implies that fewer sugars were degraded and limited inhibitors were generated, which will benefit the fermentation of the liquors.

3.3. Enzymatic hydrolyzability of SPORL and DA-pretreated spruce

The substrate characteristics that affect enzymatic hydrolysis of cellulose include hemicellulose content, lignin structure, distribution and content, cellulose crystallinity and degree of polymerization, and surface area, pore size and particle size of the substrate (Mansfield et al., 1999; Zhu et al., 2009b). Generally speaking, removing hemicellulose and lignin, swelling cellulose to destroy crystallinity, pre-hydrolyzing cellulose to shorten chain length (increasing the number of chain ends for enzymes to attack), and increasing surface area or decreasing particle size are favorable for enzymatic digestibility of cellulosic substrates.

The enzymatic hydrolyzability of SPORL- and DA-pretreated and original spruce wood is compared in Fig. 4. At enzyme loadings of 15 FPU (Filter Paper Units) cellulase and 30 IU (International Units) β-glucosidase per gram cellulose (Fig. 4A), the SPORL pretreated spruce displayed much greater hydrolysis than did the DA-pretreated spruce and untreated spruce. For example, only 25% and 55% of the cellulose in untreated spruce and the DA substrate, respectively, was hydrolyzed to glucose after a 2-d hydrolysis, whereas 93% of the cellulose in SPORL substrates was saccharified within the same time. When the enzyme loadings were reduced to 5 FPU cellulase and 10 IU β-glucosidase per gram cellulose (Fig. 4B), the cellulose-to-glucose conversion yield after a 2-d hydrolysis of the SPORL substrate (71%) was still substantially higher than those of the DA substrate and untreated spruce (49 and 17%, respectively). The results clearly indicated that the SPORL substrate had substantially better enzymatic digestibility than did the DA substrate under the same hydrolysis conditions. As discussed above, the DA substrate contained no hemicellulose and had slightly lower viscosity (lower cellulose degree of polymerization), compared to the SPORL substrate. Based on these factors alone, the DA substrate might be assumed to have a better hydrolysability, however, lignin (both content and nature), and other factors, resulted in quite the opposite situation. The DA substrate contained approximately 50% lignin, and the lignin was highly condensed, ex-
tremely hydrophobic, and covered the surface of the substrate, all of which enhanced the effect of lignin as physical barrier and non-productive enzyme adsorbent. The SPORL substrate contained less lignin, and the sulfonation made the lignin less hydrophobic, which may have reduced the non-productive hydrophobic adsorption of enzymes onto the lignin. Approximately one third (~33%, Table 1) of the SPORL substrate was lignin, but this lignin retarded the hydrolysis little. The observation implies that the residual lignin in the SPORL substrate was enzyme-friendly and behaved differently from the acid-condensed DA lignin. This suggests that costly delignification is not the only way to remove the recalcitrance (attributable to lignin) to enzymatic degradation of cellulose; less expensive lignin modification may be more promising. The results also suggest that the action of lignin as a purely physical barrier played a less important role in its impacts on enzymes, compared to other interactions such as non-productive adsorption, which agrees with our previous results (Pan et al., 2005b).

### 3.4. Fermentability of SPORL and DA spent pretreatment liquors

Potential fermentation inhibitors formed during the DA and SPORL pretreatments are listed in Table 3, including the soluble lignin, acetic acid released from acetyl groups on hemicelluloses, furfural derived from pentoses, HMF from degradation of hexoses, and levulinic and formic acids from successive decomposition of HMF. The formation of the furfurals and their degradation products (levulinic and formic acids) is shown in Scheme 1. The data in Table 3 clearly indicated that fewer inhibitors were formed from degradation of saccharides during the SPORL pretreatment than the DA pretreatment. The total of known inhibitors (furfural, 5-hydroxymethylfurfural, and formic, acetic and levulinic acids) formed in SPORL were only 35% of those formed in DA pretreatment. As discussed above, this was owing to the addition of sulfite in the SPORL pretreatment, which increased the pH value of the pretreatment liquor, limiting extensive degradation of saccharides. The high lignin concentration in SPORL liquor is due to the formation of water-soluble lignosulfonate. Low amounts of traditional inhibitors (Table 3) and high sugar (Table 1) concentrations suggest that good fermentability can be expected for SPORL liquor.

The fermentability of SPORL and DA liquors was evaluated by an in vitro ruminal gas production assay, as shown in Fig. 5. Surprisingly, the two liquors did not show difference in net gas yield within the first 24 h, while the gas yield of SPORL liquor decreased beyond this point. In tests with various perennial grasses, in vitro ruminal gas production has been shown to provide a reasonable surrogate estimate of ethanol production potential by an en-

![Fig. 4.](image-url) Comparison of time-dependent enzymatic hydrolysability of SPORL- and DA-pretreated spruce at different levels of enzyme loading (A) 15 FPU cellulase + 30 IU β-glucosidase per gram of cellulose; (B) 5 FPU cellulase + 10 IU β-glucosidase per gram of cellulose, 50 °C, pH 4.8 and on a 250 rpm shaker. CGCY: cellulose-to-glucose conversion yield.

<table>
<thead>
<tr>
<th>Spent liquor</th>
<th>Inhibitors, g/L</th>
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<tbody>
<tr>
<td></td>
<td>Acid-soluble lignin</td>
</tr>
<tr>
<td>DA liquor</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>SPORL liquor</td>
<td>16.6 ± 0.4</td>
</tr>
</tbody>
</table>

![Scheme 1.](image-url) Inhibitors formation from cellulose and hemicellulose during pretreatment (Hemi: hemicelluloses).
zyme/yeast Simultaneous Saccharification and Fermentation (SSF) system (Weimer et al. 2005; Anderson et al., 2009). In addition, the in vitro ruminal gas production assay has displayed less inhibition than SSF for certain forages that contain natural fermentation inhibitors (e.g., some varieties of switchgrass; Weimer et al. 2005). It was thus a surprise that in vitro ruminal gas production from the SPORL pretreatment liquor was less than that of DA pretreatment liquor that apparently had higher concentrations of classical pretreatment-derived inhibitors (e.g., furfurals). This inhibition may have been due to lignosulfonate (present in much higher concentrations in the SPORL liquor). Alternatively, inhibition could have been due to the presence of other metabolites that may be released from wood upon pretreatment. For example, Varel and Jung (1986) have shown that certain phenolic acids inhibit degradation of purified cellulose by mixed ruminal microflora; these acids were not measured in our study. Yeast fermentation of SPORL liquor is in progress to verify the results reported here.

4. Conclusion

SPORL pretreatment significantly removed the recalcitrance of spruce wood and allowed nearly complete enzymatic hydrolysis (>90%) within 24 h with a cellulose loading of 15 FPU/g cellulose. SPORL removed the recalcitrance not only by dissolving hemicellulose and depolymerizing cellulose, but also by partially (32%) dissolving lignin and saponifying the residual lignin in substrate, which presumably reduced the hydrophobic interactions between lignin and the enzymes. SPORL achieved a significantly higher sugar recovery and produced much lower levels of traditional fermentation inhibitors (e.g., furfurals). This inhibition may have been due to lignosulfonate (present in much higher concentrations in the SPORL liquor). Alternatively, inhibition could have been due to the presence of other metabolites that may be released from wood upon pretreatment. For example, Varel and Jung (1986) have shown that certain phenolic acids inhibit degradation of purified cellulose by mixed ruminal microflora; these acids were not measured in our study. Yeast fermentation of SPORL liquor is in progress to verify the results reported here.

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