

Comparisons of SPORL and Dilute Acid Pretreatments for Sugar and Ethanol Productions from Aspen

S. Tian

College of Life Science, Capital Normal University, Beijing, China

U.S. Forest Service, Forest Products Laboratory, Madison, WI 53726

W. Zhu

State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, China

U.S. Forest Service, Forest Products Laboratory, Madison, WI 53726

R. Gleisner

U.S. Forest Service, Forest Products Laboratory, Madison, WI 53726

X.J. Pan

Dept. of Biological Systems Engineering, University of Wisconsin–Madison, Madison, WI 53726

J.Y. Zhu

U.S. Forest Service, Forest Products Laboratory, Madison, WI 53726

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*This study reports comparative evaluations of sugar and ethanol production from a native aspen (*Populus tremuloides*) between sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) and dilute acid (DA) pretreatments. All aqueous pretreatments were carried out in a laboratory wood pulping digester using wood chips at 170°C with a liquid to oven dry (od) wood ratio (L/W) of 3:1 at two levels of acid charge on wood of 0.56 and 1.11%. Sodium bisulfite charge on od wood was 0 for DA and 1.5 or 3.0% for SPORL. All substrates produced by both pretreatments (except DA with pretreatment duration of 0) had good enzymatic digestibility of over 80%. However, SPORL produced higher enzymatic digestibility than its corresponding DA pretreatment for all the experiments conducted. As a result, SPORL produced higher ethanol yield from simultaneous saccharification and fermentation of cellulosic substrate than its corresponding DA pretreatment. SPORL was more effective than its corresponding DA pretreatment in reducing energy consumption for postpretreatment wood chip size-reduction. SPORL, with lower energy input and higher sugar and ethanol yield, produced higher sugar and ethanol production energy efficiencies than the corresponding DA pretreatment. Published 2011 American Institute of Chemical Engineers *Biotechnol. Prog.*, 27: 419–427, 2011*

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Introduction

Woody biomass is a very important feedstock for the future biobased economy. It can be sustainably produced from forest land and intensively managed plantations in large quantities in many regions of the world such as Scandinavia, New Zealand, South America, Canada, and the United States.¹ Intensive short rotation and culture can provide almost unlimited opportunity for woody biomass production.^{2,3} Woody biomass has several advantages in terms of its high density to reduce transportation cost, flexible har-

vesting to eliminate storage cost, and high cellulose and lignin content to increase energy density.⁴ However, barriers in woody biomass bioconversion need to be removed to translate these advantages to tangible economic benefits. Compared with other plant biomass, woody biomass is especially recalcitrant to enzyme and microbial actions because of its large physical size, strong physical structure, and high lignin content. Few pretreatment methods have been proven to be effective in removing the recalcitrance of woody biomass for efficient biochemical processing for cellulosic ethanol production. Dilute acid (DA) pretreatment is the most studied process for agricultural biomass.^{5,6} It is effective in producing high sugar yields when applied to certain hardwood species, such as poplar, using significantly size-reduced materials.^{7,8} DA also has excellent scalability for large-scale

Correspondence concerning this article should be addressed to J.Y. Zhu at jzhu@fs.fed.us.

production. Therefore, it has great potential for commercial applications.

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL), a novel process recently developed at the U.S. Forest Service, Forest Products Laboratory and the University of Wisconsin, demonstrated robust performance in terms of enzymatic digestibility of woody lignocellulose, sugar and ethanol yields, pretreatment energy efficiency, and net energy output.^{4,9–11} Using SPORL on lodgepole pine (softwood),^{11,12} one of the most recalcitrant feedstocks, achieved an ethanol yield of 270 L/ton wood; this is equivalent to 70% theoretical yield, and gives a net energy output (from ethanol only without considering lignin) of over 4 GJ/ton wood (before distillation). SPORL is an acidic pretreatment and is very similar to DA pretreatment in terms of process flow (configuration) and ranges of pretreatment temperature, duration, and acid dosage but with the addition of sulfite or bisulfite. Just like DA, SPORL also has excellent scalability for commercialization, as it can use mature capital equipment and infrastructure similar to those used in pulp and paper mills for commercial implementations. In view of the excellent performance and scalability of SPORL, it is of great interest to compare SPORL with DA, the most studied process, for cellulosic ethanol production from woody biomass. Previously, we compared SPORL with DA for pretreatment of spruce,¹³ a softwood. The study provided some insights into the key differences between these two processes. DA is limited in removing the recalcitrance of softwoods, and the advantages of SPORL over DA for softwood pretreatment were clearly shown in terms of improving recovery of fermentable sugars and enzymatic cellulose saccharification of pretreated substrate (more than double) and reducing the formation of fermentation inhibitors. Future studies could compare SPORL with DA for pretreatment of relatively less recalcitrant biomass feedstocks, such as hardwoods, to demonstrate the merits of SPORL over DA.

The objective of the present study was to evaluate the performance of SPORL and DA for sugar and cellulosic ethanol production from native aspen (*Populus tremuloides*), one of the least recalcitrant kinds of woody biomass, based on our unpublished laboratory study that shows over 85% cellulose conversion to glucose using hot-water pretreatment on commercial-size wood chips at 180°C. The present study, therefore, establishes the minimal advantages of SPORL over DA by applying SPORL to a very low-recalcitrance feedstock while the maximal advantages of SPORL over DA were demonstrated when these treatments were applied to very recalcitrant softwoods.^{9,12,13} Both SPORL and DA pretreatments will be directly applied to commercial-sized aspen wood chips, rather than significantly size-reduced materials.^{7,8} The pretreated wood chips are then disk milled to produce a solid substrate for simultaneous enzymatic saccharification and fermentation (SSF). This approach of post-chemical-pretreatment size reduction can significantly reduce energy consumption in wood size reduction by a factor of 10,^{3,14} critically important to net energy output and overall process energy efficiency in commercial production. The present study compared fermentable sugar and ethanol yields from the SPORL and DA processes under similar pretreatment conditions. The study also examined the formation of fermentation inhibitors during SPORL and DA pretreatments in the water-soluble pretreatment hydrolysates that mainly contain the hemicelluloses sugars. The goal of the present

study is to provide an objective laboratory comparison of two promising technologies for woody biomass bioconversion to biofuel.

Experimental

Raw material and chemicals

Fresh aspen (*Populus tremuloides*) wood logs were obtained from northern Wisconsin, USA. The logs were hand-debarked and then chipped at the U.S. Forest Products Laboratory using a laboratory chipper. The wood chips were then screened to remove all particles greater than 38 mm and less than 6 mm in length. The thickness of the accepted chips ranged from 1 to 5 mm. The chips were kept frozen at a temperature of about –16°C until used.

Celluclast 1.5 L and Novozyme 188 (β -glucosidase) were generously provided by Novozymes North America (Franklinton, NC). Sodium acetate, sulfuric acid, and sodium bisulfite were used as received from Sigma-Aldrich (St. Louis, MO). All other chemicals, including culture media ingredients, were received from Fisher Scientific (Hanover Park, IL). All chemicals were of analytical quality.

Microorganism and culture

Saccharomyces cerevisiae Y5 (Strain preserved No. CGMCC2660, China General Microbiological Culture Collection Center) was obtained from Capital Normal University of Beijing, China. Details of the inhibitor-tolerance profiles of this yeast have been reported.¹⁵ To prepare seed culture, the strain was grown at 30°C for 2 days on YPD-agar plates containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 20 g/L agar. A colony from the plate was then transferred by loop to a liquid YP medium supplemented with 30 g/L glucose in a flask. The *S. cerevisiae* Y5 seed was grown overnight at 30°C with agitation at 90 rpm on a shaking bed until the biomass concentration reached ~2 g/L as monitored by optical density OD_{600nm} measurements (Agilent 8453, UV-visible spectroscopy system, Agilent Technologies, Santa Clara, CA).

SPORL and DA substrate production

Substrate production follows the schematic flow diagram shown in Figure 1. Subprocesses connected with dashed lines were not carried out in this study. SPORL and DA pretreatments were directly applied to wood chips by placing 150-g oven dry (od) wood chips and pretreatment solutions in a 1-L pressure vessel (manufactured in-house). Three 1-L vessels were mounted inside of a wood pulping digester (pressure vessel) in an autoclave configuration as described elsewhere.⁹ The 1-L pressure vessels were heated externally using steam while the wood pulping digester was rotating at the speed of 2 rpm for mixing. The pretreatment liquid to wood ratio (L/W) was kept at 3:1 (v/w) and the temperature was fixed at 170°C for all pretreatments conducted. The temperature ramping time to 170°C was about 7 min. Pretreatment duration at 170°C was varied from 0 to 30 min with an increment of 10 min. Zero pretreatment duration means that reactions were taking place during temperature ramping to 170°C, so actual pretreatment temperature was less than 170°C. Sulfuric acid charges of 0.55 and 1.10% (w/w) on od wood were used for both SPORL and DA pretreatments. Sodium bisulfite charges of 1.5 and 3.0% (w/w) on od wood

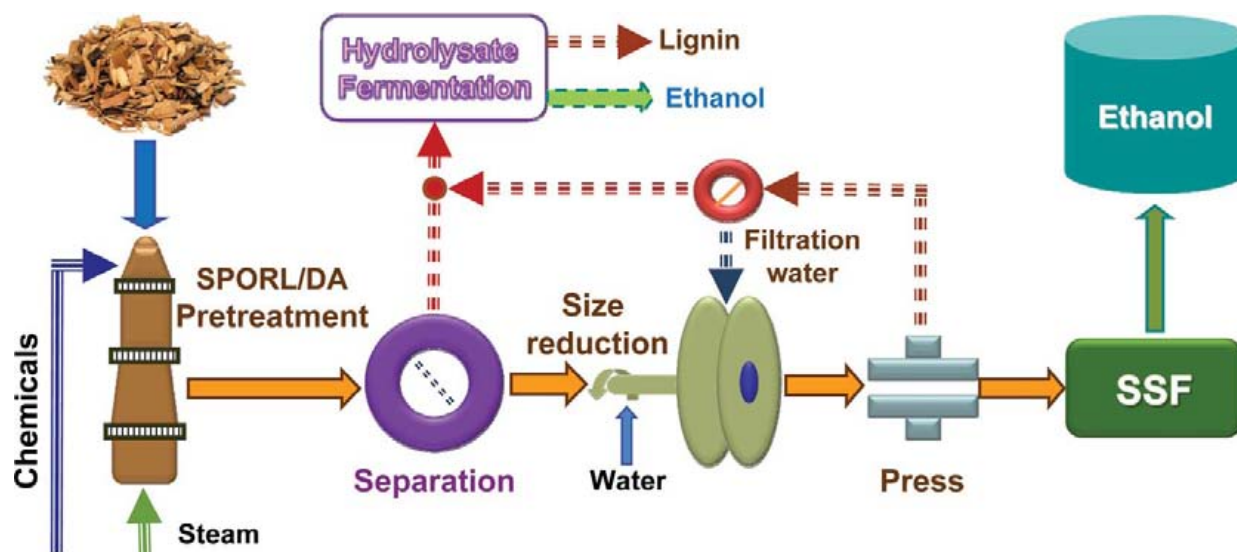


Figure 1. A schematic flow diagram of the SPORL process completed with simultaneous saccharification and fermentation (SSF) of solid substrate.

Table 1. Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL) (Bisulfite > 0) and Dilute Acid (DA) (Bisulfite = 0) Comparative Experiments and Corresponding Chemical Loadings and Pretreatment Duration Conducted at 170°C with Liquor to Wood Ratio (L/W) of 3:1

Experiment Number	Sample Label*	Acid Dosage (mL/L; wt % Wood)	Bisulfite Charge (wt % Wood)	Pretreatment Duration at 170°C (min)	Initial Liquor pH	Final Liquor pH
T7-1: SPORL	A2B3t0	2; 1.10	3	0	1.45	1.77
T7-2: DA	A2B0t0	2; 1.10	0	0	1.20	1.60
T7-4: SPORL	A2B3t10	2; 1.10	3	10	1.45	1.57
T7-5: DA	A2B0t10	2; 1.10	0	10	1.20	1.74
T7-7: SPORL	A2B3t20	2; 1.10	3	20	1.45	1.60
T7-8: DA	A2B0t20	2; 1.10	0	20	1.20	1.76
T7-10: SPORL	A2B3t30	2; 1.10	3	30	1.45	1.65
T7-11: DA	A2B0t30	2; 1.10	0	30	1.20	1.82
T7-B1: SPORL	A1B1t20	1; 0.55	1.5	20	1.58	1.31
T7-B2: SPORL	A1B3t20	1; 0.55	3	20	1.73	1.32
T7-A1: DA	A1B0t20	1; 0.55	0	20	1.44	1.93

* Sample label: A number is acid volumetric concentration in pretreatment liquor (mL/L); B number is sodium bisulfite charge (B0 is DA; B1 and B3 are SPORL); t# is pretreatment duration in minutes (min).

were applied for SPORL pretreatment. These pretreatment conditions were selected (as optimal) from a total of 51 different SPORL and DA pretreatments using a temperature range of 160–180°C, sulfuric acid and sodium bisulfite charges of 0–3.3% and 0–4.5%, respectively, and pretreatment duration of 0–30 min. Maximal sugar yields were achieved for both SPORL and DA pretreatments at 170°C under the selected pretreatment conditions reported in this work, based on the results of the 51 experiments. Experiments were designed so that comparisons of sugar and ethanol productions between SPORL and DA can be made under the exact same conditions except for sodium bisulfite applied in all SPORL runs (Table 1). The initial liquor pH was measured before wood chips were added.

Sulfite is an inexpensive chemical and the cost of sodium bisulfite is similar to that of sulfuric acid. Several bases, such as sodium (used in this study), calcium, magnesium, and ammonia can be used.^{16,17} In industrial operations, such as in sulfite pulp mills, sulfite is generated by using SO₂ and metal oxide or ammonia. The recovery of sulfur is in the form SO₂ through stripping and scrubbing as practiced in industry. The recovery of metal (sodium and magnesium) from lignin (enzymatic hydrolysis lignin only in biorefinery applications) are practiced using a fluidized bed reactor.^{18,19}

The dissolved lignin (lignosulfonate) that can be separated and directly marketed as practiced in some sulfite pulp mills without recovering the metal base (usually sodium). Although sulfite pulping is an old technology, a new modern magnesium sulfite pulp mill with full recovery of magnesium was recently built in South Africa.

Solid substrate was produced from the pretreated wood chips, which remained intact, by disk milling. The disk mill was equipped with plates of pattern D2-B505. The disk plate gap was set at 0.76 mm. Water was added during disk milling, which resulted in a solids discharge consistency of 10%. The energy consumption for disk milling was recorded as described elsewhere.^{14,20} The size-reduced solids were directly dewatered by pressing using a canvas bag to a solids content of about 30%, without a separate washing step. The yield of solid (substrate) in the form of fibers or fiber bundles was then determined from the weight and moisture content of the collected substrate. The moisture content was determined gravimetrically by drying the collected solids in an oven at 105°C overnight.

Separate enzymatic hydrolysis

Separate enzymatic hydrolysis experiments of the pretreated SPORL and DA substrates were conducted to

Table 2. Yields of Substrate Solids and Major Wood Components (wt %) in the Solid Substrates After Different SPORL and DA Pretreatments Listed in Table 1

Sample Label	K. Lignin	Glucan	Xylan	Mannan	Solid Substrate Yield (wt %)
Untreated aspen	20.8	43.8	16.4	1.6	100.0
A2B3t0	19.8	58.6	6.4	0.6	67.5
A2B0t0	22.7	56.5	10.7	1.00	72.3
A2B3t10	22.9	68.0	3.6	0.5	64.9
A2B0t10	25.4	62.2	4.5	0.4	66.7
A2B3t20	24.4	68.5	2.4	0.2	63.3
A2B0t20	29.6	64.7	2.8	0.1	66.5
A2B3t30	25.6	68.9	1.9	0.2	62.7
A2B0t30	29.2	62.9	2.0	0.0	66.4
A1B1t20	24.8	66.5	4.0	0.7	65.3
A1B3t20	23.2	67.9	3.9	0.5	63.9
A1B0t20	26.4	63.7	4.6	0.6	70.6
RSTD (%)*	0.8	1.2	1.4	2.1	0.7

* Relative standard deviation.

measure the enzymatic hydrolysis glucose yield (EHGY) in terms of kg/ton untreated wood. EHGY was used to determine total sugar recovery. Enzymatic hydrolysis was conducted using commercial enzymes at 2% substrate solids (w/v) in 50-mL of sodium acetate buffer (pH 4.8, concentration 50 mM) on a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 50°C and 200 rpm. Enzyme loading was Celluclast 1.5 L at 7.5 FPU/g substrate and Novozyme 188 (β -glucosidase) at 11.25 CBU/g substrate. Enzyme loadings were based on od substrate and not cellulose because hydrolysis was conducted before carbohydrate analyses were completed. The actual enzyme loading based on cellulose for a DA substrate was slightly higher than its corresponding SPORL substrate because of the higher glucan content of the SPORL substrate than its corresponding DA substrate (Table 2). Hydrolysate was sampled periodically for glucose concentration analysis. Each data point is the average of two replicates. The average relative standard deviation was about 2%.

Simultaneous enzymatic saccharification and fermentation (SSF) of solid substrates

SSFs were carried out in 250-mL Erlenmeyer flasks using a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) set at 35°C and 90 rpm with 10% substrate (water insoluble). The enzyme loading was Celluclast 1.5 L at 10 FPU/g and Novozyme 188 at 15 CBU/g cellulose (based on measured glucan content, Table 2) for both SPORL and DA substrates. Initial cell concentration for all SSF experiments was 2 g/L (wet base). No additional nutrients were added during fermentation. Samples of the fermentation broth were taken every 24 h and centrifuged at 10,000 rpm for 5 min and were stored at -4°C until analyzed for sugar and ethanol. Reported results are the average of duplicates with an average relative standard deviation of about 4%.

Analytical methods

The chemical compositions of the original and pretreated biomass were analyzed by the Analytical and Microscopy Laboratory of the Forest Products Laboratory.^{11,21} Ethanol analysis in the cellulosic substrate fermentation broth was carried out using a gas chromatograph (GC, model 7890, Agilent Technologies, Palo Alto, CA) through direct sample

injection using an external standard for calibration. The sample was centrifuged and the supernatant was filtered before injection to the GC column. The GC is equipped with a flame ionization detector and Agilent DB Wax column of 30 m with an ID 0.32 mm. A universal guard column was used to reduce column contamination. Inhibitor concentrations in the pretreatment hydrolysates were measured using an HPLC equipped with an Econosphere™ C18 column (5-mm particle size, 250 mm × 4.6 mm, Alltech, Deerfield, IL) and a UV1000 ultraviolet detector (277 nm; Thermo Finnigan, San Jose, CA). Samples were run at ambient temperature and eluted at 0.8 mL/min with a linear gradient of 50–100% acidified methanol (containing 0.25% acetic acid) over 15 min. All analyses were carried out in duplicate at a minimum. The average data were reported. The standard deviations were calculated as a measurements error. For fast analysis, glucose in the enzymatic hydrolysate was measured in duplicate using a commercial glucose analyzer (YSI 2700S, YSI, Yellow Springs, OH).

Results and Discussions

Comparisons of cell wall component between SPORL and DA pretreated solid substrates

The cell wall chemical composition can provide some indications of the effect of chemical pretreatment on biomass chemical structure. The untreated aspen has cellulose, lignin, and hemicelluloses contents of about 44, 21, and 20%, respectively (Table 2). Both SPORL and DA pretreatments were very effective in removing hemicelluloses, especially at pretreatment duration greater than 10 min (Table 2). As a result, glucan and lignin were enriched in the pretreated substrates despite the fact that both pretreatments partially hydrolyzed glucan and removed lignin. All the SPORL-pretreated substrates have a higher glucan and lower lignin and xylan content than the corresponding DA-pretreated substrate does (Table 2). This suggests that the SPORL-pretreated substrate may have a better enzymatic digestibility than its corresponding DA-pretreated substrate. The quantitative comparisons of xylan and lignin removal between the two pretreatments are presented in Figure 2. More than 80% of the xylan was removed by both pretreatments of duration greater than 10 min. However, SPORL pretreatment is more effective in removing xylan than DA pretreatment is, especially at short pretreatment durations. Glucan losses were minimal through both pretreatments with typical glucan loss of about 5% by DA and close to zero by SPORL pretreatments (Figure 2), respectively. SPORL removed about double the amount of lignin (~20 to 40%) than DA (~10 to 20%) for the four pairs of pretreatment shown (Figure 2). An increase in pretreatment duration reduced lignin removal for both SPORL and DA pretreatments because of lignin condensation.²² The higher pH of SPORL pretreatment would expect to decrease the extent of lignin condensation more than the corresponding DA pretreatment with a lower pH (Table 1).

Comparisons of inhibitor formation and hemicellulose sugar recovery between SPORL and DA pretreatments

The hemicelluloses removed by pretreatments were partially hydrolyzed to fermentable sugars and can be further degraded to furfurals, fermentation inhibitors. The measured concentrations in the pretreatment spent liquor collected right after solid/liquid separation (before disk refining) were

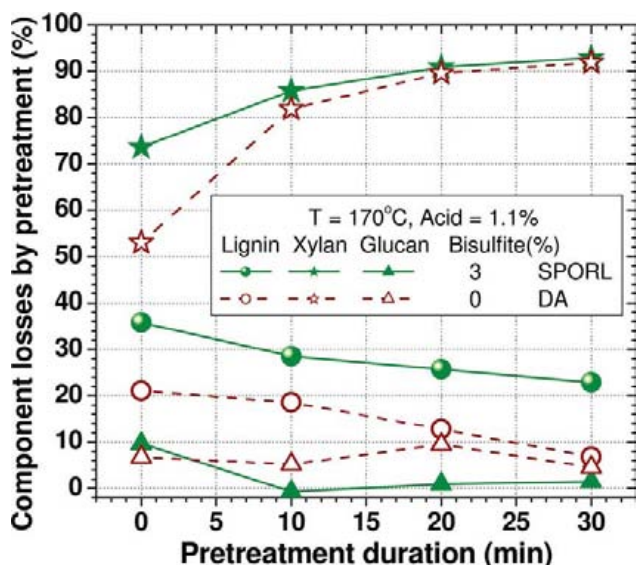


Figure 2. Comparisons of wood component losses by SPORL and DA pretreatments under different pretreatment durations.

reported and discussed in this section. Furfural formations were close to zero for both pretreatments with a duration of about 10 min or shorter (Figure 3a) and then increased rapidly. Although SPORL pretreatment removed more xylan than DA did (Figure 2), it produced a slightly lower amount of furfural than the corresponding DA pretreatment (Figure 3a). This can be clearly seen for the pretreatment duration of 30 min with measured furfural concentrations of 4.5 and 5.2 g/L in the SPORL and DA pretreatment hydrolysates, respectively. HMF formation was low (Figure 3a) because aspen has a very low mannan content, and glucan loss was minimal in both pretreatments. Acetic acid is another toxic compound to certain microbes in fermentation. The acetyl groups in aspen can be easily released to acetic acid during pretreatment as can be seen from Figure 3b. SPORL produced more acetic acid than DA did. The acetic acid concentration in the SPORL hydrolysate reached an asymptotic value of about 22.5 g/L with pretreatment duration of 20 min while it continuously increased in the DA hydrolysate with pretreatment time to about 16.5 g/L at 30 min.

Most of the hemicelluloses removed were converted to monomer sugars by pretreatments. The reported data in this section are from directly analyzing the collected pretreatment spent liquor before disk milling without further acid hydrolysis. SPORL produced slightly more (about 10%) xylose and mannose than DA did at a pretreatment duration of longer than 20 min (Figure 4). This most likely is due to less degradation to furfural (Figure 3a) because xylan removal between each pair of SPORL and DA pretreatment of 20 min or longer were about the same (Figure 2). At short pretreatment duration (zero duration means $T < 170^{\circ}\text{C}$), the DA produced more xylose because of its stronger acid hydrolysis reactions at a lower pH than SPORL, though SPORL removed more xylan than DA (Figure 2). The highest yield of xylose and mannose was 13.6 and 1.2 weight percent (wt%) of wood, or 76% and 67% theoretical yield, for SPORL and DA, respectively, obtained at pretreatment duration of 30 min. The reported yields (wt %) of hemicellulose sugars can be easily converted into concentrations in g/L simply dividing by the pretreatment liquid to wood ratio of 3:1.

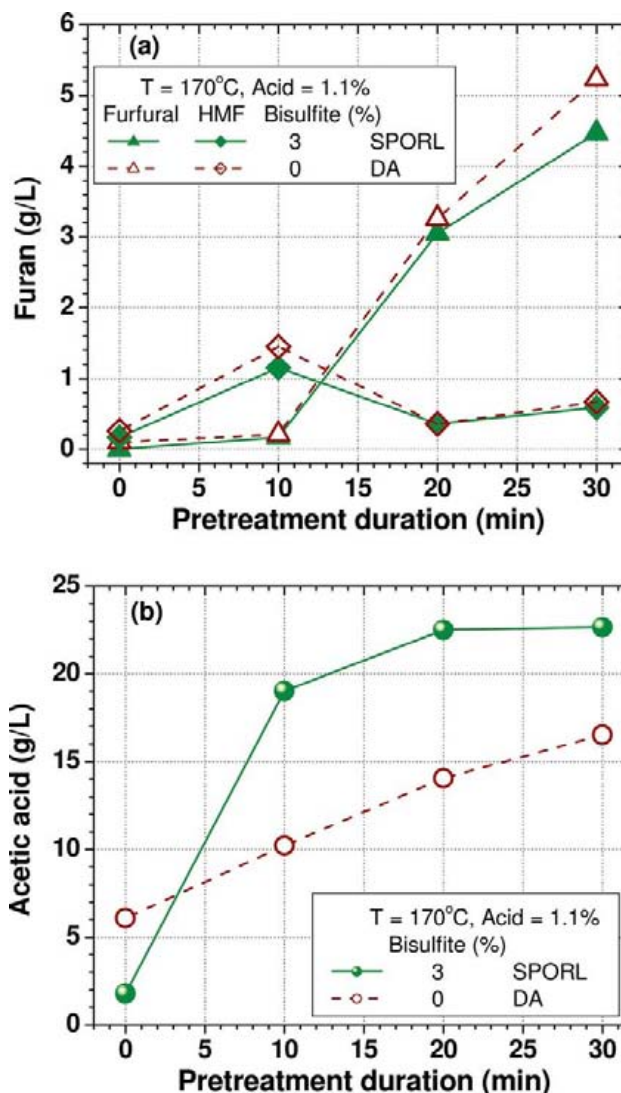


Figure 3. Comparisons of the formation of fermentation inhibitors by SPORL and DA pretreatments under different pretreatment durations.

(a) Furan: furfural and HMF; (b) Acetic acid.

Comparisons of substrate enzymatic digestibility (SED) and enzymatic hydrolysis glucose yield from SPORL and DA pretreatments

SPORL is very effective in removing the strong recalcitrance of woody biomass, including softwood species.^{9,11} The improved performance of SPORL pretreatment over DA in removing woody biomass recalcitrance can be seen by comparing its substrate enzymatic digestibility (SED) with the SED of the corresponding DA substrate (Figure 5a). SED is defined as the percentage of glucan in a substrate enzymatically hydrolyzed to glucose. The rate of hydrolysis of the SPORL substrate is slightly higher than that of the corresponding DA substrate in the first 12 h (Figure 5a), which resulted in a higher final SED after 72 h hydrolysis. Similar results were obtained when comparing other pairs of SPORL and DA substrates as can be seen from Figure 5b. Although DA pretreatments were very effective with SED close to 90% at a 30 min pretreatment, a similar SED can be achieved by SPORL with a shorter duration of about 15 min (Figure 5b). A shorter pretreatment time can significantly reduce the production of furfurals and acetic acid (Figure 3a,b) for favorable fermentation of the hemicellulose

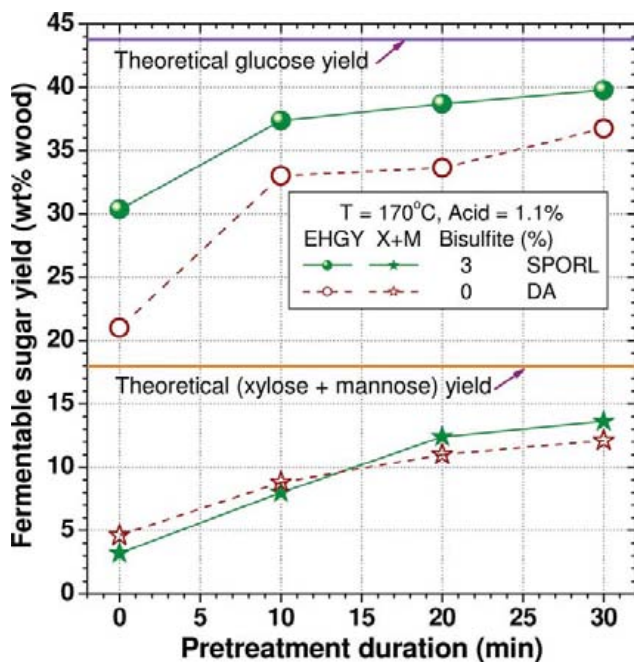


Figure 4. Comparisons of enzymatic hydrolysis glucose yield (EHGY) and yield of xylose and mannose from pretreatment hydrolysate between SPORL and DA pretreatments under different pretreatment durations.

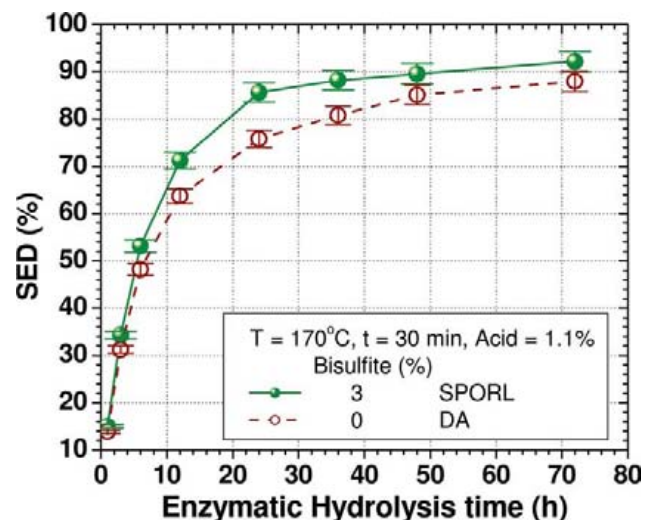
sugar stream. The relative effectiveness of SPORL is more pronounced at a pretreatment duration less than 10 min. The addition of sulfite (SPORL) improved SED by about 50% at zero duration (actual temperature less than 170°C).

Comparisons of ethanol production from SPORL and DA solid substrates through SSF

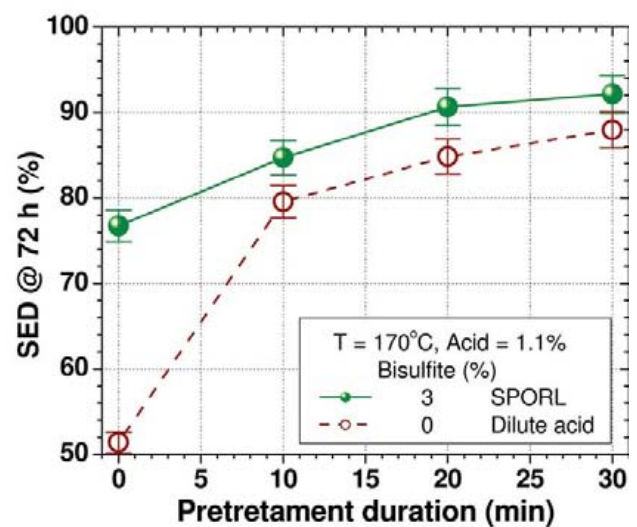
The relative performance of SPORL to DA pretreatment in removing woody biomass recalcitrance can also be seen from the results of SSF. For the pair of substrates pretreated for 30 min, the ethanol productivity in the first 24 h was 0.88 g/L/h for the SPORL substrate vs. 0.59 g/L/h for the DA substrate determined using the data shown in Figure 6. The maximal ethanol concentration was 30.0 g/L produced from the SPORL substrate vs. 25.4 g/L from the DA substrate, both achieved after 48 h of SSF (Figure 6). The SPORL substrate (A2B3t30) has a slightly higher glucan content of 68.9% than DA substrate (A2B0t30) of 64.7% (Table 2). For comparison, we calculated maximal SSF efficiencies using the glucan contents of the substrates and the measured maximal ethanol concentrations in the fermentation broth. The results clearly show that the SSF efficiency of SPORL substrate is consistently higher than that of the corresponding DA substrate (Figure 7). The maximal fermentation efficiency of 85% was achieved for the SPORL substrate pretreated for 30 min. The results also show that pretreatment duration has a weak effect on the SSF efficiency of SPORL pretreatment. The DA substrate produced at pretreatment temperature below 170°C (zero holding time at 170°C) was not fermentable, whereas the fermentation efficiency of the corresponding SPORL substrate is about 70%.

Preliminary evaluation of mass balance and energy efficiency between SPORL and DA pretreatments

Direct comparisons of yields and energy efficiencies of sugar and ethanol production between SPORL and DA



(a)



(b)

Figure 5. Comparisons of substrate enzymatic digestibility between SPORL and DA pretreatments.

(a) Time-dependent SED for a pair of SPORL and DA pretreatments with 30-min duration; (b) SEDs after 72-h hydrolysis for pretreatments conducted under different durations. Cellulase = 7.5 FPU/g substrate and β -glucosidase = 11.25 CBU/g substrate for all experiments.

pretreatment can provide objective information about these two processes for practical applications. The glucose yields from enzymatic hydrolysis of cellulosic substrate, EHG Y, monomeric sugar yields from pretreatment hydrolysate (water soluble), and ethanol yields from SSF of the cellulose fraction from different SPORL and DA pretreatment experiments are listed in Table 3. The results show that EHG Y from a SPORL substrate is higher than its corresponding DA substrate for all the experiments conducted except for A1B0t20. The maximal EHG Y was 91% of theoretical wood glucose for SPORL (A2B3t30) vs. 84% for DA (A2B0t30) as a result of the improved SED (Figures 5a,b) resulting from the application of bisulfite in SPORL pretreatments. The improved SED resulted in improved fermentation efficiencies defined as the percentage of theoretical sugars in the substrate converted to ethanol (Figure 7). The high fermentation efficiency together with low degradation of glucan (Figure 2) increased SSF ethanol yields from SPORL. The

maximal ethanol yield from the cellulosic fraction was 238 L/ton wood for SPORL (A2B3t30) vs. 214 L/ton for DA (A2B0t30), equivalent to 76% and 68% theoretical value (from SSF only) based on aspen glucan content of 43.8%. The higher EHGy from SPORL resulted in a higher total sugar yield than from DA. The maximal total sugar recovery was 86% theoretical value for SPORL (A2B3t30) vs. 79% for DA (A2B0t30) based on total wood glucan + xylan + mannan content of 61.8%.

Energy consumption for wood size-reduction is significant and can affect the overall energy efficiency in ethanol production.^{3,4} Our previous studies found that chemical pretreatment can reduce energy consumption for wood-size reduction, and different pretreatments have different effects on postpretreatment size reduction through disk milling.^{3,14} The results obtained in the present study further validated our previous work. The mechanical energy consumption for disk milling of SPORL-pretreated wood chips was significantly lower than that for milling the corresponding DA-pretreated wood chips for all the experiments conducted (Table 3). The longer the pretreatment, the lower the energy consumption for disk milling is for both SPORL and DA pretreatments. For pretreatment duration of 20 min or longer, disk-milling energy consumption was reduced to less than

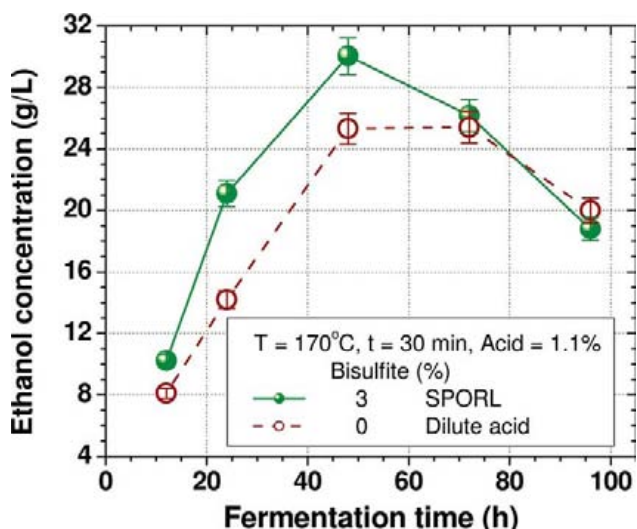


Figure 6. Comparisons of ethanol production from simultaneous saccharification and fermentation (SSF) between a pair of SPORL and DA pretreatments with 30-min duration.

Enzyme loading: Cellulase = 10 FPU/g cellulose and β -glucosidase = 15 CBU/g cellulose for all experiments.

0.09 GJ (25 kWh)/ton wood for SPORL, equivalent to energy consumption used for size-reduction of herbaceous biomass.²³

In evaluating energy efficiencies of sugar and ethanol productions, the thermal energy consumption for pretreatment was determined to be 1.25 GJ/ton wood using thermodynamic calculations, based of saturated pulp suspension at 25% solids (L/W = 3:1) and temperature 170°C with the consideration of thermal energy recovery of 50%. Wood chipping energy was estimated at 0.18 GJ (50 kWh)/ton based on pulp and paper industrial experience. Distillation was not conducted in this study and therefore not included in the energy balance analysis. Likewise, the energy consumed in SSF of solid substrates was not included in the preliminary energy balance as it was conducted using a laboratory bench scale shaker as a batch process, which does not reflect industrial operations. According to our previous description,^{3,4,11} the pretreatment and ethanol energy efficiencies are defined as follows:

$$\eta_{\text{Pretreatment}} = \frac{\text{Total monomeric sugar recovery} \left(\frac{\text{kg}}{\text{ton wood}} \right)}{\text{Total energy consumption for pretreatment} \left(\frac{\text{GJ}}{\text{ton wood}} \right)} \quad (1)$$

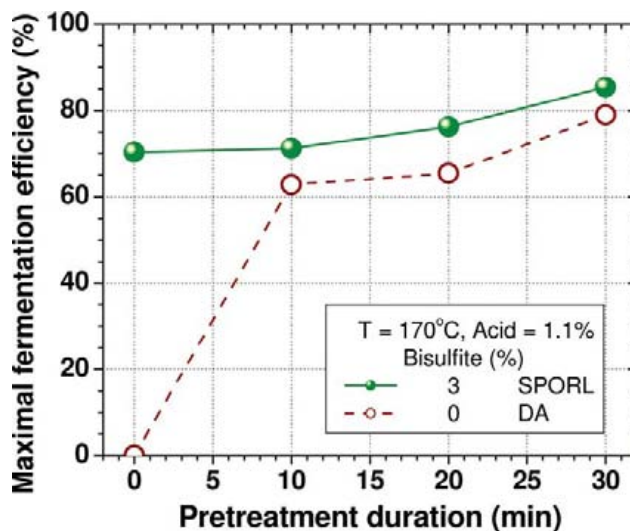


Figure 7. Comparisons of the efficiencies of simultaneous saccharification and fermentation (SSF) between substrates produced by SPORL and DA pretreatments under different pretreatment durations.

Enzyme loading: Cellulase = 10 FPU/g cellulose and β -glucosidase = 15 CBU/g cellulose for all experiments.

Table 3. Comparisons of Yields and Energy Efficiencies of Sugar and Ethanol (SSF of Cellulose Fraction Only) Production Between SPORL and DA Pretreatments

Sample Label	EHGY at 72 h*	SSF Ethanol (L/ton)	Hydrolysate Sugar (wt % Wood)	Total Sugar*	Wood Chip Milling Energy (GJ/ton)	Total Energy Input (GJ/ton)	$\eta_{\text{Pretreatment}}$ (kg Sugar/GJ)	SSF Ethanol Energy (GJ/ton Wood)	η_{Energy} (%)
A2B3t0	33.7/69.3	166.8	3.2	36.9/53.8	0.764	2.192	168.3	3.904	78.1
A2B0t0	23.3/47.9	0	4.8	28.1/41.0	1.159	2.587	108.6	0.000	NA
A2B3t10	41.5/85.3	204.0	8.4	49.9/72.7	0.159	1.587	314.4	4.773	200.8
A2B0t10	36.7/75.4	187.7	9.6	46.3/67.4	0.654	2.082	222.4	4.392	110.9
A2B3t20	43.0/88.4	215.0	13.1	56.1/81.8	0.084	1.512	371.0	5.031	232.7
A2B0t20	40.6/83.4	182.1	12.0	52.5/76.6	0.343	1.771	296.5	3.927	140.6
A2B3t30	44.2/90.8	238.4	14.8	59.0/86.0	0.066	1.494	394.9	5.579	273.4
A2B0t30	40.8/83.9	213.7	13.5	54.3/79.1	0.172	1.600	339.3	5.002	212.6
A1B1t20	40.1/82.5	178.8	10.0	50.2/73.1	0.338	1.766	284.3	4.183	136.9
A1B3t20	40.9/84.1	160.4	9.1	50.0/72.8	0.325	1.754	285.1	3.752	114.0
A1B0t20	41.0/84.2	152.1	8.9	49.9/72.7	0.706	2.134	233.9	3.560	66.8

*The first numbers are in weight percent (wt%) of untreated wood. The second numbers after the slash are the percentage of theoretical value based on untreated wood glucan content of 43.8% for EHGy and glucan + xylan + mannan content of 61.8% for total sugar.

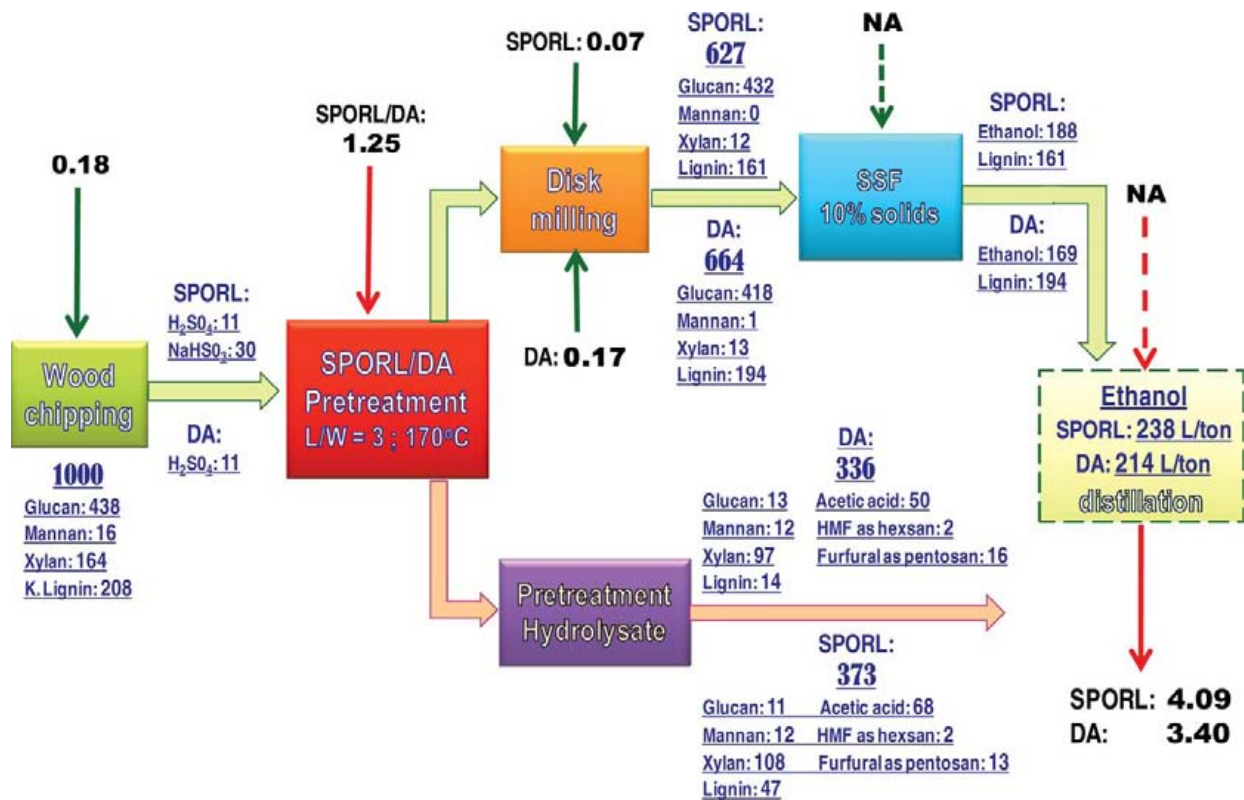


Figure 8. A block diagram showing process mass and energy balance between a pair of SPORL and DA pretreatments with 30-min duration at 170 °C and acid charge 1.1% on wood.

Unless indicated, energy data (bold font) are in GJ/ton wood and mass data (underlined) are in kg.

$$\eta_{\text{Energy}} = \frac{\text{Net energy output} \left(\frac{\text{GJ}}{\text{ton wood}} \right)}{\text{Total energy input} \left(\frac{\text{GJ}}{\text{ton wood}} \right)} \quad (2)$$

For the present study, only the ethanol energy was used in calculating the net energy output. SPORL pretreatment had a higher total sugar recovery and lower energy input than the corresponding DA pretreatment, which resulted in a higher pretreatment energy efficiency. The maximal pretreatment energy efficiency was 395 kg sugar/GJ for SPORL (A2B3t30) vs. 340 kg sugar/GJ for DA (A2B0t30) (Table 3). Similarly, the higher ethanol yields from and lower energy input of SPORL than those of the corresponding DA pretreatment resulted in a higher net energy output. The maximal net energy output (from SSF ethanol only excluding lignin and water soluble sugar stream) was 4.085 GJ/ton wood for SPORL (A2B3t30) vs. 3.402 (GJ/ton wood) for DA (A2B0t30) before distillation (Table 3), corresponding to ethanol production energy efficiencies of 273% and 213% for SPORL and DA, respectively. A side by side comparison between the pair of SPORL (A2B3t30) and DA (A2B0t30) pretreatments conducted for 30 min is presented in Figure 8 to provide a clear picture about component mass and process energy balance. This pair of pretreatments represents the best performance for SPORL and DA, respectively.

Conclusions

This study established the minimal advantages of SPORL over DA by applying these two pretreatments to native aspen (*Populus tremuloides*), which has very low recalcitrance. Both SPORL and DA pretreatments can remove recalcitrance

of native aspen for efficient enzymatic cellulose saccharification and hemicellulosic sugar recovery. However, SPORL is more effective, resulting in a higher substrate enzymatic digestibility (SED) and enzymatic hydrolysis glucose yield (EHGY) than the corresponding DA pretreatment under the same conditions except bisulfite charge. The maximal EHGY for SPORL was 91% of theoretical value, higher than the 84% for DA, because of improved SED and a lower acid hydrolysis glucan loss during SPORL pretreatment. The improved SED of the SPORL cellulosic substrate also produced a higher ethanol yield in simultaneous saccharification and fermentation (SSF) than that from DA. The maximal ethanol yield from SSF with an enzyme dosage of 10 FPU/g cellulose was 238 L/ton wood for SPORL vs. 214 L/ton for the corresponding DA pretreatment, or about 11% higher. Both pretreatments reduced energy consumption for size-reduction of wood chips. However, SPORL is more effective. SPORL demonstrated higher sugar and ethanol production energy efficiencies than the corresponding DA pretreatment because of the reduced energy input and increased sugar and ethanol yields. The maximal sugar and ethanol production energy efficiency was 395 kg/GJ and 273% for SPORL vs. 339 kg/GJ and 213% for DA, respectively. Although SPORL produced more acetic acid than the corresponding DA pretreatment, the effectiveness of SPORL pretreatment affords using milder pretreatment conditions (such as short pretreatment duration) than DA to achieve similar sugar yield. Milder pretreatment can lead to reduced production of fermentation inhibitors, such as furfural and HMF, which would be favorable for the fermentation of the pretreatment hydrolysate that consists mainly of hemicellulosic sugars. Whereas the significant advantages of SPORL

over DA for pretreating very recalcitrant softwoods are obvious as reported in the literature, the fact that SPORL produced about 10% more sugar and ethanol yields than DA did from aspen, suggests that the application of sulfite in SPORL is advantageous even for a feedstock with considerable recalcitrance.

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