Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL) for Robust Enzymatic Saccharification of Hardwoods

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This study demonstrates sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust bioconversion of hardwoods. With only about 4% sodium bisulfite charge on aspen and 30-min pretreatment at temperature 180°C, SPORL can achieve near-complete cellulose conversion to glucose in a wide range of pretreatment liquor of pH 2.0–4.5 in only about 10 h enzymatic hydrolysis. The enzyme loading was about 20 FPU cellulase plus 30 CBU β-glucosidase per gram of cellulose. The production of fermentation inhibitor furfural was less than 20 mg/g of aspen wood at pH 4.5. With pH 4.5, SPORL avoided reactor corrosion problem and eliminated the need for substrate neutralization prior to enzymatic hydrolysis. Similar results were obtained from maple and eucalyptus. © 2009 American Institute of Chemical Engineers *Biotechnol. Prog.*, 25: 1086–1093, 2009
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Introduction

Feedstock pretreatment is one of the most critical steps in biochemical conversion of lignocellulose for commercial production of biofuel and bioproducts.[1] Efficient processes for pretreating woody biomass are still lacking despite much research efforts and progresses.[2,3] However, woody biomass is an important and sustainable source of renewable feedstock for the future bioeconomy. Because woody biomass can be harvested at any time, it offers significant advantages over agriculture residues in terms of storage. Furthermore, short-rotation intensive culture or tree farming offers an almost unlimited opportunity for biomass production.[4] Of all the existing pretreatment processes, the organosolv, acid-catalyzed steam explosion, and dilute-acid prehydrolysis are considered to have the most commercial potential for woody biomass biorefining.[5] These processes also have major pitfalls. The steam explosion process is energy-intensive (e.g., 1.8 MJ natural gas/kg oven dry (od) wood at steam explosion temperature of 215°C), with the recovery of low-quality steam of 144°C based on our own estimation, which significantly increases production cost. Furthermore, commercial scale-up of the steam explosion device to over 1,000 ton/day needs to be proven. Dilute-acid prehydrolysis can achieve satisfactory cellulose conversion for most feedstocks except softwood, when the feedstock size is significantly reduced to the scale of millimeters, as demonstrated in the literature.[6] Unfortunately, electric energy consumption in mechanical size reduction of woody biomass is very energy-intensive (200–400 Wh/kg (0.72–1.54 MJ/kg) of od wood),[7] which has not been addressed. Furthermore, the low pH of the dilute acid process causes serious equipment corrosion problems. The organosolv process requires the high-value use of all fractions from the pretreatment to be economically viable.

We reported a novel process using sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) and achieved robust bioconversion of softwoods in a previous study.[8] SPORL was very effective even when it was directly applied to chip-size ($\sim 2 \times 3 \times 0.5$ cm^3) woody biomass and without wood chip impregnation with chemicals. The

This work was conducted on official government employee time by Zhu and Gleisner and while Wang was a visiting scientist at the USDA Forest Products Laboratory.

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pretreated wood chips can be easily pulverized mechanically with electric energy consumption of only about 20 Wh (0.07 MJ)/kg od untreated wood through disk milling,[8] which significantly reduced pretreatment cost. Substrates from SPORL have excellent digestibility, about 95% cellulose conversion and enzymatic hydrolysis glucose yield of 40 (wt % od wood) from red pine (glucan content of 45 g) after only 48 h enzymatic hydrolysis using normal enzyme dosage of 20 FPU/g cellulose. Currently, only the organosolv process can achieve such excellent enzymatic digestion performance. With low pretreatment cost, excellent substrate digestibility, along with sulfite pulping and chemical recovery, and disk refining technologies that have long been practiced in the pulp and paper industry, and existing industry infrastructure and commercial markets for high-value coproducts from pretreatment dissolved hemicellulose sugars and lignin, SPORL offers many advantages over existing processes for commercialization, with low environmental and technological barriers and risks.

As discussed in our previous study,[8] the terms sulfite and bisulfite are used interchangeably in SPORL because active reagents in the pretreatment liquor can be sulfite (SO_3^{-2}), bisulfite (HSO_3^{-1}), or the combination of sulfite (SO_3^{-2}) or bisulfite (HSO_3^{-1}) with an acid medium such as sulfur dioxide (SO_2), depending on the pH of the pretreatment liquor at pretreatment temperatures.[9] Hardwoods contain significant amounts of acetyl groups (3–5%). The formation of acetic acid from acetyl groups during pretreatment[10,11] can maintain the pH value required for effective SPORL pretreatment without the application of additional acid. Unlike traditional sulfite pulping whose goal is delignification while preserving cellulose for strong pulp, SPORL is focused on (1) significantly degrading or depolymerizing cellulose, (2) completely removing hemicellulose, and (3) preventing excessive lignin condensation through proper control of pretreatment pH, temperature, and sulfite dosage to achieve good digestibility of the pretreated substrate. Therefore, SPORL is different from traditional sulfite pulping in terms of objectives and process operating conditions (such as pH, temperature, chemical dosage).

The objective of this study is to demonstrate the advantages of SPORL for bioconversion of three hardwoods—aspen, maple, and a short-rotation planted eucalyptus. In general, hardwoods have lower lignin and higher glucan contents than do softwoods.[12,13] Species like poplar are particularly attractive for bioconversion for its high cellulose content and relatively weak recalcitrance compared with softwoods. Short-rotation, fast-growing *Populus* and *Eucalyptus* species provides sustainable feedstock for biorefining in many regions of the world, such as South America, Australia, and southeastern Unites States, because they grow very fast and can be harvested in 3–5 years.[14] Previous studies on bioconversion of hardwoods were mainly carried out using poplar species by organosolv[15,16] and acid-catalyzed steam explosion.[17–19] Dilute acid pretreatment can achieve satisfactory conversion after significant size reduction prior to pretreatment.[6,20] We believe that SPORL, with its excellent performance in overcoming softwood recalcitrance, is more effective than existing processes for pretreating hardwoods. Furthermore, SPORL can achieve rapid enzymatic saccharification even when directly applied to wood chips prior to size reduction. This allows significantly reduced energy consumption in pretreatment and enzyme loading to reduce production cost or improve fermentation yield using simultaneous saccharification and fermentation (SSF).[21]

Experimental

Materials

Fresh aspen and maple wood chips were from the Wisconsin Rapids mill of Stora Enso North America (now New Page Corporation, OH). Fresh clone eucalyptus wood logs were supplied by the University of Florida (Gainsville, FL) and chipped at the USDA Forest Products Laboratory using a laboratory chipper. Chips were kept frozen at about $-16°C$ until used. The wood chips were screened to remove all particles greater than 38 mm and less than 6 mm in length, which gives accepted wood chips with dimensions of about 2×3 cm^2 and thickness ranging from 2 to 6 mm.

Commercial enzymes, Celluclast 1.5 L (cellulase) and Novozym 188 (β-glucosidase), were used as received from Sigma-Aldrich (St. Louis, MO). Sodium bisulfite and sulfuric acid were used as received from Sigma-Aldrich (St. Louis, MO).

Pretreatment chemical solution preparation

As discussed in our previous study,[8] there are several ways to prepare the sulfite chemical solution for pretreatment applications. Calcium, sodium, magnesium, ammonia, potassium, and other counter-ion bisulfite chemistries have been practiced in the pulp and paper industry.[9] The standard industry method for preparing sulfite-pulping liquor using sulfur and counter-ion oxide[22] can be readily applied to SPORL. Magnesium bisulfite eases chemical recovery.[9,22] Calcium bisulfite has the advantage of low cost. Sodium bisulfite has excellent solubility over a wide pH range. To demonstrate SPORL, the industry practice of producing sulfite pulping liquor was not used in this study. Commercially purchased sodium and magnesium bisulfite were directly used as received with or without addition of sulfuric acid. As mentioned previously, hardwood contains acetyl groups that can be converted to acetic acid during pretreatment. As a result, effective SPORL may be achieved even without the addition of the acid.

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL)

SPORL experiments were conducted according to the process flow diagram shown in Figure 1. SPORL pretreatment was carried out in a laboratory pulping digester. The digester has three 1-L sealed stainless steel cylinder reactors in an autoclave-type arrangement. The digester was heated by steam. About 130 g of od wood chips were loaded in each reactor. Wood chips were subjected to pretreatment using bisulfite with or without sulfuric acid prior to size reduction. The pretreatment liquor to od wood chip solid ratio was 5. The sodium bisulfite charge on od untreated wood (wt bisulfite/wt od wood) varied from 0 to 6%, and sulfuric acid charge on od untreated wood (wt acid/wt od wood) varied from 0 to 2.76%, which resulted in pH of the pretreatment solution ranging from 1.9 to 4.5. During pretreatment, the reaction temperature was raised to 180°C in about 30 min and then maintained for another 30 min at this temperature. SPORL experiments were conducted at 180°C due to the limit of the steam heating capacity. At the end of the pretreatment, the pretreatment spent liquor was separated from the pretreated wood chips using an ordinary screen. The solid was collected. Solid loss was determined from the measured wet weight and moisture content of the collected solid.

The collected solid from pretreatment was fed directly into a laboratory 8-inch disk refiner (Andritz Sprout-Bauer

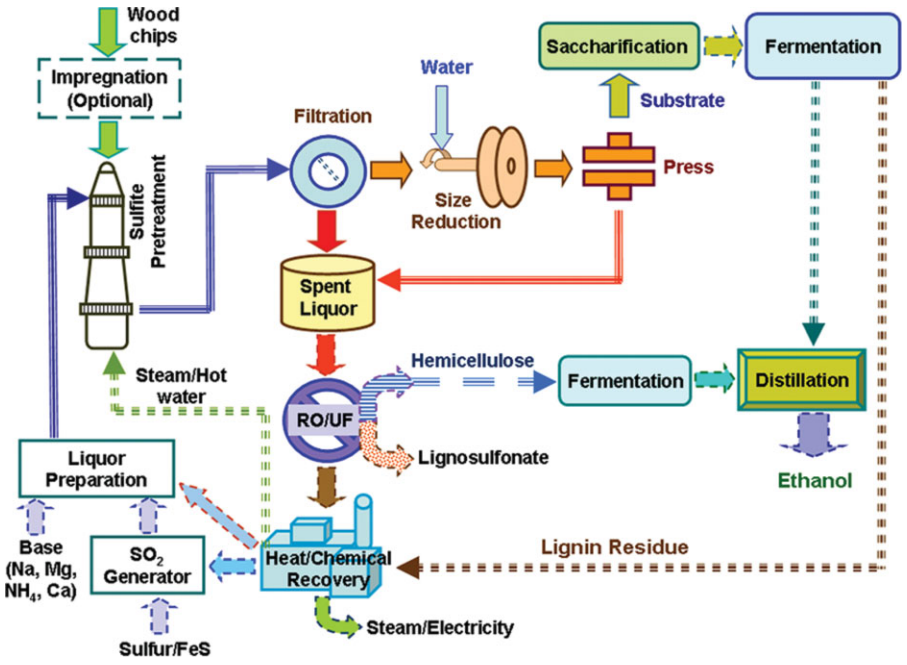

Figure 1. The schematic flow diagram of the SPORL process used in the present study.

Pressurized Refiner, Springfield, OH) to produce substrate for enzymatic hydrolysis. Water was added into the disk refiner with the pretreated wood chips. The mechanical disk milling was carried out in ambient conditions with disk gap of 0.25 mm. The biomass collected from size reduction was dewatered using a Buchner funnel to obtain the substrate for enzymatic hydrolysis. Fast draining filter paper (Grade 617, Ahlstrom, Mt Holly Springs, PA) was used for dewatering. The dewatering process acts as washing of the substrate. No additional substrate washing step was employed.

Enzymatic hydrolysis

Enzymatic hydrolyses of the substrates from SPORL were carried out at 2% of substrate (w/v) in 50 mL sodium acetate buffer using an shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 200 rpm. The pH and temperature were adjusted to 4.8 and 50°C, respectively. A mixture of Celluclast 1.5 L (cellulase) and Novozyme 188 (β-glucosidase) was used in enzymatic hydrolysis. Because carbohydrate analyses of the substrates were not completed before enzymatic hydrolysis, the actual enzyme loadings were based on od weight of substrate in hydrolysis, that is, Cellulclast 1.5 L of 14.6 FPU/g substrate and Novozyme 188 of 22.5 CBU/g substrate. This enzyme loading is approximately 20 FPU cellulase plus 30 CBU β-glucosidase per gram of cellulose based on the measured glucan contents of the substrates. The enzyme activity ratio between cellulase to β-glucosidase was maintained at about 1 FPU:1.5 CBU for all the hydrolysis experiments conducted. The excess of Novozym 188 (β-glucosidase) was used to prevent cellobiose accumulation.[23] Hydrolyzates were sampled periodically for glucose analysis. Each data point was averaged from two replicates.

Analytical methods

The chemical components of the original and pretreated samples were measured using an improved high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) method[24] by the Analytical and Microscopy Laboratory (US Forest Service, Forest Products Laboratory). For fast analysis, glucose in the enzymatic hydrolysates was measured using a commercial glucose analyzer (YSI 2700S, YSI, Yellow Springs, OH). The analyzer has an auto-calibration procedure that calibrates the system every five tests. The calibration solution was provided by the manufacturer. The manufacturer-specified precision for glucose measurements was 2%. The YSI 2700 system was recommended by the Laboratory Analytical Procedure (LAP-009) of National Renewable Energy Laboratory (Golden, CO) to analyze glucose of enzymatic hydrolysate of lignocellulose substrates. Hydroxymethyl furfural (HMF) and furfural in the SPORL spent liquors were measured by HPLC (HP 1090 Series II, Hewlett-Packard, Now Agilent Technologies, Palo Alto, CA) with UV detection at 280 nm using external standards. A reverse phase column (C18, Grace Vydac, Deerfield, IL) was used for separation.

Results and Discussions

Different terms have been used in the literature for describing substrate enzymatic digestibility and pretreatment process performance (for example, cellulose-to-glucose conversion yield[16] and enzymatic hydrolysis yield[17]). To more easily compare our results with published data (as listed in Table 1), we define the following terminologies. The term process enzymatic cellulose conversion (PECC) is preferred over enzymatic cellulose conversion (or digestibility) of substrate (ECCS), which is frequently used in most publications to represent substrate enzymatic digestibility (SED), because PECC can be added to glucose yield from pretreatment spent liquor (GYSL) as shown in Table 1 to obtain process overall cellulose conversion (OCC), also called overall glucose yield.[19] Ultimately, overall cellulose conversion (OCC) determines overall glucose recovery.

Aspen

Table 2 lists the major wood chemical component removal through four SPORL experiments using different acid charge

Table 1. Definitions of Terminologies Used for Evaluating Pretreatment Process Performance

Parameter		Unit	Definition
Enzymatic hydrolysis glucose yield	EHGY	wt % od wood	Amount of glucose in gram obtained from enzymatic hydrolysis 100 gram oven dry (od) untreated wood
Enzymatic cellulose conversion of substrate; Substrate enzymatic digestibility	ECCS, SED	%	90*EHGY / glucan content in substrate times substrate mass yield from 100 gram od untreated wood
Process enzymatic cellulose conversion	PECC	%	90*EHGY / amount of glucan in 100 gram od untreated wood
Glucose yield from pretreatment spent liquor	GYSL	wt % od wood	Amount of glucose in spent liquor resulted from pretreating 100 gram od wood
Overall cellulose conversion	OCC	%	PECC + 90*GYSL / amount of glucan in 100 gram od untreated wood

Table 2. Weights of Major Wood (Aspen) Components and Percentage Loss After SPORL at Different Sulfuric Acid Charges at 180°C and Sodium Bisulfite Charge on od Untreated Wood of 4%

Acid Charge on Wood (%)	K. lignin (g) (% loss)	Glucan (g) (% loss)	Xylan (g) (% loss)	Mannan (g) (% loss)	Sum (g)	Total (g)
Untreated sample	23.0	45.9	16.7	1.2	86.8	100
2.76 (pH 1.9)	16.8 (27.0)	37.7 (17.9)	0.3 (98.2)	N/A	54.8	58.2
1.84 (pH 2.0)	16.1 (29.8)	39.7 (13.6)	0.7 (95.8)	N/A	56.5	60.7
0.92 (pH 2.3)	13.8 (40.0)	41.1 (10.5)	1.5 (91.3)	N/A	56.4	60.2
0 (pH 4.5)	13.0 (43.5)	44.2 (3.8)	2.9 (82.7)	N/A	60.1	64.7

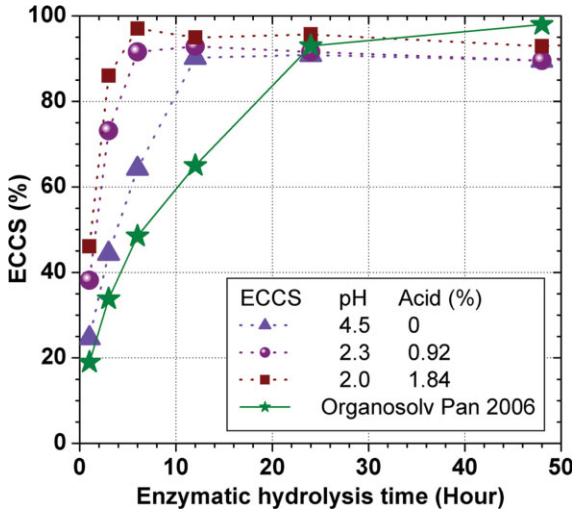

Figure 2. Comparison of time-dependent enzymatic cellulose conversion of substrates (ECCS) produced from SPORL and Organosolv pretreatments of aspen.

(or pH) and sodium bisulfite charge of 4% on od wood at 180°C. Xylan is the major hemicellulose (16.7%) in the aspen sample. Other hemicellulose components are approximately 2% in total in the untreated wood and therefore are not reported. The results indicate that xylan can be completely removed with an acid charge greater than 1% on od wood. At acid charge zero, or pH about 4.5 (i.e., using bisulfite only), xylan removal was about 80%. Lignin removal increased with the decrease in acid charge or increase in pH. About 30% of the lignin was removed at pH around 2.0, or acid charge between 1.8 and 2.8%, on od wood, while more than 40% of the lignin was removed at zero acid charge, or pH about 4.5. Glucan loss decreases with the decrease of acid charge. Glucan loss was less than 4% at zero acid charge, or pH 4.5.

Figure 2 shows that the SPORL substrates can be easily digested with commercial enzymes at normal dosage of 14.6 FPU/g substrate. The experimental uncertainty was 3% based on triplicate runs. For comparison, we also plotted the data from an ethanol organosolv substrate, one of the most readily digestible substrates[16] in Figure 2 (error bars are not shown for clarity). Enzyme loading was 20 FPU/g cellulose for the organosolv substrate.[16] We converted the enzyme loading of 14.6 FPU/g substrate used for the three SPORL substrates shown in Figure 2 to the same base unit of FPU/g cellulose. Based on the measured glucan contents of the substrates, enzyme loadings for the three SPORL substrates obtained at pH 4.5, 2.3, and 2.0 are 21.4, 21.4, and 22.3 FPU/g cellulose, respectively. Therefore, enzyme loadings used for the SPORL samples are equivalent to that used by Pan et al.[16] Enzymatic hydrolysis rates for the SPORL substrates are much faster than that for the organosolv substrate. Enzymatic cellulose conversion of substrate (ECCS) can reach over 90% in just 5–10 h for the SPORL substrates, depending on acid charge. It takes more than 20 h for the organosolv substrate to achieve equivalent ECCS. The difference in wood species used in the work of Pan et al.[16] may partially contribute to the low rate of enzymatic hydrolysis. Nevertheless, the data in Figure 2 showed the excellent digestibility of SPORL substrates.

Table 3 compares the enzymatic hydrolysis efficiencies of SPORL with those of organosolv and steam explosion processes. The steam explosion[17] used a shorter pretreatment time but a higher pretreatment temperature than the present SPORL. The steam-exploded aspen[17] achieved a lower enzymatic cellulose conversion of substrate (ECCS), about 80% after about 72 h, when compared with the more than 90% conversion of the present SPORL substrates in 4–12 h, even though the steam explosion used slightly higher enzyme dosages. The ethanol organosolv process[16] achieved an equivalent enzymatic hydrolysis conversion of about 95% after 24 h enzymatic hydrolysis that the present SPORL process achieved at the same pretreatment temperature of 180°C and similar enzyme application dosages in just 4–12 h hydrolysis, however, the ethanol organosolv process[16] used a relatively long pretreatment time of 1 h at acid charge of 1.25%. Table 3 also indicates that the terminal process enzymatic cellulose conversion (PECC) of SPORL was equivalent to that achieved by the ethanol organosolv process,[16] between 85 and 90%. The PECC of the steam explosion study[17] was below 70%. The PECC of sulfuric acid catalyzed steam

Table 3. Comparisons of Enzymatic Cellulose Conversion Among SPORL, Steam Explosion, and Organosolv Processes at 48 h Enzymatic Hydrolysis

Process and Source	Wood Species and Glucan Content	Pretreatment Conditions	Cellulase FPU[a]	β-Glucosidase CBU[a]	Time to Achieve Conversion Rate Listed (h)	ECCS (%)	PECC (%)
Steam explosion[17]	Aspen chips (47.7%)	10 min@ 205°C SO_2 0.9% on wood	21.9/11.5	112.5/58.8	72	84	70
		3 min@205°C SO_2 0.7% on wood	19.6/11.5	100.3/58.8	72	75	67
		10 min@205°C SO_2 0	21.0/11.2	107.8/57.5	72	57	54
Steam explosion[19]	Salix (4 years old) (41.4%)	8 min@200°C H_2SO_4 0.25%	–/13.6	–/21.3	96		81
		8 min@200°C H_2SO_4 0.5%	–/13.4	–/20.9	96		80
Organosolv[16]	Aspen chips (44.1%)	60 min@180°C H_2SO_4 1.25% on wood, ethanol 50%	20/8.6	40/17.3	24	97	87
SPORL ($NaHSO_3$ 4%) (Present study)	Aspen chips (45.9%)	30 min@180°C H_2SO_4 0	21.4/9.7	32.1/14.6	12	92	89
		30 min@180°C H_2SO_4 0.92% on od wood	21.4/9.0	32.1/13.5	6	94	84
		30 min@180°C H_2SO_4 1.84% on od wood	22.3/9.1	33.5/13.7	4	96	83

*The data are reported in two bases: per g cellulose (first number) and per g wood (second number).

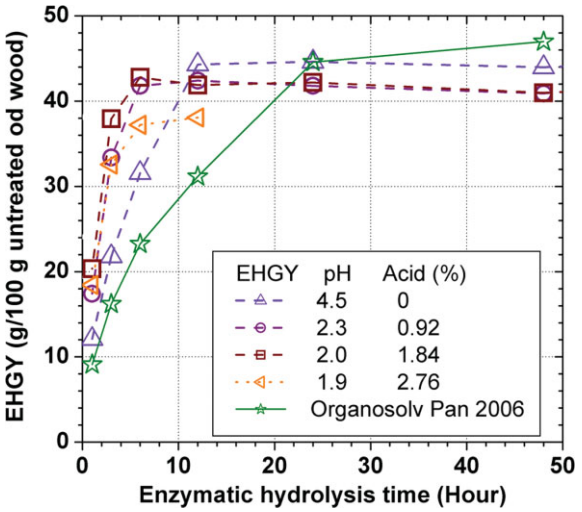

Figure 3. Comparison of time-dependent enzymatic hydrolysis glucose yield (EHGY) obtained from SPORL and Organosolv pretreatments of aspen.

explosion of Salix[19] was about 80% after about 96 h of hydrolysis, higher than that of SO_2-catalyzed steam explosion of aspen[17] in 72 h but lower than that achieved by the SPORL process in just 4–12 h.

Figure 2 indicates that increasing the pH of pretreatment liquor by reducing acid application in SPORL pretreatment decreased enzymatic hydrolysis rate and cellulose conversion of SPORL substrate because of less hemicellulose removal and insignificant depolymerization (prehydrolysis) of cellulose, which agrees with finding in our previous study with spruce.[8] Because aspen (hardwood) has a relatively low degree of recalcitrance compared with spruce (softwood), satisfactory ECCS (>90%) was achieved even at pH 4.5 (zero acid charge on wood) in just about 12 h of hydrolysis, as shown in Figure 2. Furthermore, glucan loss through pretreatment is reduced at high pH (low acid charges), as shown in Table 1. As a result, a greater enzymatic hydrolysis glucose yield (EHGY) was obtained at a high pH condition (i.e., zero acid charge at pH 4.5) than those obtained at low pH values (Figure 3). The measurement uncertainty in EHGY was 3% based on triplicate runs. In other words, the significantly low loss of glucan through pretreatment is more than sufficient to compensate the slightly low ECCS obtained at pH 4.5 (zero acid charge). The greater EHGY translates to a greater process enzymatic cellulose conversion (PECC) at high pH conditions, as PECC is directly calculated from EHGY (Table 1). This is very significant from the practical point of view. A high pH (~4) pretreatment can significantly reduce pretreatment reactor corrosion problem and even use less expensive materials for the reactor. Reactor corrosion is a major problem of the dilute acid process. It also eliminates the need of neutralization of the substrate prior to enzymatic saccharification. Both of these advantages can significantly reduce the cost of ethanol production and the barriers for commercialization. Figure 3 also indicates that the final EHGY from the SPORL substrates are equivalent to that obtained from the organosolv substrate. Furthermore, there is a wide pH range (operating window of acid charge) in which SPORL can produce satisfactory EHGY. The transition point below which EHGY decreases sharply is pH 2.

Similar results were obtained for maple under similar pretreatment and enzymatic hydrolysis conditions. The results of maple are not reported here.

Short-rotation eucalyptus

We applied SPORL to pretreat a short-rotation fast-growing eucalyptus (hardwood). Two SPORL pretreatments were carried out using sodium bisulfite only and bisulfite with the addition of sulfuric acid. The sodium bisulfite charge on wood was 6%, higher than the 4% used for aspen. This is because eucalyptus has a much higher lignin content of 32% (Table 4) than aspen of 23% (Table 2). The high lignin content may pose more barriers to cellulose conversion (recalcitrance). Increased bisulfite charge on wood can overcome lignocellulose recalcitrance, as reported in our previous study using softwood.[8] For comparison, eucalyptus chips were also pretreated with hot water and dilute acid processes. The acid charge was 1.84% on od wood for dilute acid and SPORL pretreatment. The initial pH values of the pretreatment liquors were about 4.5 for both hot water (we used RO water, pH was as measured) and bisulfite only SPORL and 2.1 for both dilute acid and SPORL with acid. The pretreatments were carried out at 180°C for all four experiments.

Table 4. Weights of Major Wood (Eucalyptus) Components and Percentage Loss After Different Pretreatments at 180°C for 30 min

Pretreatment	Klason. Lignin (g) (% loss)	Acid Soluble Lignin (g) (% loss)	Glucan (g) (% loss)	Xylan (g) (% loss)	Sum (g)	Total Solid Yield (g)
Untreated sample	32.4	3.4	39.7	11.4	86.9	100
SPORL (pH = 2.1)	24.2 (25.1)	1.5 (55.9)	36.0 (9.4)	0.4 (96.6)	62.1	64.2
Dilute Acid (pH = 2.1)	32.2 (0.5)	1.6 (52.9)	34.9 (13.6)	0.3 (95.8)	69.0	70.3
SPORL (pH = 4.5)	21.8 (32.7)	1.2 (64.7)	38.9 (2.1)	2.6 (77.5)	64.5	66.1
Hot water (pH = 4.5)	31.4 (3.1)	1.7 (50.0)	38.7 (2.7)	2.9 (74.0)	74.7	77.0

Sodium bisulfite charge on wood = 6% for SPORL runs. Sulfuric acid charge on wood = 1.84% for the two runs with pH = 2.1.

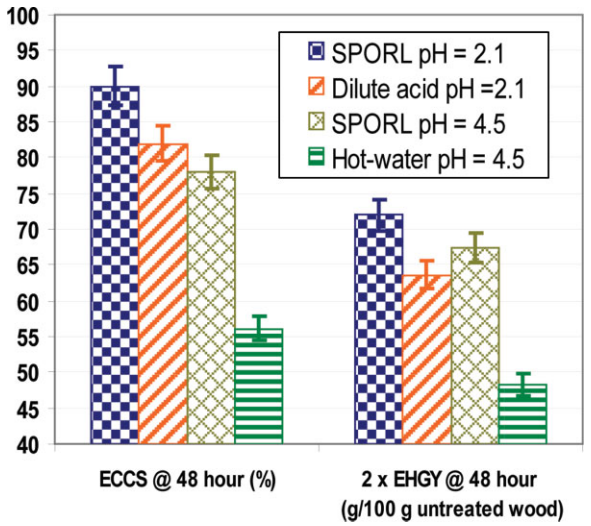

Figure 4. Comparisons of enzymatic cellulose conversion of substrates (ECCS) and enzymatic hydrolysis glucose yield (EHGY) among SPORL, dilute acid, and hot water pretreatment of short rotation eucalyptus through 48 h hydrolysis.

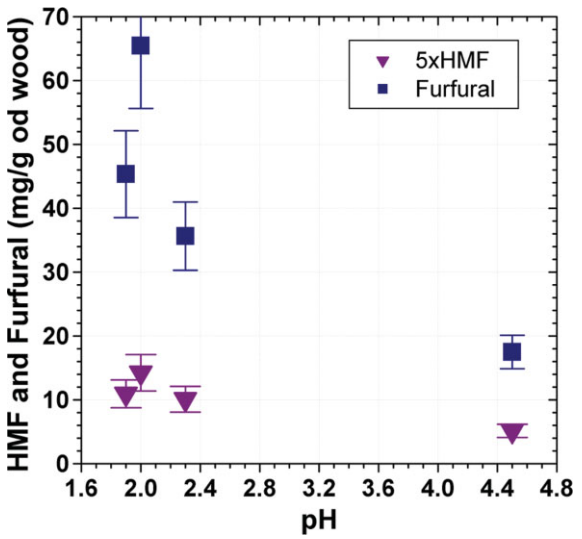

Figure 5. Effect of pH on the production of HMF and furfural from SPORL pretreatment of aspen at 180°C.

Table 4 shows the comparisons of glucan, xylan, and lignin removal among SPORL (pH 2.1 and 4.5), dilute acid (pH 2.1), and hot water (pH 4.5) pretreatments. Results indicate that about 10% of the glucan was removed with acid addition (dilute acid and SPORL with acid, pH 2.1), whereas glucan removal was negligible (<3%) without acid (hot water and bisulfite only SPORL, pH 4.5). More than 95% of the xylan was removed for pretreatments with acid addition (pH 2.1), whereas only about 75% was removed without acid addition (pH 4.5). About 25–35% of the Klason lignin was removed with bisulfite (two SPORL pretreatments) and almost none without (dilute acid and hot water).

Figure 4 shows the comparisons of enzymatic cellulose conversion of substrates (ECCS) and enzymatic hydrolysis glucose yields (EHGY) (wt % od wood). Results indicate that although dilute acid pretreatment can achieve ECCS of 82% of the theoretical value, the EHGY was lower compared with that from SPORL with or without acid. This is because of the combination of the relatively higher glucan removal of 13% and lower ECCS of 82% of the dilute acid pretreatment (pH 2.1), compared with 9% glucan removal and 90% ECCS for SPORL with acid pretreatment at the same pH. The SPORL without acid addition (pH 4.5) also yielded more glucose than did the dilute acid process due to the higher amount of glucan retained (>97%) in pretreatment compared with that for the dilute acid process (87%), even though the dilute acid process had a higher ECCS of 82% compared with 78% for the SPORL without acid. Hot water pretreatment resulted in very low ECCS of 56% and low EHGY. Results in Table 4 and Figure 4 indicate that SPORL is effective to pretreat short-rotation, fast-growing eucalyptus. Previous studies on hot water autohydrolysis of Eucalyptus[10] and mixed hardwoods[11] at 150 and 160°C, respectively, showed acetic acid formation through deacetylation. The results obtained at the SPORL run without acid addition (pH = 4.5) confirmed our hypothesis that acid addition is not necessary in applying SPORL to pretreating hardwoods because of the formation of acetic acid through deacetylation of the acetyl groups in hardwoods during pretreatment. Furthermore SPORL can be carried out at moderate pH of around 4, which avoids reactor corrosion and neutralization of the substrate prior to enzymatic hydrolysis.

Evaluation of the production of fermentation inhibitory species during SPORL

Under acid pretreatment conditions, hydroxymethyl furfural (HMF) will be produced from hexosans and furfural will be produced from pentosans. Both HMF and furfural are known to inhibit the normal fermentation process. In our previous study,[8] we found that sulfite reduced the formation of HMF and furfural significantly in SPORL pretreatment of softwood. Figure 5 shows the effect of pH (acid addition from 2.76% to 0% on wood) on the production of HMF and furfural in SPORL pretreatment of aspen at 180°C with sodium bisulfite charge of 4% on wood. Because xylan is the dominate hemicellulose of hardwoods, HMF production should be low due to limited amount of hexosans, as shown

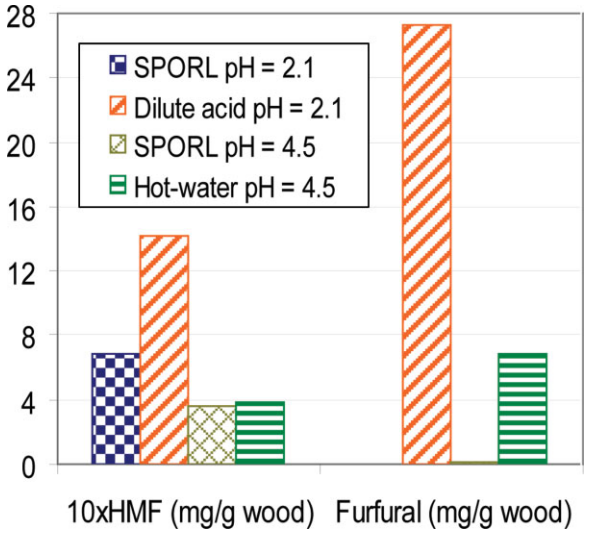

Figure 6. Comparisons of the production of HMF and furfural among SPORL, dilute acid and hot water pretreatment of short rotation Eucalyptus at 180°C.

in Figure 5. Similar to what occurs in dilute acid pretreatment, furfural production decreases significantly as pH increases in SPORL. Furfural production is less than 20 mg/g of od wood at pH 4.5, suggesting that SPORL pretreatment without acid addition not only avoided the problem of equipment corrosion and eliminated the need for neutralization for enzymatic hydrolysis, but also produced a favorable stream for fermentation.

Figure 6 compares the production of HMF and furfural during SPORL, dilute acid, and hot water pretreatments of eucalyptus. Again HMF productions are very low for all four pretreatments carried out due to the limited amount of hexosans available in the pretreatment liquor. Furfural production is almost zero for the two SPORL pretreatments (the number are too low to be seen from Figure 6), while both dilute acid and hot water pretreatments produced a fair amount of furfural even at the same pH value as their respective SPORL pretreatment.

Future studies will include pretreatment process optimization, mass balance, hemicellulose sugar recovery, and fermentation.

Conclusions

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) was very effective for direct pretreatment of hardwood chips for robust bioconversion. Under the conditions used in this study (i.e., sulfuric acid charge between 0 and 1.8% on od wood or corresponding to pH 4.5 to 2.0, sodium bisulfite charge 4% on wood, 30 min reaction time at 180°C), near complete removal of hemicellulose can be easily achieved. More than 90% of cellulose conversion to glucose for aspen substrate was achieved after 10-h enzymatic hydrolysis with enzyme loading of about 20 FPU cellulase plus 30 CBU β-glucosidase per gram of cellulose. The production of fermentation inhibitors (i.e., HMF and furfural) during SPORL pretreatment decreased significantly with the decreased acid charge. Effective SPORL pretreatment can be achieved even without acid addition. At zero acid addition, or pH about 4.5, SPORL produced a highest enzymatic hydrolysis glucose yield of about 43 (wt % od wood) from aspen (glucan content 45%). At pH 4.5, SPORL is expected to avoid reactor corrosion problem, eliminate the need for substrate neutralization prior to enzymatic hydrolysis, and produce low levels of fermentation inhibitors. Similar results were obtained from eucalyptus. On the basis of the mature equipment and technologies (such as sulfite pulping process, disk refining, and chemical recovery technologies), especially the low-capital-cost fluidized bed systems for chemical recovery,[25,26] which have long been practiced in the pulp and paper industry; SPORL can be a viable pretreatment process for commercial production of cellulosic ethanol with very low environmental and technological barriers and risks. Furthermore, high value utilization of ligninsulfonate and hemicellullose sugars in sulfite spent liquor has been in commercial practice for over half a century. SPORL offers a potentially excellent co-product pathway for biomass conversion in terms of industry infrastructure and commercial markets.

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