



## Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine

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### ABSTRACT

This study established a novel process using sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust and efficient bioconversion of softwoods. The process consists of sulfite treatment of wood chips under acidic conditions followed by mechanical size reduction using disk refining. The results indicated that after the SPORL pretreatment of spruce chips with 8–10% bisulfite and 1.8–3.7% sulfuric acid on oven dry (od) wood at 180 °C for 30 min, more than 90% cellulose conversion of substrate was achieved with enzyme loading of about 14.6 FPU cellulase plus 22.5 CBU  $\beta$ -glucosidase per gram of od substrate after 48 h hydrolysis. Glucose yield from enzymatic hydrolysis of the substrate per 100 g of untreated od spruce wood (glucan content 43%) was about 37 g (excluding the dissolved glucose during pretreatment). Hemicellulose removal was found to be as critical as lignin sulfonation for cellulose conversion in the SPORL process. Pretreatment altered the wood chips, which reduced electric energy consumption for size reduction to about 19 Wh/kg od untreated wood, or about 19 g glucose/Wh electricity. Furthermore, the SPORL produced low amounts of fermentation inhibitors, hydroxymethyl furfural (HMF) and furfural, of about 5 and 1 mg/g of untreated od wood, respectively. In addition, similar results were achieved when the SPORL was applied to red pine. By building on the mature sulfite pulping and disk refining technologies already practiced in the pulp and paper industry, the SPORL has very few technological barriers and risks for commercialization.

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### 1. Introduction

Cell walls of lignocellulosic biomass have natural resistance—often called “recalcitrance”—to microbial and enzymatic deconstruction. The recalcitrance of lignocellulose is a major barrier to the economical development of biobased fuels and products (Himmel et al., 2007). Processes and platforms that can efficiently and economically convert woody biomass, especially softwood, to fuel ethanol are still lacking. Existing alkaline, dilute acid, hot water/steam (explosion), ammonia, and organosolv pretreatment technologies (Mosier et al., 2005; Pan et al., 2005a) have achieved some level of success. However, some critical issues associated with woody biomass bioconversion remained unresolved: (1) Most existing pretreatment processes have low cellulose conversion of softwood, except for organosolv process. Softwood is the major species in several parts of the United States (such as inner Pacific Northwest and Southeast), in Canada and New Zealand, and in

Scandinavian countries. (2) These processes also require energy-intensive size reduction from wood chips to particles of millimeters or less (fiber or powder) prior to or together with chemical pretreatment to achieve satisfactory cellulose conversion efficiencies. Typical size reduction energy consumptions for fiberization wood chips are about 200–600 Wh electricity/kg oven dry (od) wood (Schell and Harwood, 1994). (3) Slow rate of enzymatic hydrolysis due to limited removal of feedstock recalcitrance affects process efficiency (Philippidis and Smith, 1995) and therefore the economics of cellulosic ethanol production (Hinman et al., 1992). (4) Most of the existing pretreatment technologies have poor scalability due to lack of commercially proven capital equipment.

This study presents a novel pretreatment process—sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL)—for robust and efficient conversion of biomass through enzymatic saccharification. The schematic flow diagram of the SPORL process is shown in Fig. 1. To demonstrate the SPORL concept, softwood wood chips were pretreated in an aqueous sulfite solution followed by mechanical size reduction using disk refining. The subprocesses connected with dashed lines in Fig. 1 were not carried out in the present study. The terms sulfite and bisulfite are used interchangeably in the SPORL because the active reagents

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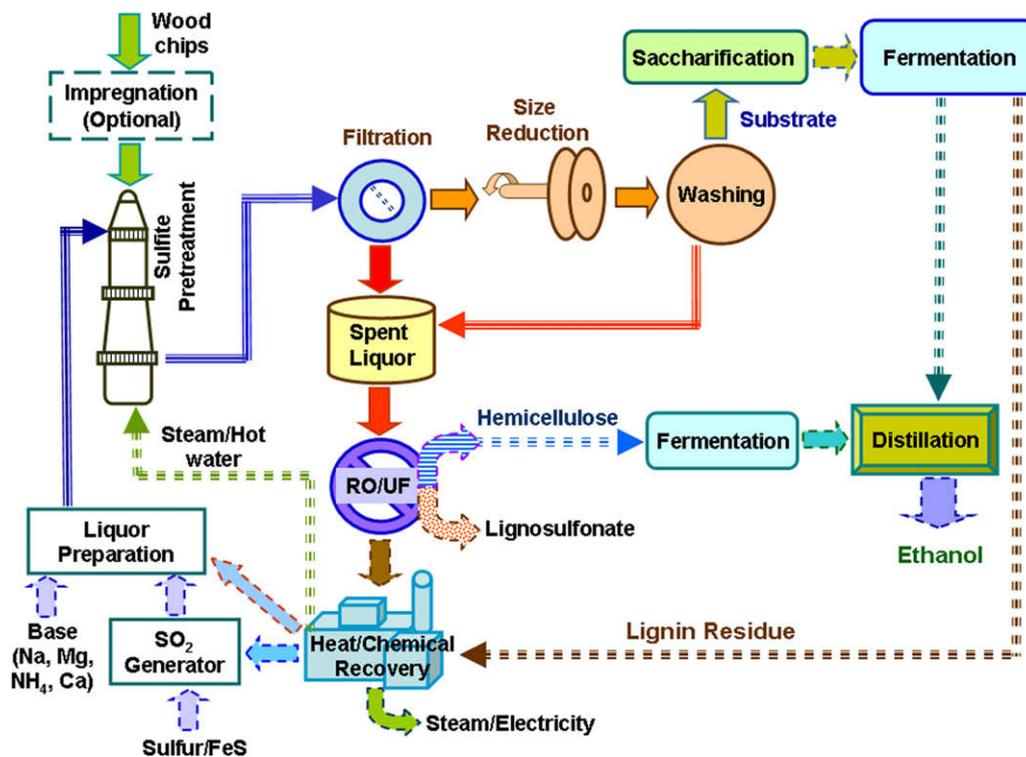


Fig. 1. Schematic process flow diagram of the SPORL.

in the pretreatment liquor can be sulfite ( $\text{SO}_3^{2-}$ ), bisulfite  $\text{HSO}_3^-$ , or a combination of two of the three reagents sulfite ( $\text{SO}_3^{2-}$ ), bisulfite ( $\text{SO}_3^{2-}$ ), and sulfur dioxide ( $\text{SO}_2$ , or  $\text{H}_2\text{SO}_3$ ), depending on the pH of the pretreatment liquor at a pretreatment temperature (Ingruber, 1985). The pretreatment liquor can be prepared and recovered using existing industrial practices as described elsewhere (Bryce, 1980). The pH of the solution can be easily controlled by the amount of  $\text{SO}_2$  absorbed.  $\text{SO}_2$  can be substituted by other acids, such as sulfuric acid, hydrochloric acid, oxalic acid, and acetic acid (such as the acetic acid released from acetyl groups during pretreatment of hardwood or agricultural residues).

Traditional sulfite pulping has been in industry practice for more than a century, but only a few sulfite pulp mills are in production today. It has been replaced by kraft pulping for robust pulping of various wood species to produce strong fibers for papermaking. Sulfite pulping can operate in a wide range of pH and temperature, as well described in textbooks (Bryce, 1980). The goal of pulping is to remove as much lignin as possible without concurrent loss and degradation of hemicellulose and cellulose, leading to a pulp with high yield and strength. Sulfite process has also been used for pretreating wood chips for the production of chemithermomechanical pulp (CTMP), semi-chemical pulp (SCP), or neutral sulfite semi-chemical (NSSC) pulp prior to or during mechanical refining, as described in numerous textbooks (Kurdin, 1980; Marteny, 1980).

The development of the SPORL process is based on the following fundamental understandings of sulfite pulping and considerations: (1) a considerable amount of hemicellulose degradation and removal takes place during sulfite pulping, as evidenced by the predominant xylose content in pulping spent liquor (Janson and Sjostrom, 1964); (2) the degrees of polymerization of xylan (Meier, 1962a,b; Pfister and Sjostrom, 1977; Sundman, 1950) and cellulose (Heuser, 1950) are reduced; (3) sulfonation of lignin increases the hydrophilicity of lignin, which may promote the aqueous enzyme process; (4) the degrees of dissolution of hemicellulose, degrada-

tion of cellulose, and sulfonation and condensation of lignin are increased as reaction time and temperature increases, and pH decreases (Bryce, 1980; Hall and Stockman, 1958). We hypothesize that a certain degree of lignin condensation using sulfite pretreatment can be tolerated without affecting satisfactory enzymatic cellulose conversion as long as the pretreatment significantly degrades cellulose and removes hemicellulose at temperatures significantly higher than those used for sulfite pulping. The objective of this study is to demonstrate the process and performance of the SPORL as a pretreatment for bioconversion of softwoods (spruce and red pine) through enzymatic saccharification.

The pulp and paper industry has over 100 years of experience in resource management, raw materials handling, wood pulping, and mill operation logistics with capacity of 1000–2000 od wood ton/day, equivalent to that of a future biomass refining plant of capacity 30–60 million gallons/year. It has accumulated a wealth of knowledge and highly skilled human capital in lignocellulose chemistry, engineering design, and operation. By making full use of existing infrastructure, capital, processes, resource, and knowledge base of the pulp and paper industry, the SPORL has low technological and environmental barriers for commercialization. For example, the existing wood pulping digester can be used for carrying out pretreatment, mechanical disk refiner for reducing the size of pretreated wood chips, and low capital cost fluidized bed type pulping spent liquor recovery systems (Arvold et al., 1968; Miles, 1970) for recovering energy (heat and steam) and chemicals from spent pretreatment liquor and enzymatic hydrolysis residues (lignin).

## 2. Experimental

### 2.1. Materials

Fresh spruce and red pine chips were generously provided by the Wisconsin Rapids mill of Stora Enso North America (now

New Page Corporation, Miamisburg, Ohio). The purities of the wood chips were not provided. However, the chemical composition of the two wood chip samples are very close to those of pure spruce and red pine, respectively, obtained in our laboratory previously (Zhu et al., 2007). The chips were frozen at  $-16^{\circ}\text{C}$  until used. The wood chips were screened to remove all chips greater than 38 mm and less than 6 mm in length. The thickness of the accepted chips ranged from 2 to 6 mm. Commercial enzymes, Celluclast 1.5 L (cellulase) and Novozyme 188 ( $\beta$ -glucosidase), were used as received from Sigma–Aldrich (St. Louis, Missouri). Sodium and magnesium bisulfite and sulfuric and hydrochloric acids were used as received from Sigma–Aldrich (St. Louis, Missouri).

## 2.2. Pretreatment chemical solution preparation

The pretreatment liquor was prepared by mixing either sulfuric or hydrochloric acid with sulfite or bisulfite in this study. The pretreatment solution for the SPORL can be prepared in several ways. The standard industry practices for preparing sulfite pulping liquor using sulfur and counter-ion oxide (Bryce, 1980) can be readily adapted and applied to the SPORL.

## 2.3. Sulfite pretreatment

The SPORL experiments were conducted according to the process flow diagram shown in Fig. 1. Two lab-scale pulping digesters were used with batch capacity of oven dry wood chip of 130 and 2000 g, respectively. In the case of the 2000 g batch runs, the wood chips and pretreatment solution were placed into a 23 l stainless steel, steam-jacketed rotating pressure vessel (unknown manufacturer). The contents were heated via the external steam jacket and rotated at a speed of 2 rpm to provide the mixing. In the case of the 130 g batch runs, the wood chips and pretreatment solutions were placed in sealed stainless steel 1 L pressure vessels (manufactured in-house). These 1 L vessels were mounted inside of the larger pressure vessel described above and heated externally via steam while rotating at the speed of 2 rpm. Wood chips were directly subjected to pretreatment using sodium bisulfite with or without sulfuric acid prior to size reduction described below. The ratio of pretreatment liquor to wood chip (od) was 5 (v/w). The bisulfite charge on od wood chips varied from 0 to 12% (w/w), and sulfuric acid charge on od wood varied from 0 to 7.36% (w/w), which resulted in pH values of the pretreatment solution varying from 1.7 to 4.5. Sodium sulfite instead of sodium bisulfite was used in experiments simulating neutral and alkaline pretreatment for mechanical and semichemical pulping. Sodium sulfite charge on od wood was 10.9% (w/w) with acid charges of 0.92% and 0% (w/w), corresponding to pH values of 7.6 and 10.2, respectively. The wood chips were first impregnated with the pretreatment liquor at  $90^{\circ}\text{C}$ . Impregnation time varied from 0 to 3 h. Immediately after impregnation, the temperature was raised to  $180^{\circ}\text{C}$  in about 30 min and maintained for an additional 30 min. The steam heating capacity of the two laboratory digesters employed limited the temperature of the SPORL experiments to  $180^{\circ}\text{C}$ . All pretreatments were carried out at  $180^{\circ}\text{C}$  for 30 min in this study. At the end of the pretreatment, the solid was collected and directly transferred to disk refiners for size reduction without washing. Solid loss was determined from the measured wet weight and moisture content of the collected solid. The pretreatment spent liquor (hydrolysate) was collected for analysis and future fermentation study.

## 2.4. Mechanical size reduction

A laboratory 8 in. disk refiner and a 12 in. disk refiner (Andritz Sprout–Bauer Pressurized Refiner, Springfield, Ohio) were used to defiberize the pretreated wood chips to produce substrates. The

pretreated wood chips collected from the 1 L digester were refined using an 8 in. disk refiner. For the experiments conducted using the 23 l digester, the pretreated wood chips were fed into the 12 in. disk refiner for size reduction. Mechanical disk milling was carried out under atmospheric conditions with disk gap of 0.25 mm. The electrical energy consumption for size reduction was recorded. The materials collected from size reduction were filtered on a Buchner funnel to separate solid (substrate) and liquid. The solids material from filtration was stored for characterization and enzymatic hydrolysis.

## 2.5. Enzymatic hydrolysis

Enzymatic hydrolyses of the substrates were carried out at substrate consistency of 2% (w/v) in 50 mL sodium acetate buffer (pH 4.8) at  $50^{\circ}\text{C}$  using a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, Massachusetts) at 200 rpm. A mixture of Celluclast 1.5 l with an activity loading of 14.6 FPU/g substrate and Novozyme 188 with an activity loading of 22.5 CBU/g substrate (equivalent to about 20 FPU and 30 CBU/g cellulose) was used for enzymatic hydrolysis. Excessive Novozyme 188 was used to prevent cellobiose accumulation (Emmel et al., 2003). Hydrolysates were sampled periodically for glucose analysis. Each data point was the average of duplicate experiments. For clarity, error bars calculated based on the relative difference of duplicate experiments are not shown in time-dependent cellulose conversion plots in this paper.

## 2.6. Analytical methods

Cellulase activity of Celluclast 1.5 l was determined by the filter paper method (Wood and Bhat, 1988). Whatmann No. 1 filter paper was used as a standard substrate. Duplicate calibration experiments showed cellulase activity of 56.4 FPU/g. Manufacturer-specified activity of 250 CBU/g of Novozyme 188 was directly used to calculate loading.

Carbohydrate compositions of the original and pretreated wood samples were measured by the Analytical Chemistry and Microscopy Laboratory (USDA Forest Products Laboratory) using an improved high-performance anion exchange chromatography (ICS-3000, Dionex, Sunnyvale, California) with pulsed amperometric detection (HPAEC-PAD) method (Davis, 1998). The solid materials were milled using a Wiley mill (Thomas Scientific, Swedesboro, New Jersey) before acid hydrolysis (acid-insoluble lignin procedure). The supernatant of the acid hydrolysate was directly used for ion chromatographic (IC) analysis. The measurement relative standard deviations (RSD) were reported based on internal regular quality assurance (QA) and quality control (QC). The monomeric sugar concentrations in the pretreatment spent liquor (hydrolysate) were also measured by the same HPAEC-PAD technique. The liquor was first centrifuged to remove solid particles. The supernatant was injected into IC column after dilution. The average of triplicate measurements was used to determine the hemicellulose sugar recovery from the pretreatment.

Hydroxymethylfurfural (HMF) and furfural in the pretreatment spent liquor (hydrolysate) were measured by HPLC (HP 1090 Series II, Hewlett–Packard, Now Agilent Technologies, Palo Alto, California) with UV detection at 280 nm using external standards. A reverse phase column (C18, Grace Vydac, Deerfield, Illinois) was used for separation. Results were obtained from the average of duplicate measurements. The standard deviations of selected samples reported were calculated from four sets of duplicate measurements.

The enzymatic hydrolytic reactions of pretreated substrates were monitored by measuring the time-dependent glucose concentrations in the hydrolysates. For fast analysis, glucose in

hydrolysates was measured using a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, Ohio). The instrument precision is about 2% based on manufacturer specifications. The average of duplicate runs was used in reporting.

### 3. Results and discussion

#### 3.1. Effect of impregnation time on enzymatic hydrolysis of spruce substrate

Wood chip impregnation allows the pretreatment chemicals to uniformly penetrate the wood chips prior to reactions at the final pretreatment temperature. Impregnation is very important to acid sulfite and bisulfite chemical pulping to get uniform delignification. It is also important for dilute acid and steam explosion pretreatments for biorefining. Typical impregnation is conducted by soaking the wood chips in the pretreatment solution below the pretreatment or pulping temperature for several minutes to several hours. In acid sulfite pulping, impregnating the wood chips requires 3–6 h. Such a long impregnation time costs productivity and energy but is necessary in sulfite pulping.

In this study, spruce wood chips were soaked in a digester with the pretreatment solution (liquor) at 90 °C, with an impregnation time varied from 0 to 180 min. After this, the digester temperature was raised to 180 °C. The results indicate that impregnation time has no effect on the digestibility of substrates obtained from the SPORL process with acid charge 1.84% and sodium bisulfite charge of 9%, 6%, and 3% (Fig. 2). However, when the sodium bisulfite charge was reduced to 0 at the same sulfuric acid charge of 1.84%, then the process became dilute acid pretreatment, impregnation showed some effect on substrate digestibility. For example, impregnation increased cellulose conversion by 4.5% after 48 h of enzymatic hydrolysis. Further examination of the chemical composition of the substrates indicates that impregnation time has no effects on the removal of major components from the wood chips during pretreatment. However, in the case of dilute acid pretreatment without sulfite addition, 30 min of impregnation removed over 11% more glucan than 0 min of impregnation (Table 1). This difference of 11% was obtained without replicated experiments, but it is significantly higher than the glucan measurement uncertainty of about 3% based on the seven SPORL experiments with sodium bisulfite charge of 3%, 6%, and 9% (Table 1). The standard deviations of enzymatic cellulose conversion and glucose yield shown in Table 1 were used as experimental uncertainties (error bars) shown in Figs. 3 and 4.

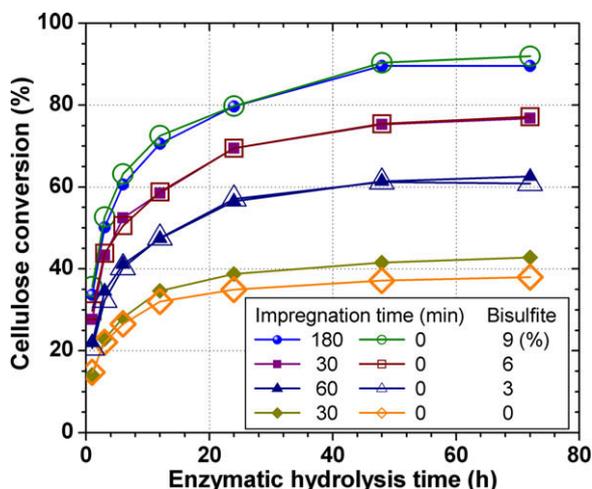


Fig. 2. Effect of wood chip impregnation time on time-dependent cellulose conversion of spruce at sulfuric acid charge of 1.84% on od wood.

Table 1

Effect of wood chip impregnation time on glucan yield, enzymatic hydrolysis conversion of cellulose and glucose yield after 48 h at 180 °C at four different bisulfite charges and sulfuric acid charge of 1.84% on od wood.

Bisulfite charge on od wood (%)	Impregnation time (min)	Enzymatic cellulose conversion at 48 h (%) <sup>a</sup>	Glucan yield from pretreatment per 100 g od wood	Enzymatic glucose yield (% wt od wood)
0	30	41.52	33.1	15.27
	0	37.12	36.9	15.22
	Mean/STD	39.32/3.11	35.00/2.69	15.24/0.04
3	60	61.21	38.4	26.12
	0	60.84	37.8	25.55
	Mean/STD	61.03/0.26	38.10/0.42	25.83/0.40
6	30	75.27	36.1	30.19
	0	75.41	37.4	31.34
	Mean/STD	75.34/0.1	36.75/0.9	30.77/0.8
9	180	89.54	35.6	35.42
	60	90.32	34.7	34.82
	0	94.02	33.3	34.79
	Mean/STD	91.29/2.39	34.53/1.16	35.01/0.35

<sup>a</sup> Based on cellulose content in pretreated substrate (glucan yield).

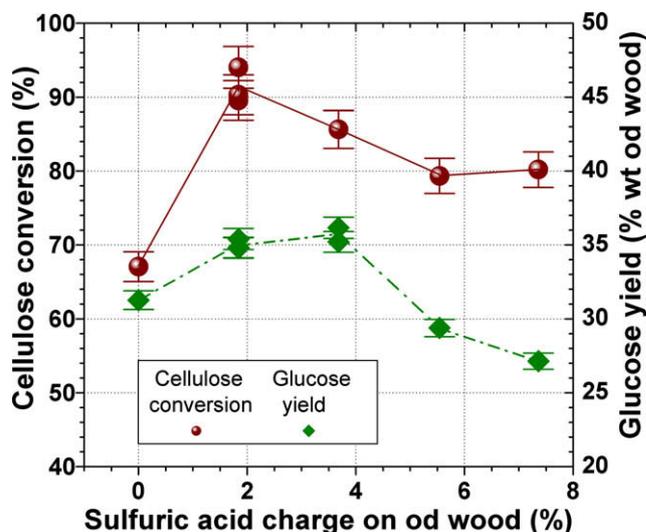
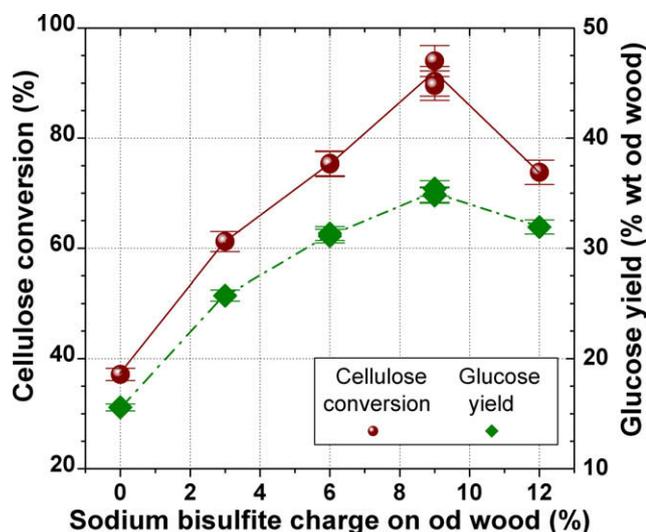


Fig. 3. Effect of sulfuric acid charge on cellulose conversion and glucose yield through 48 h enzymatic hydrolysis of spruce. Pretreatment conditions: 180 °C for 30 min with sodium bisulfite charge of 9% on od wood.

These results indicate that impregnation is not necessary for the SPORL, which will simplify the process and improve productivity. It is different from traditional sulfite pulping (acid sulfite and bisulfite) in which wood chip impregnation is critically important to avoid lignin condensation for satisfactory delignification. The results also indicate that the SPORL is very different from dilute acid pretreatment in enhancing digestibility. With about the same level of hemicellulose removal at the same acid charge and temperature, the acid pretreatment was substantially inefficient with cellulose conversion less than 40% after 48 h of enzymatic hydrolysis, whereas with the addition of only 3% sodium bisulfite charge on wood, cellulose conversion of the SPORL increased to 60%; cellulose conversion was even greater with increased bisulfite charge (Fig. 2).

#### 3.2. Effect of acid charge (pH) on enzymatic hydrolysis

The chemical compositions of spruce wood and substrates from six SPORL experiments at 180 °C for 30 min are listed in Table 2.



**Fig. 4.** Effect of sodium bisulfite charge on cellulose conversion and glucose yield through 48 h enzymatic hydrolysis of spruce. Pretreatment conditions: 180 °C for 30 min with sulfuric acid charge of 3.68% on od wood.

The first four samples were obtained with sodium bisulfite charge of 9% on od wood at different sulfuric acid charges (pH values). The last two samples were prepared using sodium sulfite at charge of 10.9% on od wood at higher pH values, simulating neutral and alkaline sulfite pretreatments for producing mechanical and semi-chemical pulps. The results indicate that hemicellulose, namely xylan and mannan, can be completely removed with acid charge on wood 3.68% or greater (pH approximately 2.2). The near complete separation of hemicellulose from cellulose is favorable not only to enzymatic hydrolysis of cellulose but also to biological conversion of hemicellulose due to lack of efficient technologies to co-ferment xylose and glucose. For the run with acid charge of 3.68% (pH approximately 2.2), most of the separated hemicellulose sugars can be recovered as monomeric sugars, as will be discussed later. Furthermore, approximately 12% glucan was dissolved in pretreatment liquor by acidic hydrolysis through pretreatment with about 20% delignification. Glucan dissolution was increased to 30% as acid charge increased to 7.36% (pH <2). The two pretreatments at higher pH values (using sodium sulfite) produced very low glucan and hemicellulose losses (dissolution), with solid yield about 90%, suggesting no significant carbohydrate hydrolysis and hemicellulose were found to be not favorable to enzymatic hydrolysis.

The effect of sulfuric acid charge (pH) on od wood on enzymatic conversion of cellulose and glucose yield after 48 h enzymatic hydrolysis is shown in Fig. 3. At a given sodium bisulfite charge

of 9% on od wood, the optimal acid charge was about 2.5%, at which both enzymatic cellulose conversion and glucose yield were maximized. The maximum enzymatic conversion of cellulose and glucose yield were about 90% and 37% wt od wood, respectively, which translates to an overall glucose yield of 78% from the enzymatic hydrolysis alone (excluding dissolved glucose during pretreatment) based on spruce wood cellulose content of 43.16% (Table 2). The two high-pH pretreatments resulted in very low cellulose conversion of less than 30% and glucose yield of less than 15% wt od wood (data not shown in Fig. 3 for clarity), implying that neutral and alkaline sulfite pretreatments are not effective for biomass conversion through enzymatic saccharification because the barriers of hemicellulose are not removed.

Pretreatment conducted at acid charge of 7.36% removed only 4.4% of Klason lignin (acid-insoluble lignin) (pH 1.72, c.f. Table 2). The enzymatic cellulose conversion of the resulting substrate was 80% (Fig. 3), which is lower than the 85% and 89% achieved at lower acid charges of 3.68% (pH 2.16) and 1.84% (pH 2.42), respectively. Lignin removal was greater than 15% for these two low acid charge runs (Table 2). Furthermore, the glucose yield at acid charge of 7.36% was only 27% wt od wood, lower than the 35% wt od wood obtained from the two runs with low acid charges of 3.68 and 1.84%. High dosage of acid at high temperature promoted lignin condensation rather than sulfonation. The condensed lignin might have a greater negative impact on enzymes through hydrophobic interference. The high lignin removal achieved at lower acid charges is likely attributable to enhanced lignin sulfonation. However, further reduction of acid charge to 0% in pretreatment did not produce additional improvement in enzymatic digestibility of substrate. Cellulose conversion and glucose yield were only about 69% and 31% wt od wood, respectively. This was because of limited hemicellulose removal at 0% acid charge. For example, only 55% xylan and 82% mannan were dissolved at 0% acid, respectively, compared with 93% and 98% at 3.68% acid charge. This indicates that hemicellulose removal is as critical as lignin sulfonation and removal to achieve satisfactory cellulose conversion. A temperature higher than 180 °C is probably needed to achieve satisfactory hemicellulose removal at acid charge 0% (pH 4.4), which needs further investigation. Limited pre-hydrolysis of cellulose at 0% acid was another reason for reduced enzymatic digestibility (resulting in lower glucose yield). These analyses support our hypothesis that achieving satisfactory substrate cellulose conversion requires partial degradation of cellulose, removal of hemicellulose, and prevention of lignin from excessive condensation.

### 3.3. Effect of bisulfite charge on enzymatic hydrolysis

The effect of bisulfite charge on enzymatic digestibility of the SPORL substrates is shown in Fig. 4. At the pretreatment temperature of 180 °C and sulfuric acid charge of 1.84%, there was an opti-

**Table 2**

Weights of wood components and percentage loss after the SPORL at different sulfuric acid charges at 180 °C for 30 min with 9% sodium bisulfite charge (on od untreated wood) for the first four runs and 10.9% sodium sulfite charge for the last two runs.

Acid charge on od wood (%)	Initial liquor (pH)	Component weight (g)									
		Klason lignin	Acid soluble lignin	Arab	Galac	Rham	Glucan	Xylan	Mannan	Sum (g)	Total (g)
Untreated sample		27.6	0.71	1.35	2.65	0.11	43.16	5.72	11.46	92.76	100
7.36	1.72	26.38	0.64	0	0	0	30.46	0.11	0.10	57.68	58.40
3.68	2.16	21.94	0.56	0	0	0	38.01	0.40	0.20	61.11	61.81
1.84	2.44	19.16	0.79	0	0	0	35.58	2.08	1.05	58.66	60.00
0	4.42	18.88	0.65	0	0.07	0	41.96	2.61	2.09	66.26	68.00
0.92	7.61	22.60	1.71	0.47	1.40	0	41.73	4.85	10.68	83.44	90.40
0	10.24	21.90	2.20	0.63	1.30	0	42.50	5.36	10.42	84.31	89.50
RSTD (%)	–	0.80	5.80	1.60	2.90	15.90	1.00	1.40	1.40	–	–

mal bisulfite charge about 9%. At this bisulfite charge, substrate enzymatic cellulose conversion and glucose yield were over 90% and 35% wt od wood through 48 h hydrolysis, respectively. On the other hand, when the bisulfite charge was reduced to 0 (sulfuric acid only), the enzymatic cellulose conversion and glucose yield were the lowest, less than 40% and 15.5% wt od wood, respectively, indicating that the enzymatic digestibility of a SPORL substrate is much better than that of a dilute acid-pretreated substrate. The addition of bisulfite dissolved a small portion of lignin. The sulfonation by sulfite makes lignin more hydrophilic, which could reduce the hydrophobic interactions with enzymes. The partial delignification and sulfonation of lignin may contribute to the significantly improved digestibility of the SPORL substrate over a dilute acid-pretreated one. Further increasing bisulfite charge to over 9%, however, resulted in decreased enzymatic cellulose conversion (Fig. 4). This is because the increase in bisulfite application increased the pH of the pretreatment liquor at a given acid charge, which may depress the dissolution of hemicellulose, depolymerization of cellulose, and even the rate of lignin sulfonation. It should be pointed out that a bisulfite charge of 9% used in the SPORL pretreatment is much lower than that for traditional sulfite chemical pulping (typically 20%). This is another difference of SPORL from traditional sulfite chemical pulping.

#### 3.4. Monomer sugar recovery from pretreatment spent liquor (hydrolysate)

Preliminary mass balance of the SPORL process at 180 °C with sodium bisulfite and sulfuric acid charges on od wood of 9% and 3.68%, respectively, is shown in Fig. 5. For 1 kg of untreated spruce wood, total yield of monomeric sugars in the pretreatment spent hydrolysate was 261 g, which includes 8, 25, 48, and 112 g, respectively, of monomeric arabinose, galactose, xylose, and mannose and an additional 68 g of glucose mainly from the dissolved glucan by acidic hydrolysis and glucose residues in hemicelluloses during the SPORL pretreatment. These results correspond to overall sugar recovery (including 372 g glucose from enzymatic hydrolysis) of the major saccharides (arabinose, galactose, xylose, mannose, and glucose) of about 56%, 86%, 76%, 88%, and 93%, respectively, based on original untreated spruce wood. The lignosulfonate content is estimated from the balance of lignin in the substrate.

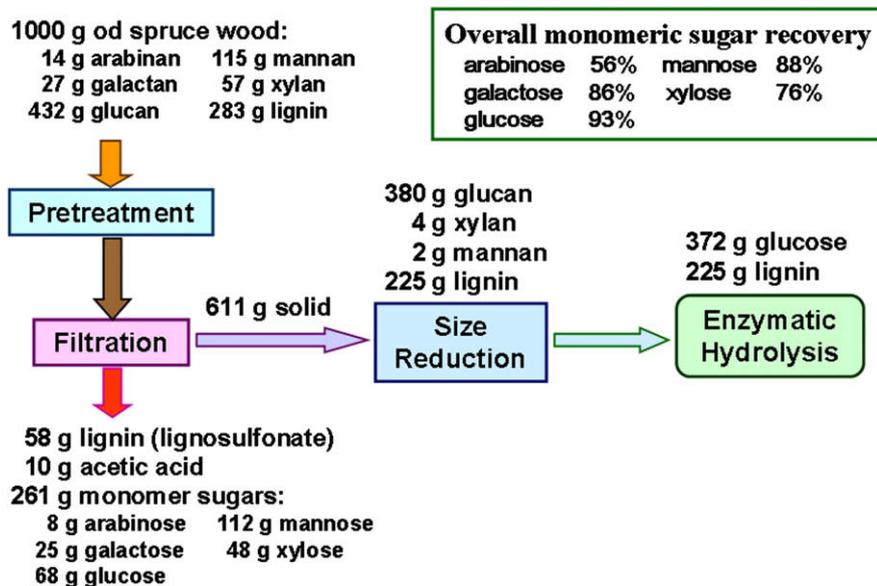
**Table 3**

Effect of chemical pretreatment on mechanical size reduction.

Sulfuric acid charge on od wood (%)	3.68 (pH 2.16)
Sodium bisulfite charge on od wood (%)	9
Electric energy used in mechanical refining (Wh/kg od chips)	19
Arithmetic mean length (mm)	0.320
Arithmetic mean width (mm)	0.0306
EH glucose yield (% wt od spruce wood)	37.2
EH glucose yield on unit electric energy consumed (g/Wh)	19.1

#### 3.5. Energy consumption in size reduction

The pretreatment experiments using a 231 digester with 2000 g wood chip capacity allowed us to conduct disk refining on the 12 in. disk refiner, which can record electric energy consumption required for size reduction. The results are summarized in Table 3. The specific mechanical refining energies have been evaluated in numerous mechanical pulping studies in our laboratory in the past decade and were found to be very close to those in pilot- and commercial-scale mechanical pulping. The measurement error with duplicates was within 2%. Therefore, the refining energy consumption reported in this study can be applied to future commercial production. With electric energy input of only 19 Wh/kg od untreated spruce wood, the pretreated wood chips with sulfuric acid and bisulfite charges of 3.68% (pH 2.16) and 9%, respectively, can be easily pulverized with a mean fiber length around 0.3 mm. The refining energy consumption of 19 Wh/kg was significantly lower than those of typical mechanical, thermomechanical, chemthermomechanical, and semichemical pulping (Kurdin, 1980; Marteny, 1980), indicating that the SPORL pretreatment is substantially effective at softening the wood chips and saving energy in size reduction. The softening of wood chips is owing to dissolution of hemicellulose, partial delignification, and lignin sulfonation. Using the data in Table 3 and glucose yield in Fig. 4, the calculated enzymatic hydrolysis (EH) glucose yield on unit electric energy consumption using the methodology described in our previous study (Zhu et al., 2008) was 19.6 g glucose/Wh.



**Fig. 5.** Preliminary mass balance of the SPORL process at 180 °C for 30 min with sodium bisulfite and sulfuric acid charge of 9% and 3.68%, respectively, on od spruce.

### 3.6. Evaluation of the production of fermentation inhibitory species during SPORL

Under acidic conditions at high temperature, hydroxymethyl furfural (HMF) and furfural will be generated from hexosans and pentosans, respectively (Larsson et al., 1999). Both HMF and furfural are species that inhibit the normal fermentation process. The total amounts of HMF and Furfural formation were determined by multiplying the HPLC-measured HMF and furfural concentrations in the pretreatment spent liquor and the liquor volume (including the moisture in the original wood chips). The HMF and furfural formations from 1 g of original od wood chips were reported in Table 4. HMF and furfural decreased with increasing bisulfite. An explanation is that at the same acid charge (1.8%), more bisulfite resulted in a higher pH, which depressed the decomposition of the sugars to HMF and furfural. The variation in pH of the pretreatment liquor resulted in the difference in the combined severity factors (Chum et al., 1990) when the pretreatment temperature and duration time were the same for all the runs listed. The amounts of HMF and furfural formation were only 7 and 3 mg/g of untreated od wood, respectively, at bisulfite and acid charges of 9% and 1.84% (combined severity factor of about 1.4) when glucose yield was maximized (Figs. 3 and 4). The productions of HMF and furfural are significantly lower than those found using dilute acid pretreatments. The amounts of HMF and furfural formation were about 50 and 25 mg/g untreated od wood during acid catalyzed steam pretreatment of spruce, respectively, when glucose yields were maximized at a combined severity factor between 3.0 and 3.4 (Larsson et al., 1999). The lower production of HMF and furfural is another advantage of the SPORL, which is favorable to the fermentation of cellulose and hemicellulose sugars in the pretreatment spent liquor.

### 3.7. Application of the SPORL to red pine

Spruce is known as an ideal softwood species for sulfite or bisulfite pulping in terms of producing strong pulp for papermaking. However, sulfite or bisulfite pulping generally does not work well with pines. Similarly, most pretreatment processes of lignocellulose for producing ethanol do not work well with softwood species. For example, the SO<sub>2</sub>-catalyzed steam explosion process performed poorly when applied to some softwood species such as Douglas-fir (Pan et al., 2005b). To demonstrate the robustness and versatility of the SPORL, we applied the SPORL to red pine, one of the dominant pine species in the northern regions of North America. Magnesium bisulfite was used to pretreat red pine at 180 °C for

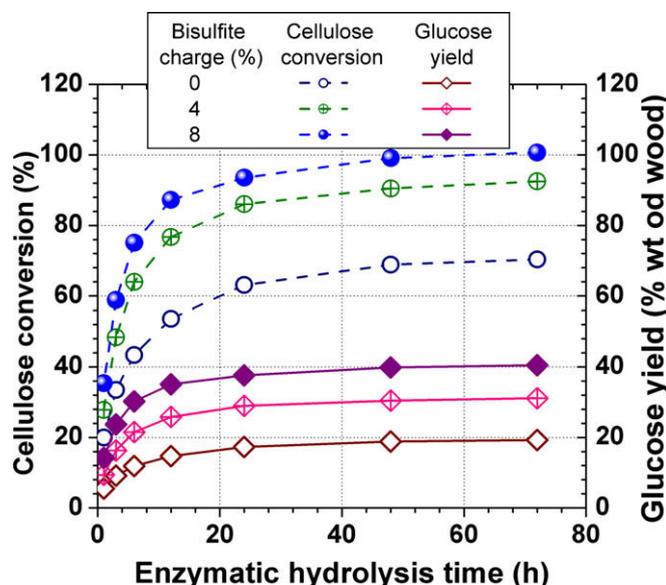


Fig. 6. Effect of magnesium bisulfite charge on time-dependent cellulose conversion and enzymatic hydrolysis glucose yield of red pine. Pretreatment conditions: 180 °C for 30 min with at sulfuric acid charge of 3.68% on od wood.

30 min. Meanwhile, the study also demonstrated that bisulfite with different counter-ions can be used in the SPORL as in sulfite pulping (sodium, magnesium, ammonium were found to be effective on red pine in this study). The sulfuric acid charge on od wood was 3.68%, while the magnesium bisulfite charge on wood was varied from 4% to 8%. The time-dependent cellulose conversion and glucose yield of red pine are shown in Fig. 6. Near 100% cellulose conversion was achieved after 48 h enzymatic hydrolysis with enzyme loadings of 14.6 FPU and 22.5 CBU per gram of substrate obtained from bisulfite charge of 8%, resulting in glucose yield of 40% wt od red pine. These results suggest the effectiveness of the SPORL for bioconversion of red pine.

### 4. Conclusions

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) solved the problem of poor digestibility of softwoods (e.g., spruce and red pine) in enzymatic saccharification. The SPORL was effective even when it was applied to directly pretreat wood chips without chip impregnation. The SPORL pretreatment under the conditions of bisulfite charge about 8–10% and sulfuric acid charge 1.8–3.7% on untreated od wood at 180 °C achieved near complete hemicellulose separation, partial delignification, and lignin sulfonation, which are favorable to subsequent mechanical size reduction and enzymatic hydrolysis. The SPORL-treated softwood chips were significantly softened and therefore readily pulverized mechanically with electric energy consumption of only about 19 Wh/kg od wood. Enzymatic cellulose conversion over 90% and enzymatic hydrolysis glucose yield of approximately 37% wt od wood, respectively, were achieved after 48 h enzymatic hydrolysis with enzyme loadings of about 14.6 FPU cellulose and 22.5 CBU β-glycosidase per gram of od substrate (or about 9.5 FPU and 14.6 CBU per gram of untreated wood, or about 20 FPU and 30 CBU per gram of cellulose). The production of fermentation inhibitors HMF and furfural in the SPORL is significantly lower than that in dilute acid, which is favorable to the fermentation of pretreatment dissolved sugars from cellulose and hemicellulose. Excellent performance of the SPORL with red pine indicates that the process may be tree species independent.

Table 4  
Effect of sodium bisulfite charge on the formation of HMF and furfural during the SPORL at T = 180 °C for t = 30 min.

Sulfuric acid charge on od wood (%)	Bisulfite charge on od wood (%)	Initial liquor pH	Combined severity factor <sup>a</sup>	HMF(mg/g od wood)	Furfural(mg/g od wood)
Spruce					
1.84	0	2.09	1.74	9.64	16.68
	6	2.25	1.58	5.55	7.51
	9	2.42	1.41	3.27 ± 1.2	6.5 ± 3.0
	12	2.54	1.29	0.08	0
3.68	0	1.90	1.93	12.1	28.55
	3	1.95	1.88	14.89 ± 3.5	30.73 ± 7.7
	6	2.04	1.79	6.86	16.55
	9	2.16	1.67	4.81	7.55
Red pine					
3.68	6	2.06	1.77	5.21	7.33
	9	2.21	1.62	3.43	1.7
	12	2.27	1.56	2.46	2.9

<sup>a</sup> Combined severity factor =  $\log[t \cdot \exp(\frac{T-100}{14.75})] - \text{pH}$  (Chum et al., 1990).

In addition, a great advantage of the SPORL is that it can directly use or adapt the existing mature equipment, technology, and infrastructure that have long been used in the pulp and paper industry, such as disk refining technology and chemical recovery, especially the low capital cost fluidized bed systems (Arvold et al., 1968; Miles, 1970). The SPORL can be a viable process for commercial biorefining of lignocellulose with very low technological barriers and risks.

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### References

- Arvold, W.V., Ray, R.K., Hanway Jr., J.E., 1968. Fluidized bed treatment of magnesia-base spent sulfite liquor. In: *Proceedings of The Recovery Symposium*, Helsinki, 429–442.
- Bryce, J.R.G., 1980. Sulfite pulping. In: Casey, J.P. (Ed.), *Pulp and Paper: Chemistry and Chemical Technology*, third ed. John Wiley & Sons, New York, pp. 291–376.
- Chum, H.L., Johnson, D.K., Black, S.K., Overend, R.P., 1990. Pretreatment-catalyst effects of the combined severity parameter. *Applied Biochemistry and Biotechnology* 24/25, 1–14.
- Davis, M.W., 1998. A rapid modified method for compositional carbohydrate analysis of lignocellulosics by high pH anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD). *Journal of Wood Chemistry and Technology* 18, 235–252.
- Emmel, A., Mathias, A.L., Wypych, F., Ramos, L.P., 2003. Fractionation of *Eucalyptus grandis* chips by dilute acid-catalysed steam explosion. *Bioresource Technology* 86, 105–115.
- Hall, L., Stockman, L., 1958. Sulfitkokning vid olika aciditet. *Svensk Papperstidning* 61, 871–880.
- Heuser, E., 1950. Trends in fundamental research in the cellulose and wood pulp field. *TAPPI* 33, 118–124.
- Himmel, M.E., Ding, S.Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D., 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science* 315, 804–807.
- Hinman, N.D., Schell, D.J., Riley, C.J., Bergeron, P.W., Walter, P.J., 1992. Preliminary estimate of the cost of ethanol-production for SSF technology. *Applied Biochemistry and Biotechnology* 34–35, 639–649.
- Ingruber, O., 1985. Sulfite science part I: sulfite pulping cooking liquor and the four bases. In: Ingruber, O., Kocurek, M., Wong, A. (Eds.), *Sulfite Science and Technology*. The Joint Textbook Committee of the Paper Industry, third ed. TAPPI/CPPA, Atlanta, pp. 3–23.
- Janson, J., Sjoström, E., 1964. Behaviour of xylan during sulphite cooking of birchwood. *Svensk Papperstidning* 67, 764–771.
- Kurdir, J.A., 1980. Refiner mechanical and thermomechanical pulping. In: Casey, J.P. (Ed.), *Pulp and Paper: Chemistry and Chemical Technology*, third ed. John Wiley & Sons, New York, pp. 197–252.
- Larsson, S., Palmqvist, E., Hahn-Hagerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., Nilvebrant, N.-O., 1999. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme and Microbial Technology* 24, 151–159.
- Marteny, W.W., 1980. Semichemical pulping: the neutral sulfite semichemical or NSSC process. In: Casey, J. (Ed.), *Pulp and Paper: Chemistry and Chemical Technology*, third ed. John Wiley & Sons, New York, pp. 252–291.
- Meier, H., 1962a. On the behavior of wood hemicelluloses under different pulping conditions, Part I Birch hemicellulose. *Svensk Papperstidning* 65, 299–305.
- Meier, H., 1962b. On the behavior of wood hemicelluloses under different pulping conditions, Part II Spruce hemicellulose. *Svensk Papperstidning* 65, 589–594.
- Miles, H., 1970. Spent liquor disposal via fluo-solids combustion at an NSSC mill. *Paper Trade Journal* 154, 26–31.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* 96, 673–686.
- Pan, X.J., Arato, C., Gilkes, N., Gregg, D., Mabee, W., Pye, K., Xiao, Z.Z., Zhang, X., Saddler, J., 2005a. Biorefining of softwoods using ethanol organosolv pulping: preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. *Biotechnology and Bioengineering* 90, 473–481.
- Pan, X.J., Xie, D., Gilkes, N., Gregg, D.J., Saddler, J.N., 2005b. Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. *Applied Biochemistry and Biotechnology* 121, 1069–1079.
- Pfister, K., Sjoström, E., 1977. The formation of monosaccharides and aldonic and uronic acids during sulphite cooking. *Paperi ja Puu* 59, 711–720.
- Philippidis, G.P., Smith, T.K., 1995. Limiting factors in the simultaneous saccharification and fermentation process for conversion of cellulosic biomass to fuel ethanol. *Applied Biochemistry and Biotechnology* 51–52, 117–124.
- Schell, D.J., Harwood, C., 1994. Milling of lignocellulosic biomass – results of pilot-scale testing. *Applied Biochemistry and Biotechnology* 45–6, 159–168.
- Sundman, J., 1950. Sockerutlösningen vid sulfitcellulosakoket. *Paperi ja Puu* 32, 267–274.
- Wood, T.M., Bhat, M., 1988. Methods for measuring cellulase activities. In: Colowick, S.P., Kaplan, N.O. (Eds.), *Methods in Enzymology*. Biomass (Part a, Cellulose and Hemicellulose), vol. 160. Academic Press, New York, pp. 87–112.
- Zhu, J.Y., Scott, C.T., Scallan, K.L., Myers, G.C., 2007. Effects of plantation density on wood density and anatomical properties of red pine (*Pinus resinosa* Ait.). *Wood and Fiber Science* 39, 502–512.
- Zhu, J.Y., Wang, G.S., Pan, X.J., Gleisner, R., 2008. Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. *Chem. Eng. Sci.*, doi:10.1016/j.ces.2008.09.026.