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# On energy consumption for size-reduction and yields from subsequent enzymatic saccharification of pretreated lodgepole pine<sup>☆</sup>

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## ABSTRACT

This study investigated the effects of chemical pretreatment and disk-milling conditions on energy consumption for size-reduction and the efficiency of enzymatic cellulose saccharification of a softwood. Lodgepole pine wood chips produced from thinnings of a 100-year-old unmanaged forest were pretreated by hot-water, dilute-acid, and two SPORL processes (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose) at acid charge on oven dry (od) wood of 0% and 2.21%. The pretreated wood chips were then milled using a laboratory disk mill under various solids-loadings and disk-plate gaps to produce substrates for enzymatic hydrolysis. We found that post-chemical-pretreatment size-reduction of forest biomass can decrease size-reduction energy consumption by 20–80% depending on the pretreatment applied under 20% solids-loading and a disk-plate gap of 0.76 mm in milling. SPORL with a sodium bisulfite charge of 8% and sulfuric acid charge of 2.21% on wood was the most effective in decreasing size-reduction energy consumption. Solids-loading had the most significant effect on disk-milling energy. When solids-loading was reduced from 30% to 3%, disk-milling energy could be decreased by more than a factor of 10 for wood chips pretreated by both SPORL and dilute-acid at an acid charge of 2.21%. The enzymatic hydrolysis glucose yields (EHGY) from the substrates produced by all pretreatments were independent of the solids-loading in milling, indicating that these energy savings in size-reduction can be realized without affecting EHGY. When wood chips were pretreated by SPORL with 2.21% acid charge, size-reduction energy consumption was decreased to less than 50 Wh/kg od wood at a practical solids-loading of approximately 10–20%, equivalent to that used in size-reduction of agriculture biomass, with excellent EHGY of about 370 g per kg od wood. Similar effects on size-reduction energy savings and excellent EHGY were also achieved when large disk-plate gaps (up to 1.52 mm studied) were applied in disk-milling of wood chips pretreated by SPORL with acid.

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## 1. Introduction

Forest biomass is an abundant and sustainable feedstock for producing biobased fuel and products. About 368 million dry tons of forest biomass can be sustainably produced each year in the United States alone (Perlack et al., 2005). This is about 30% of the total biomass that could be available annually in the United States. Furthermore, short-rotation intensive culture or tree farming can offer an almost unlimited opportunity for forest biomass production (Foody and Foody, 1991). Forest biomass is physically large and structurally very strong. It has a higher density, higher lignin and

<sup>☆</sup> This work was conducted on official government time by J.Y. Zhu and Gleisner while W. Zhu was a visiting student at the US Forest Service, Forest Products Laboratory. The work is in public domain in the US.

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cellulose, and lower hemicellulose content than most agricultural biomass. As a result, forest biomass has much greater recalcitrance to biochemical conversion than does agricultural biomass. On the other hand, the high density, high lignin, and cellulose content increase energy density and reduce transportation cost, which is favorable for advanced bioenergy production. Moreover, forest biomass can be harvested year round, which eliminates the need for long-term storage, a significant advantage over any agricultural biomass for the future biobase industry.

Physical size-reduction of forest biomass to increase enzyme-accessible surface areas is a necessary step to overcome recalcitrance in most pretreatment processes (Lynd, 1996; Zhu et al., 2009a, b). Unfortunately, the large physical size and strong structure of forest biomass make size-reduction very energy intensive. Thermal energy of about 1.8 MJ/od kg wood is needed in steam explosion (215 °C, accounting for low-quality steam recovery) to break wood chips into fibers or fiber bundles. Typical mechanical

energy consumption is between 0.72 and 2.16 MJ (200–600 Wh)/od kg wood (Schell and Harwood, 1994) in mechanical disk-milling of wood, which is about 10–30% of the ethanol energy from wood based on ethanol high heating value of 24 MJ/l and current conversion technology of 300 l/ton wood. This intensive energy consumption is a major concern not only to the economics but also to the overall energy balance of forest biomass conversion. A reduction in energy consumption for wood-size-reduction by a factor of 5–10 is required to about 0.18–0.36 MJ (or 50–100 Wh)/od kg wood, equivalent to that consumed in size-reduction of agricultural residue. Unfortunately, the issue of energy consumption in physical size-reduction has been largely overlooked in the biomass research community. The reason is likely in part because most research has been focused on agricultural residues that do not need a significant amount of mechanical energy to achieve satisfactory size-reduction. The reported work on size-reduction has only discussed energy consumption (Cadoche and Lopez, 1989; Holtzaple et al., 1989; Mani et al., 2004; Schell and Harwood, 1994) or simply carried out enzymatic hydrolysis using size-reduced wood substrates without providing any information about the energy consumed to produce the substrates (Allen et al., 2001; Nguyen et al., 2000). Recently, Wyman et al. (2009) reported a comparative study carried out by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI), through the sponsorship of US Departments of Agriculture and Energy, on sugar and ethanol yield from poplar wood from different pretreatment processes. The material used for pretreatment in their study was created from passing pulp through a ¼ inch screen. However, the work did not provide data on the energy consumed in wood size-reduction through chipping and milling before chemical pretreatments.

Four factors affect energy consumption during forest biomass size-reduction: the fiberization mechanism, chemical pretreatment (prior to size-reduction), milling process conditions, and the degree of size-reduction. All these factors also affect subsequent enzymatic cellulose saccharification of the resulting substrate. The effect of fiberization mechanism on wood-size-reduction energy consumption has been well studied in mechanical pulping (Salmen et al., 1999). Our previous study (Zhu et al., 2009a) indicated that although fiberization of wood in the lignin-rich middle lamella (ML) at a temperature above the glass transition temperature of lignin (Irvine, 1985) can save energy expended in size-reduction, the produced substrate had poor enzymatic digestibility because it was coated with lignin. It is well known in mechanical pulping that disk-refining (milling) process conditions can affect refining (milling) energy consumption (Tienvieri et al., 1999). However, the energy savings that can be realized without sacrificing pulp quality is limited. For example, only 7% in energy savings was realized by decreasing primary-stage disk-refining discharge consistency (the same as solids-loading used in this study), from 50% to 38% in commercial-scale trial runs (Alami et al., 1995). To maintain fiber quality, a pulp-discharge consistency below 24% was not studied, and energy savings were not expected based on the trend of the data with consistency above 24% (Alami et al., 1995). The effect of the degree of size-reduction on enzymatic hydrolysis has been widely studied (Chundawat et al., 2007; Dasari and Berson, 2007; Sangseethong et al., 1998). The degree of size-reduction was traditionally characterized by the sieving method in these studies. Recently, we used the specific surface of a substrate to correlate with enzymatic cellulose saccharification (Zhu et al., 2009a). All previous studies focused on maximizing or maintaining enzymatic cellulose saccharification efficiency, but none dealt with decreasing the energy consumption of size-reduction. Research presently lacks an integrated approach to comprehensively study the effect of chemical pretreatment, size-reduction process conditions, and the degree of size-reduction

on size-reduction energy consumption and enzymatic cellulose hydrolysis efficiency.

The objective of the present study is to fill this research gap on wood size-reduction for bioconversion. Specifically, we took the approach of conducting chemical pretreatment prior to wood size-reduction. We then studied the effects of chemical pretreatment and disk-milling conditions along with the degree of size-reduction on milling energy consumption and enzymatic cellulose saccharification efficiency. Our rationale for taking this approach was that chemical pretreatment can alter the chemical composition and physical structure of wood by partially removing some cell-wall components such as hemicellulose and lignin. A literature study indicates that significant energy savings of about 30% can be obtained in mechanical pulping when wood chips are pretreated by oxalate acid to partially remove wood hemicelluloses (Kenealy et al., 2007). Furthermore, direct chemical pretreatment of wood chips afford a low liquid-to-solid ratio (3) to reduce thermal energy consumption in pretreatment. In mechanical pulping of wood, the requirement for good fiber quality for papermaking limits opportunities to reduce energy through variations of fiberization conditions (Mihelich et al., 1972). The fiber qualities required for papermaking are not directly relevant to and are therefore no longer required for lignocellulose bioconversion. This provides the feasibility to optimize milling process conditions to reduce energy consumption for wood-size-reduction to about 50 Wh/kg od wood or less, equivalent to that consumed in agricultural biomass size-reduction, while maximizing the enzymatic conversion efficiency of cellulose to glucose above 90% under nominal chemical pretreatment conditions.

## 2. Experimental

### 2.1. Materials

Several lodgepole pine trees were harvested from the Pringle Falls Experimental Forest, Deschutes National Forest, Oregon. The trees were about 100 years old with a typical diameter of 12–20 cm at breast height. These trees were grown in suppressed conditions most of their life because of the lack of forest management. The logs were debarked and chipped at the US Forest Service, Forest Products Laboratory, Madison, Wisconsin. The wood chips were screened to remove all particles greater than 38 mm and less than 6 mm in length to ensure smooth operation in disk-milling. The thickness of the accepted chips ranged from 3 to 8 mm.

Commercial enzymes, Celluclast 1.5 L (cellulase) and Novozym 188 ( $\beta$ -glucosidase), were used as received from Sigma–Aldrich (St. Louis, MO). Sodium acetate, sulfuric acid, and sodium bisulfite were also used as received from Sigma–Aldrich.

### 2.2. Chemical pretreatment

Experiments were conducted according to the process-flow diagram shown in Fig. 1. The sub-processes illustrated with dashed lines, such as filtration water reuse, were not carried out in this study. One of the novelties of the present study was conducting chemical pretreatment of the feedstock in the form of wood chips before mechanical size-reduction, as opposed to using size-reduced (fiberized) materials in pretreatments as reported in the literature (Allen et al., 2001; Nguyen et al., 2000; Zhu et al., 2005; Wyman et al., 2009). A traditional 23-L stainless steel wood-pulping digester (unknown manufacturer) was used to chemically pretreat wood chips. The digester was heated by an external steam jacket and rotated at a speed of 2 rpm to provide mixing during experiments.

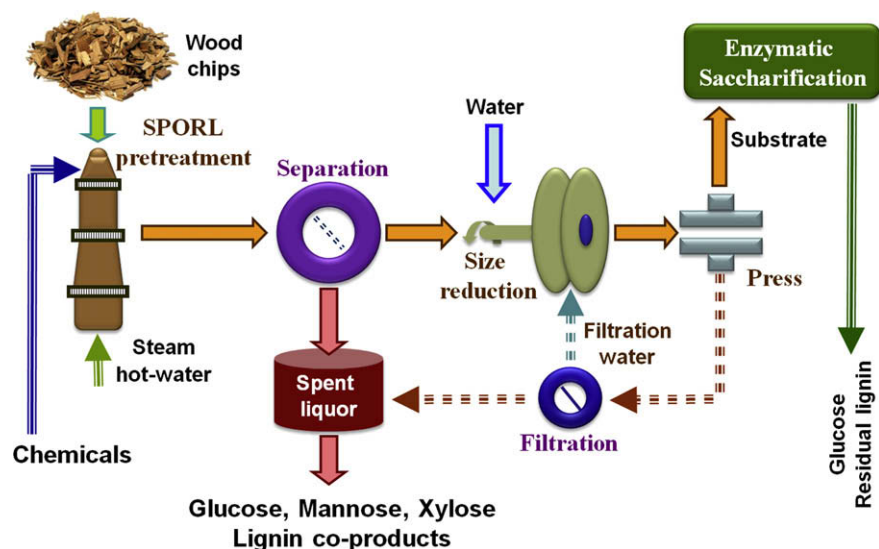


Fig. 1. Schematic process-flow diagram used in the present study.

Hot-water, dilute-acid, and two SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose) (Zhu et al., 2009b) pretreatments were conducted. About 2000 g of oven dry (od) lodgepole pine wood chips were directly subjected to each batch pretreatment using water or/and sulfuric acid or/and sodium bisulfite. The liquid to od wood ratio was 3 for all pretreatments. Sodium bisulfite of 8% on od wood was used in SPORL pretreatments without and with sulfuric acid for pH adjustment. Sulfuric acid was used in the dilute-acid pretreatment. The acid charges on od wood were 0% and 2.21%, which resulted in the initial pH of the SPORL pretreatment solutions of 4.2 and 1.9, respectively, and 1.1 in the dilute-acid pretreatment solution. During pretreatment, the temperature was raised to 180 °C in about 15 min and the temperature was maintained for 30 min.

These pretreatment conditions were chosen on the basis of our previous study using spruce wood (Zhu et al., 2009b), which achieved excellent sugar recovery 93%, 76%, and 88% of theoretical values for glucose, xylose, and mannose, respectively. The same volumetric sulfuric acid concentration in the pretreatment solution of 0.4% was also used while the liquid to wood ratio was reduced from 5 to 3, which resulted in an acid charge on wood of 2.21%, lower than 3.68% used in the spruce study (Zhu et al., 2009b). A low liquor to wood ratio reduces the thermal energy usage in pretreatment and increases sugar concentration in the pretreatment hydrolysate. Spruce and lodgepole pine are both softwoods with almost identical carbohydrate contents. The fact that good sugar recoveries were also achieved in this study, as will be discussed later, suggests that the selected pretreatment conditions for the low pH SPORL pretreatment were close to optimal. For comparison purposes, the SPORL high pH pretreatment using the same bisulfite charge of 8% without acid, and the dilute-acid pretreatment using the same acid charge of 2.21% along with a hot-water pretreatment, were all conducted at the same temperature of 180 °C for 30 min.

After the completion of the pretreatment, the pretreated feedstock remained intact as wood chips and was separated from pretreatment liquor using a simple screen device. The wood-chip solid yield was determined from the weight and moisture content of the collected wood chips. This wood-chip solid yield (Table 1) was used to convert the measured size-reduction energy consumption based on od pretreated wood chips to that on od untreated wood basis. The pretreatment spent liquor that contains mainly hemicellulose sugars was saved for future fermentation study.

### 2.3. Mechanical wood size-reduction

The collected wood chips were directly transferred to a disk mill for size-reduction (Fig. 1). Size-reduction was conducted in a laboratory 12-inch disk refiner (disk-plate pattern DB2-505, Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH) at atmospheric pressure. The disk rotating speed was 2,570 rpm. Disk-milling was carried out at varied disk gaps of 0.38–3.05 mm and solids-loadings of 3–50%. The milling solids-loading is defined as the percentage of biomass od weight as the total weight of the od biomass and the amount of water or moisture used in milling. To obtain a given solids-loading in milling, the desired water flow rate of a spray nozzle used for water addition was determined and calibrated based on the preset milling duration time (or wood-chip feeding rate) and wood-chip moisture content. To prevent potential fiber blockage, the disk mill was equipped with a separate water nozzle to wash down milled materials at the exit of disk mill chamber, which prevented the determination of milling solids-loading based on the consistency of the discharged stock. The amount of wood chips used in a batch-milling run varied from 0.15 to 1 kg. The wood-chip feeding rate was maintained at about 0.5 od kg/min for all the disk-millings conducted. The size-reduced solid was not separately washed and was directly dewatered through pressing using a canvas bag to a solids content of about 30%. The dewatering process served as a washing for subsequent enzymatic hydrolysis. Another yield of solid (substrate, Table 1) in the form of fibers or fiber bundles was then determined from the weight and moisture content of the collected substrate. This substrate yield was used to determine the losses of chemical components through pretreatment, and therefore to calculate the enzymatic cellulose saccharification of the substrate.

The electrical energy consumption of the disk refiner was recorded with a digital load monitor system (Ohio Semitronics, Inc., Hilliard, OH, model DLM-33-480-1PR). The detailed description of the system was described in a previous study (Zhu et al., 2009a). The energy consumption (watt hours) was re-set at the beginning of each run and recorded at the end of each run. These results were compared with spreadsheet-calculated energy consumption by integration of the recorded power over the processing period. Previous studies on mechanical pulping using the same disk refiner suggest that energy consumption is equivalent to those reported in commercial-scale mechanical pulp production. The milling energy consumed is divided by the total od mass of wood

chips fed to a bath run to give Wh/kg of fed chips. For pretreated wood chips, this milling energy consumption is multiplied by the wood-chip solid yield from pretreatment to obtain size-reduction energy consumption based on od untreated wood (Table 1), i.e., Wh/kg of untreated wood.

#### 2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted using commercial enzymes at 2% of substrate solid (w/v) in 50-mL sodium acetate buffer using a shaker/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 with an activity loading of approximately 15 FPU/g substrate and Novozyme 188 with an activity loading of approximately 22.5 CBU/g substrate was used for enzymatic hydrolysis. An excess of Novozyme 188 was used to prevent cellobiose accumulation (Emmel et al., 2003). Hydrolysates were sampled periodically for glucose analysis. Each data point was averaged from two replicates.

#### 2.5. Analytical methods

Cellulase activity of Celluclast 1.5 L was determined by the filter paper method (Wood and Bhat, 1988). Whatman No. 1 filter paper was used as a standard substrate. One unit (FPU) of enzyme activity is defined as the amount of reducing sugars equivalent to glucose in mM/min by 1 ml of the initial enzymatic solution. Cellobiase activity of Novozyme 188 was determined using cellobiose as substrate recommended by IUPAC Biotechnology Commission. One unit (CBU) of activity is defined as the enzyme amount, which converts 1 mol of cellobiose to 2 mol of glucose in 1 min (Wood and Bhat, 1988).

The chemical compositions of the original and pretreated biomass were measured by the Analytical and Microscopy Laboratory (US Forest Service, Forest Products Laboratory) using an improved high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) method (Davis, 1998). The reported data were the averages of duplicate measurements separated by one month apart. The same analytical method was also used to determine the concentrations of hemicellulose sugars in the pretreatment hydrolysates. For fast analysis, glucose in the enzymatic hydrolysate was measured using a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH).

#### 2.6. Substrate characterization

The morphologies of selected substrates were examined using a scanning electron microscope (SEM). Another set of selected substrates was characterized by a wet imaging technique using a Fiber Quality Analyzer (FQA) (HiRes FQA, OpTest Equipment, Hawkesbury, ON, Canada) to determine the length and diameter of each individual fiber/fiber bundle. The CCD camera of the FQA has a pixel resolution of 7  $\mu\text{m}$  for diameter/width and 14  $\mu\text{m}$  for length with 256 gray levels and zero cross talk. The specific surface of a substrate is defined as the total external surface divided by the total volume of the substrate. It is calculated using the measured pairs of length and diameter/width of each fiber/fiber bundle sampled by assuming a cylinder model for individual fiber/fiber bundle. The detailed descriptions of the wet imaging technique and its error analysis for substrate-specific surface characterization can be found in our previous study (Zhu et al., 2009a). To obtain statistically meaningful mean specific surface data, a fiber/fiber bundle count of 10,000 was used at which an asymptotic value of mean specific surface was obtained for the selected samples. Duplicate measurements were conducted. The averaged results were reported. The standard deviations were calculated as the measurement error. Because of

the limitations in the optical resolution of most commercial wet-imaging fiber analyzers (including the FQA used in this study) that were designed for characterizing papermaking fibers, accurate measurements of substrate-specific surface are difficult to obtain. Therefore, only substrates produced from the variations of disk-plate gap using wood chips pretreated by the high pH SPORL and hot-water were characterized since these substrates resemble papermaking fibers (as shown in the SEM images later) and good measurements of their length and diameter/width are possible.

### 3. Results and discussions

#### 3.1. Effect of disk-milling batch size on measured size-reduction energy consumption

A minimum amount of wood chips is required in a batch disk-milling to get a period of stable milling for consistent measurement of size-reduction energy. This is because the amount of wood chips loaded in the disk mill is always low in the beginning and ending periods, which affects the measurements of milling energy consumption in batch disk-milling. We carried out several batch disk-millings using different amounts of od wood chips from 0.15 to 1 kg. The wood chips used for these runs were pretreated by the SPORL at sulfuric acid charge of 2.21% (initial pH 1.9). Results indicate that the milling energy increases as batch size was increased (Fig. 2). The measured milling energy consumption approached an asymptotic value as the amount of wood chips in a batch run was increased over 0.5 kg.

It is understood that the batch size response curve shown in Fig. 2 may not be exactly the same for wood chips from different pretreatments and milled at different disk-plate gaps and solids-loadings. However, numerous experiments on mechanical pulping of wood chips from various chemical and biological treatments conducted at our Laboratory over the last decade suggested that a batch size of 0.5 kg can provide consistent milling energy consumption data. Therefore, a batch size of 0.5 kg was used in this study.

#### 3.2. Experimental repeatability and uncertainty

The overall repeatability of the experiments depends on the repeatability of several sub-processes (Fig. 1), i.e., pretreatment,

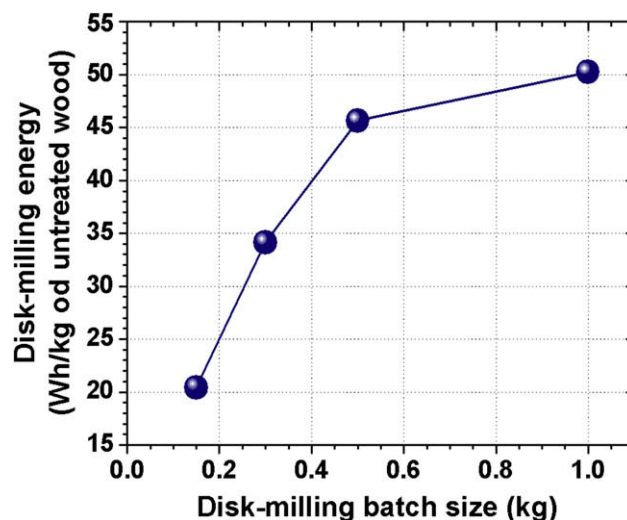


Fig. 2. Effect of the amount of wood chips used in batch disk-milling on measured size-reduction energy consumption. Wood chips were pretreated by low pH SPORL at acid charge of 2.21% (initial pH 1.9). Disk-milling solids-loading was 10% and disk-plate gap was 0.76 mm.

size-reduction, and enzymatic hydrolysis. Temperature is a key variable in pretreatment that can affect the repeatability of pretreatment, and therefore the overall experimental repeatability. Variations in steam pressure did cause some variations in pretreatment temperature from day to day. Triplicate disk-milling tests using SPORL (initial pH 1.9) pretreated wood chips from the same batch were carried out. We found that the standard deviation in disk-milling energy was only 4%, suggesting that the disk-milling experiments were repeatable. Our previous study (Zhu et al., 2009b) suggested that the enzymatic hydrolysis experiments have a 2% error from duplicate measurements. To demonstrate the overall repeatability of the experiments, two separate SPORL pretreatments (both at  $T = 180^\circ\text{C}$ , initial pH 4.2, with duration time of 30 min) were conducted. Separate disk-millings of the pretreated wood chips from each pretreatment batch were then carried out at different solids-loadings (3–30%). Enzymatic hydrolysis of each resultant substrate was also carried out separately. The maximum difference in enzymatic hydrolysis glucose yield (EHGY), defined as the wt.% of enzymatic glucose yield of untreated wood, between the two sets of data was 7.0% with the average difference of only 4.2%. The results of disk-milling energy and glucose yield on unit milling energy from the pretreated wood chips showed excellent repeatability of the experiments (Fig. 3). The data did show that the milling energy was consistently lower in milling Run II than that in milling Run I except at solids-loading of 3%. This could well be caused by the variations in pretreatment temperature for the two runs. These two experiments were conducted in two different days separated by about 3 weeks. To reduce the effect of steam pressure on pretreatment, the wood chips pretreated under the same designated conditions but from different batches were well mixed into one big batch before being used in size-reduction under various milling conditions (several batches were needed to produce enough wood chips for disk-milling runs at different solids-loadings and disk-plate gaps).

### 3.3. Effects of chemical pretreatments on size-reduction energy consumption and monosaccharide recovery

Chemical pretreatments alter the chemical composition and physical structure of wood cell walls and thereby affect size-reduction energy consumption. Lodgepole pine wood chips pretreated by hot-water, dilute-acid, and two SPORL processes were disk-milled at 20% solids-loading with a disk-plate gap of 0.76 mm. The results show that all of the four pretreatments reduced milling

energy in comparison to energy consumed in milling untreated wood chips under the same milling conditions (Table 1). However, the savings in size-reduction energy varies with the pretreatment method applied. When wood chips were pretreated with SPORL at sulfuric acid charge on od wood 2.21% (initial pH 1.9), the size-reduction energy consumption was reduced by 80%. However, when wood chips were pretreated with SPORL without acid (initial pH 4.2), size-reduction energy was reduced only by about 20%. Dilute-acid pretreatment can reduce size-reduction energy consumption by about 50%. Although the mechanism of energy savings in size-reduction is quite complex, it is intuitive that disk-milling energy is related to the changes in wood components during the chemical pretreatment. More specifically, the removal of hemicelluloses left pores in wood structure, which weakened the wood matrix and thereby reduced the energy consumption for size-reduction. This is why the wood chips treated using SPORL with acid and dilute-acid consumed less energy than those using SPORL without acid, as the former had less hemicelluloses remaining than the later (Table 2). Furthermore, it appears that removal and modification of lignin affected energy consumption as well.

As shown in Table 2, all lignin was kept in wood chips during dilute-acid pretreatment (including hot-water, a special case of acid pretreatment), while a small amount of lignin was removed during SPORL with acid (initial pH 1.9). This is one reason why the dilute-acid pretreated wood chips needed more energy for size-reduction than those pretreated by low pH SPORL with acid, although they had similar residual hemicelluloses content (Table 2). Lignin measurements from both the hot-water and dilute-acid pretreated substrates are higher than that in the original lodgepole pine wood. This type of measurement uncertainties is the limitation of current analytical methods. Similar problems were reported by the National Renewable Energy Laboratory (Dan Schell, National Renewable Energy Laboratory, Golden, CO, personal communication, 2009). Partial sulfonation of lignin during low pH SPORL with acid pretreatment is another reason for its low energy consumption for size-reduction. The sulfonation made lignin hydrophilic, which promoted swelling and softening of wood chips and consequently decreased energy consumption for size-reduction.

All pretreatments apparently improved the efficiency of enzymatic cellulose saccharification of the substrate (ECSS, defined as the percentage of glucan in substrate converted to glucose enzymatically). Both SPORL pretreatments were found to be very effective. ECSS for the low pH SPORL (2.21% acid charge) pretreated

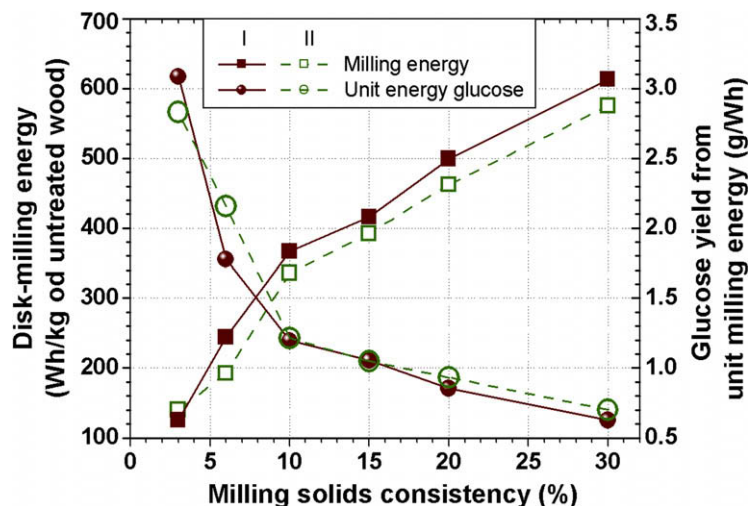


Fig. 3. Experimental repeatability of the effect of solids-loading on disk-milling energy and enzymatic hydrolysis glucose yield (EHGY) per unit size-reduction energy consumption. Wood chips were pretreated by high pH SPORL (initial pH 4.2) and disk-milling at a disk-plate gap of 0.76 mm and solids-loadings of 3–30%.

**Table 1**

Effect of chemical pretreatment on size-reduction energy consumption and enzymatic hydrolysis glucose yield. Milling solids-loading at 20% with disk-plate gap of 0.76 mm.

| Pretreatment @ 180 °C for 30 min <sup>a</sup> | Initial liquor pH | Pretreatment wood-chip yield (%) | Milling energy (Wh/kg od untreated wood) | Substrate yield from pretreatment (%) | ECSS <sup>b</sup> (%) | EHGY <sup>c</sup> (wt.% wood) |
|-----------------------------------------------|-------------------|----------------------------------|------------------------------------------|---------------------------------------|-----------------------|-------------------------------|
| None                                          |                   | 100.0                            | 615.9                                    | 100                                   | 11.3                  | 5.4                           |
| Hot-water                                     | 5.0               | 87.2                             | 537.0                                    | 74.4                                  | 33.1                  | 16.0                          |
| Acid                                          | 1.1               | 77.0                             | 335.6                                    | 71.4                                  | 39.6                  | 15.7                          |
| SPORL                                         | 4.2               | 86.1                             | 499.3                                    | 68.9                                  | 84.1                  | 43.1                          |
| SPORL                                         | 1.9               | 80.7                             | 134.5                                    | 66.7                                  | 92.2                  | 38.2                          |

<sup>a</sup> Sodium bisulfite charge was 8% on od wood for the two SPORL runs. Sulfuric acid charge was 2.21% (wt./wt.) on od wood for the dilute-acid and low pH SPORL runs, and 0 for the hot-water and high pH SPORL runs.

<sup>b</sup> wt.% of glucan in substrate converted to glucose after 48 h enzymatic hydrolysis.

<sup>c</sup> Enzymatic hydrolysis glucose yield after 48 h, in wt.% od untreated wood.

**Table 2**

Yields of key wood components (kg/ton od wood) in the solid substrate and pretreatment hydrolysate after different pretreatments at 180°C for 30 min under various chemical applications.

| K Lignin                                 | Arabinan          |                 | Galactan          |                        | Rhamnan   |       | Glucan |          | Xylan                     |                   | Mannan          |                   | G + X + M <sup>a</sup> |                    | Total                    |
|------------------------------------------|-------------------|-----------------|-------------------|------------------------|-----------|-------|--------|----------|---------------------------|-------------------|-----------------|-------------------|------------------------|--------------------|--------------------------|
| <i>1000 kg lodgepole pine wood chips</i> |                   |                 |                   |                        |           |       |        |          |                           |                   |                 |                   |                        |                    |                          |
| 270.1                                    | 15.6              |                 | 22.3              |                        | 7         |       | 425.5  |          | 69.3                      |                   | 109.9           |                   | 604.7                  |                    | 941.2                    |
| Pretreatment conditions                  |                   |                 |                   |                        | Substrate |       |        |          |                           | Hydrolysate       |                 |                   |                        |                    | Total yield <sup>e</sup> |
| Pretreatment                             | Initial liquor pH | Final liquor pH | Acid charge% wood | Bisulfite charge% wood | Glucan    | Xylan | Mannan | K Lignin | Solids total <sup>b</sup> | Glucose as glucan | Xylose as xylan | Mannose as mannan | Lignin <sup>c</sup>    | Yield <sup>d</sup> |                          |
| Hot-water                                | 5.0               | 3.3             | 0                 | 0                      | 410.8     | 27.4  | 22.1   | 283.0    | 730.4                     | 3.1               | 21.7            | 26.3              | N.D.                   | 51.1               | 781.5                    |
| Dilute-acid                              | 1.1               | 1.5             | 2.21              | 0                      | 355.8     | 1.1   | 0.5    | 334.3    | 627.5                     | 39.4              | N.D.            | 4.4               | N.D.                   | 43.8               | 671.3                    |
| SPORL                                    | 4.2               | 2.5             | 0                 | 8                      | 412.4     | 16.1  | 7.7    | 226.3    | 662.5                     | 11.2              | 23.5            | 42.6              | 43.8                   | 121.1              | 783.6                    |
| SPORL                                    | 1.9               | 1.5             | 2.21              | 8                      | 383.5     | 3.2   | 2.5    | 254.3    | 643.5                     | 40.1              | 31.8            | 67.7              | 15.8                   | 155.4              | 798.9                    |

<sup>a</sup> Glucan plus xylan and mannan content.

<sup>b</sup> Sum of listed components in solid substrate. The measured solid substrate yields after disk-milling are shown in Table 1.

<sup>c</sup> Lignin from overall mass balance of lignin.

<sup>d</sup> Sum of listed pretreatment hydrolysate components.

<sup>e</sup> Sum of substrate solids and hydrolysate yields listed.

**Table 3**

Monomeric sugar recoveries from different pretreatments and subsequent enzymatic hydrolyses.

| Pretreatment   | EHGY @ 48 h <sup>a</sup> | EHGY recovery <sup>b</sup> | Hydrolysate glucose recovery <sup>b</sup> | Hydrolysate xylose recovery <sup>b</sup> | Hydrolysate mannose recovery <sup>b</sup> | Total glucose + xylose + mannose recovery <sup>c</sup> | Total glucose + xylose + mannose recovery <sup>d</sup> |
|----------------|--------------------------|----------------------------|-------------------------------------------|------------------------------------------|-------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| Hot-water      | 16.0                     | 33.8                       | 0.7                                       | 31.3                                     | 24.0                                      | 21.7                                                   | 32.3                                                   |
| Dilute-acid    | 15.67                    | 33.1                       | 9.3                                       | N.D.                                     | 4.1                                       | 20.5                                                   | 30.6                                                   |
| SPORL pH = 4.2 | 43.06                    | 91.1                       | 2.6                                       | 33.9                                     | 38.8                                      | 51.7                                                   | 76.9                                                   |
| SPORL pH = 1.9 | 38.19                    | 80.8                       | 9.4                                       | 45.9                                     | 61.6                                      | 53.7                                                   | 79.9                                                   |

<sup>a</sup> EHGY stands for enzymatic hydrolysis glucose yield from substrate, wt.% of od wood.

<sup>b</sup> wt.% of corresponding theoretical wood sugar.

<sup>c</sup> wt.% of od wood.

<sup>d</sup> wt.% of theoretical total wood sugars based on glucan, xylan, and mannan content in wood of 60.47%.

sample was over 90% (based on data in Tables 2 and 3) after 48 h enzymatic hydrolysis. Enzymatic hydrolysis glucose yields (EHGY) were about 38, and 43 wt.% of wood for the low pH and high pH SPORL pretreatment (Table 3), respectively. The lower glucose yield for the low pH SPORL is due to the loss of glucan during pretreatment of about 10% based on carbohydrate analysis (Table 2). The glucan content in lodgepole pine wood is 42.6%, therefore, the EHGYs from the two SPORL pretreatments were excellent. The EHGY of the dilute-acid pretreatment was only about 16 wt.% of wood (Table 3), corresponding to an ECSS of about 40%. The performance of the hot-water pretreatment was about the same as that of the dilute-acid pretreatment in terms of enzymatic cellulose saccharification.

The recoveries of glucose, xylose, and mannose from pretreatment hydrolysates were determined from the measured concentrations of these sugars by HPLC. A preliminary mass balance can be conducted using the measured chemical compositions in the

substrates and sugar recoveries in the pretreatment hydrolysates shown in Table 2. For lodgepole pine, the major hemicelluloses contents are mannan of about 11% and xylan content of about 7%. Therefore, only these two hemicelluloses were included in the analyses in Tables 2 and 3. The lignin contents in the pretreatment hydrolysates were based on lignin mass balance. Total solids yield (Table 1) from pretreatment varied from 66.7% for the low pH SPORL to 74.4% for the hot-water pretreatment. Oligomeric sugars were not measured in the pretreatment hydrolysate, which resulted in a low mass yield in hydrolysates (Table 2). As listed in Table 3, excellent subsequent enzymatic glucose recoveries of 81% and 91% of theoretical wood glucose were achieved for the low and high pH SPORL pretreatments, respectively (data from substrates produced by disk-milling at 20% solids-loading and disk-plate gap of 0.76 mm). The mannose and xylose recovery from the low pH SPORL pretreatment hydrolysate were 62% and 46%, respectively. The recoveries certainly can be improved through

optimization. The hemicelluloses sugar recovery was low for the high pH SPORL pretreatment perhaps because of insufficient conversion of oligomers to monomeric sugars. Despite the lack of optimization, the overall monomeric sugar recoveries for the low pH and high pH SPORL pretreatments were 80% and 77% of theoretical wood sugars (Table 3), which is better than those reported in literature for softwoods, respectively; for example, 72% for steam explosion of spruce (Soderstrom et al., 2004) and 70% for organosolv of lodgepole pine (Pan et al., 2008). Both the hot-water and dilute-acid pretreatments showed unsatisfactory enzymatic glucose and total monomeric sugar recoveries for the conditions studied (Table 3).

#### 3.4. Effects of disk-milling conditions on size-reduction energy consumption

Alami et al. (1995) reported that disk-refining pulp-discharging consistency affects refining energy consumption and pulp properties for papermaking in thermomechanical pulping at elevated temperatures. All disk-millings were conducted under ambient conditions in this study. For the untreated wood chips, size-reduction energy consumption was over 800 Wh/kg od wood when the wood chips were directly milled at solids-loading of 50%. Adding water decreased solids-loading in milling and resulted in a reduction in milling energy consumption (Fig. 4). The size-reduction energy consumption was reduced by 34% when solids-loading was reduced to 10% from 50%. The decrease in size-reduction energy consumption was much more significant for chemically pretreated wood chips upon decreasing milling solids-loading. When solids-loading was decreased from 30% to 3%, the size-reduction energy consumption was decreased by about 80% for wood chips pretreated by the SPORL process at 0 acid charge (initial pH 4.2), and by more than 90% (or a factor of 10) for wood chips pretreated by the dilute-acid (initial pH 1.1) and SPORL (initial pH 1.9) pretreated wood chips were 4% based on the triplicate measurements conducted in the experimental repeatability tests.

Milling disk-plate gap is another milling process parameter that can be adjusted to decrease size-reduction energy consumption. Increasing disk-plate gap during milling from 0.38 mm to 2.54 mm reduced milling energy consumption by 80% and 90% for the wood chips pretreated by SPORL (initial pH 1.9) and by the dilute-acid (initial pH 1.1), respectively, as shown in Fig. 5.

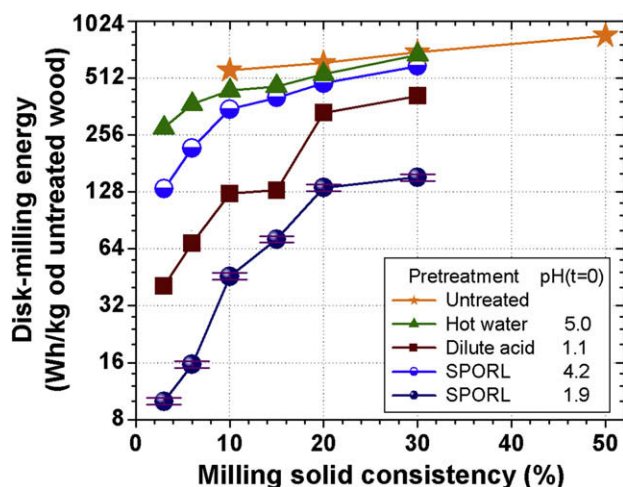


Fig. 4. Effects of solids-loading during disk-milling on size-reduction energy consumption. Disk-plate gap in milling was 0.76 mm.

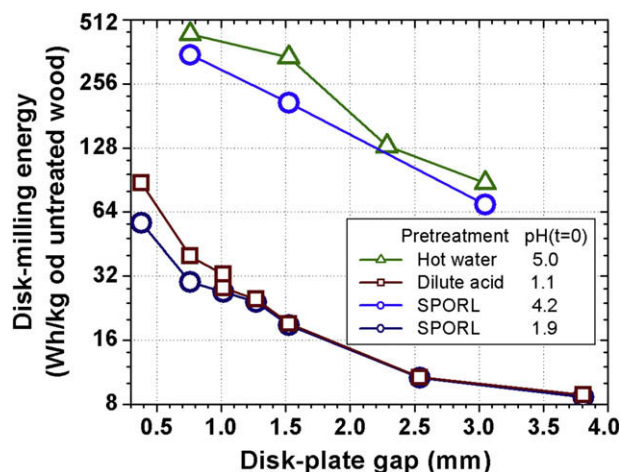


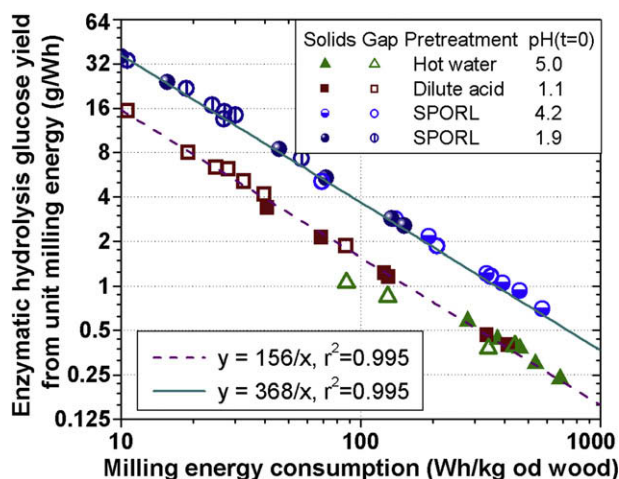
Fig. 5. Effects of disk-plate gap during disk-milling on size-reduction energy consumption. Solids-loading in milling was 10%.

Similarly, disk-milling energy was reduced by 80% for wood chips pretreated by SPORL (initial pH 4.2) and by hot-water when disk-plate gap during milling was increased from 0.76 mm to 3.05 mm (Fig. 5).

The results presented above indicate that size-reduction energy consumption can be decreased by about 95%, or about a factor between 10 and 40 by using the combination of post-pretreatment size-reduction, low solids-loading, and large disk-plate gap in disk-milling. For wood chips pretreated by the low pH SPORL (initial pH 1.9) and dilute-acid (initial pH 1.1), we can decrease size-reduction energy consumption to a very reasonable level, <50 Wh/kg od wood at solids-loading between 10% and 20%. For wood chips pretreated by the high pH SPORL with initial pH 4.2, we can achieve disk-milling energy of about 100 Wh/kg od wood under similar solids-loadings. This level of size-reduction energy consumption is similar to those used for size-reduction of agriculture residual (Cadoche and Lopez, 1989). Furthermore, using solids-loadings of 10–20% in disk-milling is realistically practical. Typical solids content of wood chips after pretreatments is about 30%.

#### 3.5. Effects of disk-milling conditions on resultant substrate enzymatic saccharification

It is important to understand whether or not the energy savings presented above can be realized when considering enzymatic glucose yields from the resultant substrates from disk-millings at low solids-loadings or large disk-plate gaps. In thermomechanical pulp production for papermaking, the realized energy savings through process variations such as low solids-loading is very limited to about 10% because of the trade-off between energy savings and pulp quality in terms of fiber length and strength (Alami et al., 1995). To evaluate the potential energy savings in disk-milling through process variations, we plotted the enzymatic hydrolysis glucose yield (EHGY) per unit disk-milling energy consumption as a function of disk-milling energy for all the substrates produced from the four pretreatment processes under various solids-loadings and disk-plate gaps. We found that EHGY on unit energy consumption from substrates pretreated by the two SPORL processes fall onto one curve and the results from substrates pretreated by both the dilute-acid and hot-water processes fall onto another curve (Fig. 6). Furthermore, each of the two data sets can be fitted by an inverse function very well; i.e.,

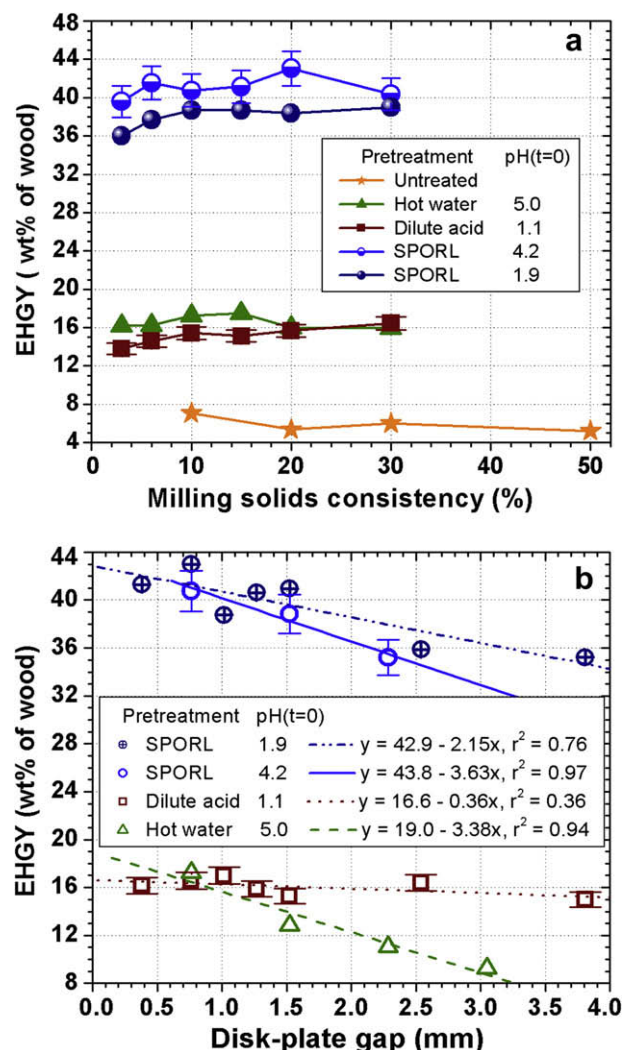


**Fig. 6.** Comparisons of enzymatic hydrolysis glucose yield (EHGY) on unit size-reduction energy consumption among different pretreatment processes under various disk-milling solids-loadings (with disk-plate gap 0.76 mm) and disk-plate gaps (with solids-loading 10%).

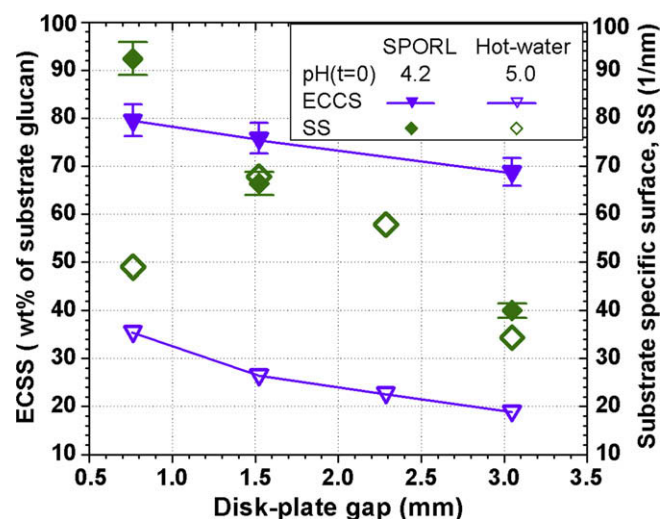
$$y = \frac{A}{x}, \quad (1)$$

where  $A$  is the EHGY in kg from 100 kg (wt.%) of untreated wood simply because the product of the two coordinates,  $x$  times  $y$ , in Fig. 6 is EHGY. The fitting results indicate that the EHGY for substrates from the two SPORL pretreatments was 368 g/kg od wood, independent of pretreatment pH, disk-milling solids-loading, and disk-plate gap. Similarly, EHGY for substrates from the dilute-acid and hot-water pretreatment was only 156 g/kg od wood. In other words, the same enzymatic hydrolysis glucose yield, EHGY, was obtained for all the substrates produced from the two SPORL pretreatments. Decreasing milling intensity and therefore energy consumption in disk-milling for size-reduction did not decrease EHGY and the efficiency of enzymatic cellulose saccharification of the substrate (ECSS) for the ranges of disk-milling conditions studied. The same is true for EHGYs from the two dilute-acid pretreatments (hot-water is a weak dilute-acid pretreatment). This indicates that the disk-milling energy savings presented in the previous section can be realized for most of the ranges of the experimental conditions tested. The most energy-efficient conditions are in the upper left corner in Fig. 6.

Careful study of the EHGY data indicates that EHGY is slightly dependent on disk-milling conditions at very low milling intensities such as very low solids-loadings and very large disk-plate gaps (Fig. 7a and b). The results shown in Fig. 7a also reveal that the EHGYs from the two SPORL pretreatments are actually slightly different. The high pH SPORL pretreatment produced a higher EHGY due to reduced loss of glucan under less acidic conditions (Table 2). The same is true for the two dilute-acid pretreatments. The linear regression of an inverse function (Fig. 6) obscured this slight dependence of EHGY on disk-milling conditions which occurred only under a few milling runs at low intensities, as well as the differences in EHGY among different pretreatments. The error bars in Fig. 7a were based on the average error of 4.2% obtained in the repeatability tests (for clarity, only two data sets are shown with error bars). The slight dependence of EHGY on disk-milling conditions can hardly be seen from the study of variations of solids-loading and is almost within the measurement uncertainty (Fig. 7a). However, it can be clearly seen from the study of variations of disk-plate-gap (Fig. 7b) for the hot-water and high pH SPORL pretreatments. Therefore, the following discussions will be focused on the study of variations of disk-plate gap. The slopes of the correla-



**Fig. 7.** Effects of disk-milling conditions on enzymatic hydrolysis glucose yield (EHGY) from substrates produced by four different pretreatment processes. (a) Variations of solids-loading at disk-plate gap 0.76 mm; (b) variations of disk-plate gap at solids-loading 10%.



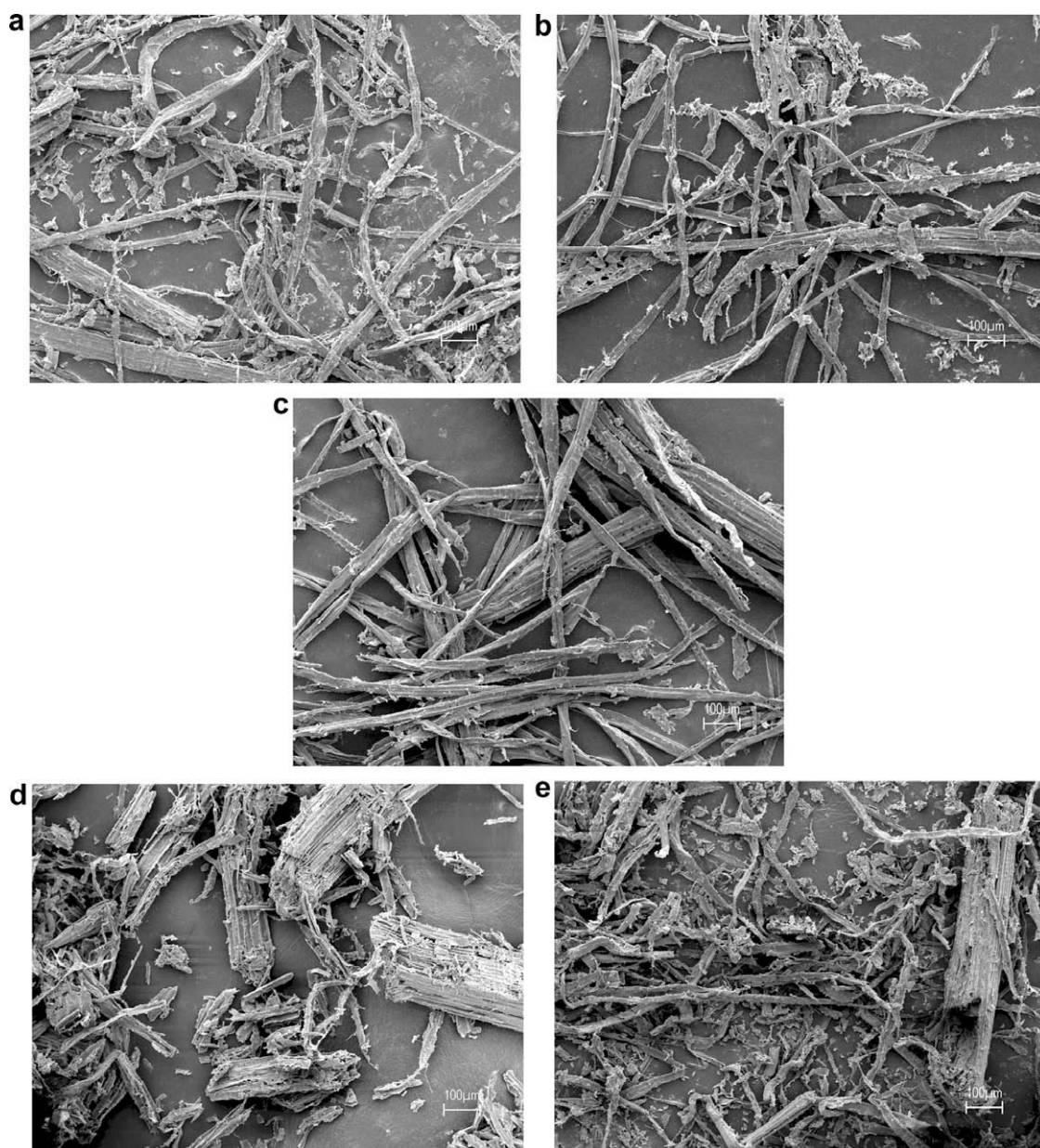
**Fig. 8.** Effect of milling disk-plate gap on the degree of size-reduction measured by substrate-specific surface and the efficiency of enzymatic cellulose saccharification of resultant substrates (ECSS).

tions between EHG and the disk-plate gap were found to vary with the pretreatment applied (Fig. 7b). The slopes for the hot-water and high pH SPORL (initial pH = 4.2) pretreatments were found to be 3.38 and 3.63, respectively. The dependence of EHG on disk-plate gap was reduced when pretreatments are conducted under strong acidic conditions. This can be clearly seen from the slopes of 2.15 and 0.36 for the low pH SPORL (initial pH 1.9) and dilute-acid (initial pH 1.1) pretreatments (Fig. 7b), respectively. When measurement uncertainty is considered, it can be said that the EHG from the dilute-acid pretreatment (initial pH 1.1) is independent of milling disk-plate gap in the range of 0.38–3.81 mm studied (Fig. 7b).

It is known that the efficiencies of enzymatic cellulose saccharification of substrates (ECSS) are affected by the enzyme accessibilities to cellulose, which can be characterized by the substrate-specific surfaces as demonstrated in our previous study (Zhu et al., 2009a). It is understood that substrate-specific surface is a measure of the degree of size-reduction and controlled by disk-

milling conditions or the milling intensity/energy. As shown in Fig. 8, the substrate-specific surface has a linear inverse relationship with disk-plate gap for both the high pH SPORL and hot-water pretreatments except one outlier in the hot-water pretreated substrates. Also shown in Fig. 8, decreasing disk-plate gap increases ECSS as expected based on the results in Fig. 7b. Therefore, ECSS increases as the degree of size-reduction, measured by substrate-specific surface, increases. The error bar for ECSS is again based on the repeatability tests reported previously. The error bar for the specific surface is the average of standard deviations of duplicate measurements in fiber characterization.

The specific surfaces of hot-water and high SPORL-pretreated substrates, as a coincidence, fall to a same line (Fig. 8), which can be validated by SEM images that show very similar morphologies and fiber/fiber bundle dimensions of the substrates from these two pretreatments (Fig. 9a and b). However, ECSSs for these two pretreatments are very different. This suggests that chemical pretreatment plays a dominant role in enhancing enzymatic cellulose



**Fig. 9.** Scanning electron microscope images of substrates produced from different pretreatments and disk-milling disk-plate gaps. Disk-milling solids-loading were all at 10%. (a) Hot-water, 1.52 mm; (b) and (c) high pH SPORL (initial pH 4.2), 1.52 mm and 3.05 mm; (d) dilute-acid (initial pH 1.1), 1.52 mm; (e) low pH SPORL (initial pH 1.9), 1.52 mm.

saccharification, which significantly reduces the effect of the degree of size-reduction (substrate-specific surface) or disk-milling intensity/conditions on ECSS. This can be clearly seen from the results shown in Fig. 7a and b. Because of this and because of the difficulties in accurate measurements of the specific surface as discussed in our comprehensive study (Zhu et al., 2009a), only the two sets of data that show clear effects of disk-milling conditions on ECSS are presented in Fig. 8.

### 3.6. Substrate morphologies and enzymatic cellulose saccharification

SEM imaging analysis reveals that the substrates produced by hot-water and high pH SPORL (initial pH 4.2) resembles semi-chemical-mechanical pulp for paper production and have very similar morphologies and fiber/fiber bundle dimensions (Fig. 9a and b). That is, the major feature is well separated individual long fibers with some degree of fibrillation when disk-milling was conducted at a disk-plate gap of 1.52 mm or smaller (comparing Fig. 9b and c). The dilute-acid pretreatment led to significantly damaged (cut) wood fibers and less fiberization, i.e., the substrate contains many very short fibers and fiber bundles (Fig. 9d). When comparing the substrate produced from same disk gap of 1.52 mm between the dilute-acid (Fig. 9d) and high pH SPORL (without acid) (Fig. 9b), the cutting action arising from acid pretreatment is very clear. Decreasing the disk-plate gap reduces the fiber bundle content for the dilute-acid pretreated substrates (not shown). The morphology of the substrate produced from low pH SPORL with acid (initial pH 1.9) reflects the combined effect of fiberization from SPORL without acid (initial pH 4.2) and cutting from the dilute-acid pretreatment (Fig. 9e).

The combined effects of fiberization and cutting observed from the substrate produced by low pH SPORL with acid (Fig. 9e) did not produce added benefit for enzymatic cellulose saccharification compared with the substrate produced by high pH SPORL without acid (Fig. 9b) under the exact same disk-milling conditions (Fig. 7b). However, low pH SPORL with acid (initial pH 1.9) did significantly reduce size-reduction energy consumption compared with that consumed for milling wood chips pretreated by high pH SPORL without acid (Table 1, Figs. 4 and 5). Based on the size-reduction energy data obtained from the four pretreatments conducted in this study, it seems that the cutting effect from acid (low pH SPORL with acid and dilute-acid) contributed to the significantly low energy consumption in size-reduction using disk-milling. For high pH SPORL (initial pH 4.2), substrate fibrillation affects enzymatic cellulose saccharification as can be seen by comparing the EHG and ECSS from substrates produced using different disk-milling gaps (Fig. 9b and c). The fibrillated substrate (Fig. 9b) produced at disk gap of 1.52 mm has a larger specific surface and achieved a higher EHG and ECSS (Figs. 7b and 8) than the unfibrillated substrate (Fig. 9c) produced at disk gap of 3.04 mm. Substrate fibrillation was increased (not shown) as disk gap further reduced to 0.76 mm, which resulted in further increase in EHG and ECSS (Figs. 7b and 8). Similar effects were also observed for substrates produced by hot-water pretreatment at the expense of increase disk-milling energy.

## 4. Conclusions

We studied the effects of chemical pretreatment and disk-milling conditions on energy consumption for wood-size-reduction and subsequent enzymatic cellulose saccharification efficiency of a softwood, lodgepole pine. We quantitatively demonstrated that post-chemical pretreatment size-reduction through disk-milling can reduce size-reduction energy consumption by as much as 95% (or a factor of more than 10) depending on the chemical pre-

treatments and milling conditions employed. Although research has shown that energy consumption can be reduced in mechanical pulping through chemical pretreatment of wood chips and by adjusting disk-refining conditions, energy savings is limited to 10–30% without affecting pulp quality in mechanical pulping. For the four pretreatments studied, we found that SPORL with sulfuric acid charge on wood of 2.21% was the most effective in saving size-reduction energy consumption while producing readily digestible substrates at all disk-milling conditions tested. These substrates have excellent enzymatic cellulose saccharification efficiencies (ECSS) of over 90%, or enzymatic hydrolysis glucose yields (EHG) of about 37 wt.% od wood, in 48 h at a cellulase loading of only 15 FPU/od g substrate. We also found that solids-loading in milling has the most significant effect on disk-milling energy. By decreasing solids-loading from 30% to 3%, disk-milling energy can be reduced by more than a factor of 10 for wood chips pretreated by SPORL and dilute-acid both at 2.21 acid charge on wood. However, the dilute-acid pretreatment produced unsatisfactory enzymatic hydrolysis conversion of only about 40% under the conditions studied, or glucose yield of about 15 wt.% od wood. The enzymatic hydrolysis glucose yields (EHG) from the substrates produced by all pretreatments were independent of the disk-milling solids-loading in the ranges studied. The exception were those under very low milling intensities using very low solids-loadings (3–6%) and large disk-plate gaps (>1.5 mm), indicating that energy savings in size-reduction by a factor of more than 10 achieved through disk-millings of wood chips pretreated by SPORL at 2.21% acid charge can be realized without affecting excellent ECSS and EHG. Finally, by using the combinations of low solids-loading and large disk-plate gaps, it is possible to decrease size-reduction energy consumption for milling lodgepole pine chips to less than 50 (after SPORL with 2.21% acid charge) or to less than 100 Wh/kg od wood (after SPORL without acid), which is equivalent to that for the size-reduction of agriculture biomass, while still producing excellent enzymatic hydrolysis conversion of over 90% and EHG of about 37 wt.% od wood.

The results obtained in this study indicate that SPORL can effectively remove the recalcitrance of lodgepole pine, a softwood, with very low size-reduction energy consumption and excellent subsequent enzymatic cellulose saccharification efficiency and glucose yield. The post-chemical-pretreatment size-reduction approach may be preferred to the current common practice of size-reduction prior to chemical pretreatment for forest biomass conversion to decrease size-reduction energy consumption. The approach also affords a reduced liquid-to-solid ratio in chemical pretreatment to save thermal energy otherwise not possible because of the increased liquid uptake of size-reduced biomass in the form of fibers or fiber bundles due to its porous and hydrophilic nature.

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