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Ethanol production from SPORL-pretreated lodgepole pine: preliminary evaluation of mass balance and process energy efficiency

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Abstract Lodgepole pine from forest thinnings is a potential feedstock for ethanol production. In this study, lodgepole pine was converted to ethanol with a yield of 276 L per metric ton of wood or 72% of theoretical yield. The lodgepole pine chips were directly subjected to sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) pretreatment and then disk-milled; the recovered cellulose substrate was quais-simultaneously saccharified enzymatically and fermented to ethanol using commercial cellulases and *Saccharomyces cerevisiae* D5A. The liquor stream from the pretreatment containing hydrolyzed sugars mainly from hemicelluloses was fermented by the same yeast strain after detoxification using an XAD resin column. The SPORL pretreatment was conducted at 180°C for a period of 25 min with a liquor-to-wood ratio of 3:1 (*v*/*w*) in a laboratory digester. Three levels of sulfuric acid charge (0.0%, 1.4%, and 2.2% on an oven dried wood basis in *w*/*w*) and three levels of sodium bisulfite charge (0.0%, 4.0%, and 8.0% in *w*/*w*) were applied. Mechanical and thermal energy consumption for milling and pretreatment were determined. These data were used to determine the efficiency of sugar recoveries and net ethanol energy production values and to formulate a preliminary mass and energy balance.

Keywords Cellulosic ethanol · SPORL · Fermentation/saccharification · Woody biomass · Pretreatment · Ethanol yield

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Introduction

Renewed global interest in using biofuels to meet regional energy needs and to reduce carbon dioxide emissions for sustainable economic development (Farrell et al. 2006) have spurred research efforts for biochemical conversion of lignocellulose into ethanol. Technical issues associated with some key sub-processes in cellulosic ethanol production, such as pretreatment (Gable and Zacchi 2007; Wyman et al. 2005; Zhu and Pan 2010), xylose fermentation (Jeffries and Jin 2004; Sedlak and Ho 2004; van Vleet and Jeffries 2009), high solids substrate saccharification, and fermentation (Hoyer et al. 2008; Zhang et al. 2009), have been investigated. Techno-economic models for specific cellulosic ethanol processes have also been conducted, which provided some predictive capabilities for qualitative analysis (Aden et al. 2002; Wingren et al. 2003).

However, few studies reported complete mass and energy balances for the process technologies examined, including overall ethanol yields from both the pretreatment hydrolysate and cellulosic substrate and energy consumed for pretreatment and high-solids enzymatic saccharification. This is a consequence of the complexities in achieving complete mass and energy balances for an entire process, which presents many technical difficulties to overcome. Most prior studies only reported the ethanol yield from enzymatic saccharification and fermentation of the pretreated and washed cellulosic substrate without including the sugars recovered in pretreated hydrolysates (De Bari et al. 2007; Munoz et al. 2007; Sassner et al. 2008; Wyman et al. 2009). Alternately, numerous studies focused exclusively on the fermentation of hemicellulosic sugars (Jeffries et al. 2007; Liu et al. 2005). Studies reporting ethanol fermentation data for both the pretreated liquor and solids generally did not provide key process data necessary from determining ethanol productivity (liter per ton biomass) as required for techno-economic mass balance analysis (Lee et al. 2009). Furthermore, very few studies carried out a comprehensive energy balance spanning the entire process. Recently, a complete mass balance of ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A (LNH-ST) capable of fermenting xylose was reported (Lau and Dale 2009). The study achieved an ethanol yield of 191.5 g/kg of corn stover on an oven-dried basis (od), equivalent to 242.7 L/ton corn stover and a conversion efficiency of 62.5% based upon a total fermentable carbohydrate (i.e., glucan and xylan) content of 54.5%. The study also provided the necessary information for determining thermal energy consumption in AFEX pretreatment. However, mechanical energy consumptions in the two most energy-intensive sub-processes, i.e., milling the feedstock to pass a 4-mm screen and mixing the pretreated substrate for enzymatic saccharification at 17.6% solids (*w*/*w*), were not reported. As such, there is a need for more comprehensive mass and energy balance to promote more detailed techno-economic modeling of processes.

Comparatively less research has focused on woody biomass, especially softwoods versus other cellulosic feedstocks for ethanol production. Meeting transportation fuel needs in a sustainable manner will best be achieved using a diversity of feedstocks. Woody biomass can be sustainably produced in large quantities in many regions of the world. About 370 million tons of woody biomass, accounting for 30% of the total biomass, can be sustainably produced annually in the USA (Perlack et al. 2005). Woody biomass also has many advantages over agricultural biomass, (e.g., corn stover and switch grass) such as flexible harvesting times, that greatly reduces the need for long-term storage, high density that reduces transportation costs, and very low ash contents, which eliminates dead load in transportation and processing. However, few pretreatment technologies have achieved satisfactory enzymatic saccharification efficiencies when applied to woody biomass (Wyman et al. 2009) due to its strong recalcitrance to biological conversion. Pretreating with organosolv can result in efficient enzymatic cellulose saccharification of both hardwoods and softwoods but at the price of lower hemicellulosic sugar yields (Pan et al. 2006). Acid-catalyzed steam explosion produces only about 55% glucose recoveries from enzymatic hydrolysis (Monavari et al. 2009). These are the two most widely reported technologies for processing woody biomass into ethanol (Ewanick et al. 2007; Munoz et al. 2007; Sassner et al. 2008). We recently reported a novel process, sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL), that exhibits robust enzymatic saccharification efficiency when applied to both softwoods (Zhu et al. 2009a; Zhu et al. 2010) and hardwoods (Wang et al. 2009). This work extends the earlier analysis to the entire process, i.e., ethanol production from both solid substrate and pretreatment hydrolysate.

The present study attempts to address two shortcomings in the current literature discussed above. Specific objectives of this study are to (1) examine the performance of SPORL for ethanol production from a softwood, lodgepole pine, a particularly recalcitrant feedstock; and (2) take the first step towards producing complete experimental data on mass and energy balances for accurate economic analysis. The energy balance will be extended to both chemical pretreatment and size reduction through milling. Lodgepole pine used in this study represents a major wood species from forest thinning of the unmanaged forests that is available in large volumes and requires value-added utilizations to mitigate expensive thinning costs for sustainable healthy forest and ecosystem management in the USA.

Materials and methods

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 spans because of the lack of forest management. The logs were debarked and chipped at the U.S. Forest Service, Forest Products Laboratory, Madison, Wisconsin. The wood chips were screened to remove the particles greater than 38 mm and less than 6 mm in length to ensure smooth operation in disk milling for size reduction. The accepted chips (Fig. 1) have thickness ranging from 3 to 8 mm.

Fig. 1 Lodgepole pine wood chips directly used in SPORL chemical pretreatment process

Accellerase 1500 and Celluclast 1.5 L and Novozyme 188 (β-glucosidase) were generously provided by Genencor (Palo Alto, CA) and Novozymes North America (Franklinton, NC), respectively. Sodium acetate, sulfuric acid, and sodium bisulfite were used as received from Sigma-Aldrich (St. Louis, MO). All other chemicals, including culture media ingredients, were received from Fisher Scientific (Hanover Park, IL). All chemicals were of analytical quality. The Amberlite™ XAD-4 was also purchased from Sigma-Aldrich (St. Louis, MO). The yeast strain used was *Saccharomyces cerevisiae* D5A, which is available from ATCC culture collections (ATCC® Number: 200062).

SPORL pretreatment

The SPORL experiments were conducted according to the process flow diagram shown in Fig. 2. Sub-processes connected with dashed lines were not carried out in this study. 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 a larger pressure vessel as described elsewhere (Zhu et al. 2009a) and heated externally via steam while rotating at the speed of 2 rpm. Based upon our prior study (Zhu et al. 2009a), pretreatment temperature and duration time were fixed at 180°C and 25 min, respectively, and the ratio of pretreatment liquor to od wood chip (L/W) was 3 (v/w). The pretreatment liquors were dilute solutions of sodium bisulfite and sulfuric acid. Three levels of bisulfite charge on od wood chips were used: 0%, 4%, and 8% (*w*/*w*), and three levels of sulfuric acid charges on od wood were also used: 0%, 1.40%, and 2.21% (*w*/*w*). These variations in chemical applications, though not sufficient for process optimization, represent a wide operating range to judge post-SPORL pretreatment energy requirements for disk-milling and sugar and ethanol yields in subsequent saccharification and fermentation. SPORL pretreatment conditions are detailed in Table 1. Following pretreatment, residual solids remained as wood chips, which allowed an easy separation from the hydrolysate liquor using a simple screen. The yield of the wood-chip solids was determined from the weight and moisture content of the collected wood chips. This wood-chip solids yield was used to convert the measured energy consumption on pretreated wood chips in the subsequent size reduction to that on od untreated wood basis. The pretreatment spent liquor, which mainly contains hemicellulosic sugars, was recovered and stored at 4°C until used for analysis and fermentation.

Mechanical wood-size reduction

The collected wood chips were directly transferred to a laboratory disk mill for size-reduction under atmospheric pressure (Fig. 2). The 12-inch disk mill was equipped with disk-plates of pattern D2-B505 (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH). Disk-milling was operated at 2,570 rpm with a disk gap of 1.0 mm and at milling solids-loading of 20%. The milling solids-loading is defined as the percentage of pretreated wood chip (od basis) in the total feed into the mill, where the total feed includes chips "as is" and added water. The size-reduced solids (substrate) was not separately washed and was directly dewatered through pressing using a canvas bag to a solids content of about 30%. The yield of solid (substrate) in the form of fibers or fiber bundles was then determined from the weight and moisture content of the collected substrate. The moisture content was determined gravimetrically by drying the collected solids in an oven at 105°C overnight. This solid substrate yield was used to convert the measured substrate glucan content and enzymatic hydrolysis glucose yield (EHGY) from substrate base to untreated wood base for process mass balance analysis.

The electrical energy consumption for the disk-milling was recorded with a digital load monitor system (Ohio Semitronics, Inc., Hilliard, OH, model DLM-33-480-1PR) as previously described (Zhu et al. 2009b). The milling energy was divided by the od mass of wood chips fed into the mill to give energy consumption in Wh/kg od fed chips, which was further converted to energy consumption for size-reduction, in Wh/kg od untreated wood, by multiplying the yield of wood-chip solids after the chemical pretreatment.

Enzymatic hydrolysis

Separate enzymatic hydrolysis experiments of the pretreated substrates were conducted to measure the EHGY in terms of kilograms per ton untreated wood. EHGY was used to calculate fermentation efficiency to ethanol from a simulta-

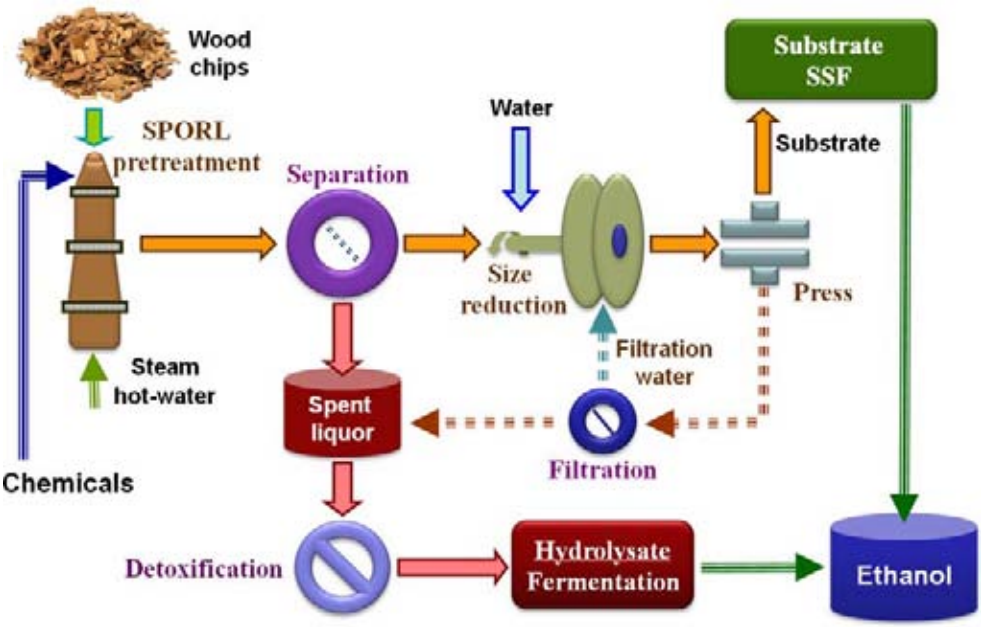

Fig. 2 A schematic process flow diagram of the SPORL process complete with ethanol fermentations. All steps, except those related to water recycling (*dashed lines*), were included in the present study

neous saccharification and fermentation (SSF). Enzymatic hydrolysis was conducted using commercial enzymes at 2% substrate solids (*w*/*v*) in 50-mL of sodium acetate buffer (pH 4.8, concentration 50 mM) on a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) set at 50°C and 200 rpm. An enzyme mixture of Celluclast 1.5 L cellulase (15 FPU/g substrate) and Novozyme 188 β-glucosidase (22.5 CBU/g substrate) was used for hydrolysis. Hydrolysate was sampled periodically for glucose concentration. Each data point is the average of two replicates.

Pretreatment hydrolysate fermentation

Hydrolysates were preconditioned for fermentation using XAD-4 to absorb furan inhibitors. Amberlite™ XAD-4 (15 g, Rohm and Haas, Philadelphia, PA) was loaded into a 1.5×15 cm glass column and washed with ×3 volume of water. The SPORL hydrolysate (50 mL/batch) was pumped onto the column at ambient temperature and the elution was only collected once the hydrolysate began to exit the column as judged by color. The XAD-4 was replaced for each batch of hydrolysate. Following absorption, the hydrolysate was neutralized with $Ca(OH)_2$ to pH 5 and filter sterilized by passing through a 0.22 micron filter.

Ethanol fermentation was conducted using Corning Pyrex™ solution bottles (25 ml) sealed with a silicone septa held in place using open top screw caps. The septum was pierced with a 22-gauge needle for venting CO_2. The bottle was filled with 8.5 ml of hydrolysate and 1.5 ml of sterile sodium citrate (pH 4.8, 50 mM final concentration), yeast extract (10 g/L final concentration), and peptone (20 g/L final concentration) stocks. The solution was

Table 1 A list of pretreatment runs and corresponding conditions for the nine experiments conducted at 180°C for 25 min with liquor to wood ratio of 3

Experiment no.	Sample label	Acid charge (wt.% wood)	Bisulfite charge (wt.% wood)	Liquor initial pH	Final liquor pH
1	1-A2B0-1	2.21	0	1.0	1.18
2	1-A2B4-1	2.21	4	1.8	1.43
3	1-A2B8-1	2.21	8	1.9	1.51
4	2-A2B8-2	2.21	8	1.9	1.63
5	3-A2B8-3	2.21	8	1.9	1.61
6	2-A1B8-1	1.40	8	2.3	1.80
7	3-A1B8-2	1.40	8	2.3	1.70
8	2-A0B8-1	0	8	4.2	2.87
9	3-A0B8-2	0	8	4.2	2.78

Sample label: the first number is the batch number of the run; A# is acid charge level; B# is bisulfite charge; the last number is replicate number. Batch 1 was conducted 2 weeks earlier than batches 2 and 3. Batches 2 and 3 were conducted sequentially on the same day. All replicate runs were from different batches

inoculated with *S. cerevisiae* D5A to an $OD_{600\ nm}$ of 1.0 and the culture incubated at 32°C and agitated at 100 rpm for 72 h using a shaker/incubator. Samples were stored at −20°C until analyzed for sugars and ethanol.

S. cerevisiae D5A was maintained on solid YPD medium (10 g/L yeast extract, 20 g/L Bacto peptone, 20 g/L dextrose, and 15 g/L agar), which were incubated at 32°C for growth and stored at 4°C. A single colony was transferred to 25 ml of YPD and incubated at 32°C and mixed at 200 rpm for 18 h, sub-culture made to YP+5% glucose and grown again under similar conditions for another 18 h. The cells were recovered by centrifugation and resuspended in saline-phosphate-peptone diluent as a concentrated stock for inoculation.

quasi-Simultaneous enzymatic saccharification and fermentation

SSFs were carried out in 250 ml Erlenmeyer flasks using a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) set at 35°C and 90 rpm with 8% substrate (water insoluble). The enzyme loading of Accellerase 1500 was 3.2 mL/g substrate, or about 15 FPU/g substrate. Liquefaction of the solid substrates was initiated in about 2–12 hours at 50°C and 200 rpm before adding yeast. No additional nutrients were added during fermentation. The *S. cerevisiae* D5A culture was grown in YPD medium at 30°C for at least 24 h prior to harvesting by centrifuge. The initial cell concentration for SSF was 2 g/L (wet base). Samples of the fermentation broth were taken every 24 h for ethanol analysis. Reported results are the average of duplicates.

Analytical methods

The chemical compositions of the original and pretreated biomass were measured by the Analytical and Microscopy Laboratory of the Forest Products Laboratory. The solid biomass substrates were Wiley milled to a size passing a 20 mesh (~1 mm) screen. The resulting materials were hydrolyzed using sulfuric acid in two stages. The hydrolysis conditions were acid concentration of 72% (*v*/*v*) at 30°C and 3.6% (*v*/*v*) at 120°C for the first and second stage, respectively. The hydrolysis duration time was 1 h for both stages. The hydrolysate was analyzed for carbohydrates using an improved high-performance anion exchange chromatography with pulsed amperometric detection method (Davis 1998). The Klason lignin content was measured gravimetrically after washing and drying the solid residue from the acid hydrolysis. The reported data are the average of replicate experiments conducted one month apart. For fast analysis, glucose in the enzymatic hydrolysate was measured using a commercial glucose analyzer (YSI 2700 S, YSI Inc., Yellow Springs, OH). Ethanol analysis in the cellulosic substrate fermentation broth was carried out using a gas chromatograph (model 7890, Agilent Technologies, Palo Alto, CA) through direct sample injection using an external standard for calibration. The chromatograph is equipped with a flame ionization detector and Agilent DB-Wax column of 30 m with an ID 0.32 mm. A universal guard column was used to reduce column contamination.

Liquid hydrolysate samples, both pre- and post-fermentation, were analyzed for sugars and fermentation products by the Biofuel Research Unit at the NCAUR laboratory (USDA Agricultural Research Service, Peoria, IL). Samples were analyzed for fermentation productions using a Spectra-system liquid chromatography system equipped with a RI-150 a refractive index detector (Thermo Electron Corporation, Waltham, MA) and with an organic acids column (Aminex HPX-87H Column, 300 mm× 7.8 mm, Bio-Rad Laboratories, Inc., Hercules, CA). The samples were injected on the column at 65°C and eluted isocratically with 5 mM sulfuric acid at 0.60 mL/min. Sugar concentrations were measured using a similar arrangement, but equipped with a carbohydrate column (Aminex HPX-87P Column, 300 mm×7.8 mm, Bio-Rad Laboratories, Inc., Hercules, CA) that was maintained at 80°C and run isocratically using distilled water as the mobile phase. Furfural and 5-HMF concentrations were measured using an HPLC equipped with an Econosphere™ C18 column (5-mm particle size, 250 mm×4.6 mm, Alltech, Deerfield, IL) and a UV1000 ultraviolet detector (277 nm; Thermo Finnigan, San Jose, CA). Samples were run at ambient temperature and eluted at 0.8 mL/min with a linear gradient of 50% to 100% acidified methanol (containing 0.25% acetic acid) run over 15 min.

Results

Saccharide recovery by SPORL pretreatment

Following chemical pretreatment, the solids and liquid fractions were separated using a screen and analyzed for chemical composition (Table 2) separately. Solids were analyzed for polysaccharides and the liquid hydrolysate for monosaccharides, which included almost all of the sugars detected in the hydrolysates (data not shown). For consistency, all of the sugars listed in Table 2 are expressed in terms of polysaccharides. The maximum glucan recovered in the solids following SPORL pretreatment was 408 kg/ton wood, or about 96% of theoretical, which was achieved without acid addition (A0B8). As more acid was added for pretreatment, glucose, released by direct acid-hydrolysis, began to be detected in the liquor and total glucan recovered in the solids consequently decreased (Table 2). When combining the yields from both the solids and liquors, the overall glucan recoveries were 364–422 kg/ton

Table 2 Yields of key wood components in the recovered solids and liquid hydrolysate after pretreatment at 180°C for 25 min with various chemical applications

1000 kg lodgepole pine wood chips									
K Lignin	Arabinan	Galactan	Rhamnan	Glucan	Xylan	Mannan	Glucan+Mannn		Total
270.9	15.6	22.3	7.0	425.5	69.3	109.9	535.4		941.2

	SPORL substrate 					SPORL hydrolysate 							Total Yield
Sample	Glucan	Xylan	Mannan	K Lignin	Solids yields[a]	Glucose as glucan	Xylose as xylan	Mannose as mannan	K Lignin[b]	Furfural as pentosan	HMF as hexosan	Yield[c]	
1-A2B0-1	326.7	0.8	1.0	285.7	639.6	36.9	5.4	18.4	0	37.9	19.4	118.0	757.6
1-A2B4-1	353.5	2.2	1.1	231.0	594.9	40.5	32.9	79.9	39.9	21.4	7.1	221.7	816.6
1-A2B8-1	385.3	7.8	3.8	194.9	602.9	32.4	26.9	86.1	76.0	12.6	5.6	239.6	842.5
2-A2B8-2	365.3	4.9	3.3	188.8	592.4	19.8	20.9	70.1	82.1	8.2	6.9	208.0	800.4
3-A2B8-3	396.3	8.1	3.5	189.1	597.8	21.0	23.4	67.7	81.8	8.9	4.4	207.2	805.0
2-A1B8-1	401.9	10.4	5.2	178.6	613.1	19.4	19.1	61.1	92.3	10.1	6.1	208.1	821.2
3-A1B8-2	407.0	13.7	8.2	183.7	619.6	15.2	17.8	56.6	87.2	6.5	3.4	186.7	806.3
2-A0B8-1	405.3	24.9	19.3	153.7	626.1	6.0	8.6	17.4	117.2	3.3	1.3	153.8	779.9
3-A0B8-2	410.3	25.1	25.2	166.3	649.2	2.6	7.4	14.6	104.6	1.9	0.5	131.6	780.8

[a] As measured after disk-milling.
[b] Based on balance of lignin.
[c] Sum of listed pretreatment hydrolysate components.

wood or recovery efficiencies of 85–99%. The lowest recovery was obtained in the dilute-acid pretreatment without addition of bisulfite (A2B0), and the highest in the pretreatment with the lower acid charge (A1B8). Duplicate experiments (Table 2) consistently showed that the SPORL pretreatments at zero acid charge produced a slightly lower glucan recovery of 97% theoretical than the 99% achieved at acid charge 1.4%.

As expected, the removal of hemicelluloses by acid hydrolysis during pretreatment (Table 2) was strongly dependent on pretreatment pH (Table 1). Almost all of the xylan and mannan were removed by the dilute acid pretreatment (A2B0). The amounts of xylan and mannan extracted from the chips were reduced to about 64% and 80%, respectively, by the SPORL pretreatments with zero acid charge (A0B8). However, higher extraction of hemicelluloses does not necessarily translate into high sugar recoveries if the released sugars are subsequently degraded into fermentation inhibitors under acidic conditions (Larsson et al. 1999). For the dilute-acid pretreatment (A2B0), the recoveries of xylose and mannose in the pretreatment hydrolysate were only 7% and 17% of theoretical because of their degradation (in part) into furfural and HMF (Table 2). The furfural and HMF measured in the pretreatment hydrolysate corresponded to 55% and 18%, respectively, of the beginning xylan and mannan contents in wood. The SPORL with the lowest pH (A2B4) produced the maximum xylan and mannan recoveries of 33 and 80 kg/ton wood from pretreatment liquor, which are 47% and 74% of theoretical, respectively. A2B8 experiment had lower mean recoveries of xylan and mannan, 24 and 75 kg/ton wood, or 34% and 68% of theoretical (Table 2), respectively. However, xylan and mannan recoveries varied widely (Table 2) between batch 1 (1-A2B8-1), 39% and 78% of theoretical, respectively, and batches 2 and 3 (2-A2B8-2 and 3-A2B8-3 conducted sequentially on the same day, 2 weeks later), 32% and 63% of theoretical on average, respectively.

The maximum overall saccharide recovery (glucan, xylan, and mannan) was 505 kg/ton wood, or 84% theoretical, and was achieved with 2.2% acid and 8% bisulfite charges (mean of 1-A2B8-1, 2-A2B8-2, and 3-A2B8-3 in Table 2). Because the yeast used (*S. cerevisiae*) is incapable of fermenting xylose, xylose was excluded when calculating the fermentable saccharides (glucan and mannan) recovery, which was 481 kg/ton wood, or 90% theoretical based on glucan and mannan content of 53.5% in wood. Reducing the acid charge to 1.4% had little effect on overall saccharide recovery

(499 kg/ton wood, mean of 2-A1B8-1 and 3-A1-B8-2 in Table 2). The amount of glucan retained in the solids increased slightly whereas the amount of hemicelluloses sugars extracted to the liquid hydrolysate was reduced. Furan production (HMF + Furfural) was also slightly reduced from 15% to 13%, respectively, of the available xylan and mannan.

SPORL pretreatment also extracted a small amount of lignin, presumably as lignosulfonate from the wood chips, resulting from delignification by bisulfite. The amount of lignin removed decreased with the increase of acid charge at the same bisulfite loading, probably due to the condensation of lignin at low pH (Gierer 1985). The maximum lignin removal was about 40% at zero acid charge (2-A0B8-1 and 3-A0B8-2).

Effect of SPORL pretreatment on substrate enzymatic digestibility

To maximize the glucose yield from the lodgepole pine wood, it is desirable to minimize extraction of glucan during pretreatment and maximize enzymatic digestibility of the pretreated cellulosic substrate. The former criterion was met, as 90–99% of the recovered glucan was retained in the pretreated chips (Table 2). The substrate enzymatic digestibility (SED) was determined as the amount of glucan enzymatically hydrolyzed to glucose (as measured in the enzymatic hydrolysate) as the percentage of glucan initially present in the pretreated substrate. Our previous results demonstrated very high SED values of SPORL substrates (Zhu et al. 2009a). Likewise, SED values of up to 90% were achieved for the SPORL substrates from lodgepole pine in this study (Fig. 3). The error bars in Fig. 3 were based on the standard deviation of SEDs from replicate experiments listed in Table 1. SED reached 90% or higher

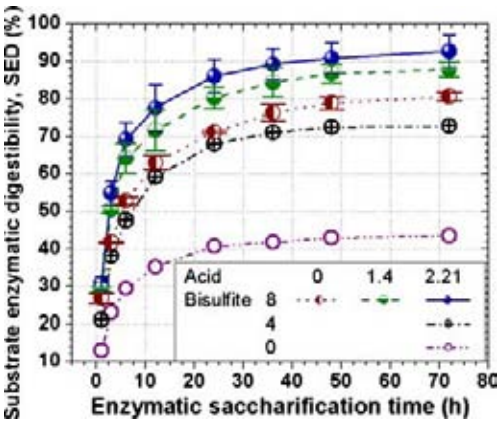

Fig. 3 Time dependent glucose production from cellulase digestions of pretreated and milled wood chips. Substrate enzymatic digestibilities (% theoretical) are plotted for five pretreatment conditions along with *error bars* (standard deviations)

Table 3 Monomeric sugar recoveries and ethanol yields from different pretreatment runs and enzymatic hydrolyses

Sample label	EHGY @72h[a]	SSF ethanol[b]	SSF fermentation efficiency[c]	Hydrolysate glucose[a]	Hydrolysate mannose[a]	Hydrolysate glucose+ mannose[a]	Hydrolysate ethanol[b]	Hydrolysate fermentation efficiency[c]	Total glucose+ mannose[d]	Total ethanol yield[e]
1-A2B0-1	158.0									
1-A2B4-1	286.2	136.7	73.7	39.9	85.5	125.4	52.8	65.1	420/69.7	189.5/49.2
1-A2B8-1	385.9	209.4	83.8	35.1	74.6	109.7	66.9	94.2	517.6/85.9	276.3/71.7
2-A2B8-2	396.9	229.8	89.4	28.5	71.0	99.5	1.9	2.9	496.8/82.5	231.6/60.1
3-A2B8-3	397.5	207.9	80.7	25.1	66.7	91.8	44.5	74.9	496.1/82.4	252.4/65.5
2-A1B8-1	398.5	227.9	88.3	24.3	63.7	88	38.5	67.6	487.9/81.0	266.4/69.1
3-A1B8-2	390.5	193.2	76.4	19.6	60.0	79.6	36.3	70.4	470.3/78.1	229.5/59.6
2-A0B8-1	366.3	165.6	69.6	5.3	17.7	23	1.7	11.3	392.3/65.1	166.7/43.3
3-A0B8-2	363.6	167.2	71.0	3.7	17.6	21.2	0.6	4.2	382.7/63.5	167.8/43.6

[a] EHGY stands for enzymatic hydrolysis glucose yield from substrate; in kg/od metric ton wood. Data for hydrolysate were measured after detoxification

[b] In L/od metric ton wood

[c] Percentage of theoretical ethanol (0.511 g ethanol/g hexose) yield from experimentally measured EHGY or detoxified hydrolysate glucose and mannose

[d] The first number is in kg/od metric ton wood and the second number is wt.% of theoretical, based on glucan and mannan content in wood of 53.5%. Undetoxified hydrolysate glucose and mannose were used

[e] The first number is in L/od metric ton wood and the second number is wt.% of theoretical based on glucan and mannan content in wood of 53.5%

with an acid charge greater than 1.4% and a bisulfite charge of 8%. Reducing the bisulfite charge decreased SED (Fig. 3); adding no bisulfite (A2B0) reduced the SED to only 40%. Adding 4% bisulfite with the same amount of acid (A2B4) increased the SED to 70%.

Fermentable sugar recoveries and ethanol yields

Enzymatic hydrolysis glucose yield (EHGY) is the amount of glucose yield from 1,000 kg of wood through enzymatic saccharification. The maximum EHGY was obtained by the pretreatment with acid charge of 1.4% and 2.21%. The average EHGY for the five independent experiments (three A2B8 and two A1B8 runs, Table 3) was 394 kg glucose per ton wood, or 83% theoretical. When the pretreated solids were converted to ethanol through SSF, the average ethanol yield was 214 L/ton wood, which gave an average fermentation efficiency of 84% (calculated based on the measured EHGY in Table 3). The maximum ethanol yield of 230 L/ton wood, or 90% fermentation efficiency, was obtained from sample 1-A2B8-2. The EHGY was reduced to about 365 kg glucose/ton wood due to reduced substrate enzymatic digestibility at zero acid charge (Fig. 3), which dramatically reduced the final ethanol yield to 166 L/ton with a 70% fermentation efficiency (Table 3).

As presented earlier, the hemicellulosic sugars were hydrolyzed mainly as monosaccharides in the hydrolysate liquor. Initially, the hydrolysate was neutralized to pH 5 with Ca $(OH)_2$ and fermented to ethanol using *S. cerevisiae* D5A. No enzymes were added to the fermentations because the sugars were already in the form of monomers. However, these fermentations failed and only succeeded when the hydrolysate was diluted in half with distilled water (data not shown). It was suspected that the cause of the failure was the high concentration of furans present in the hydrolysates (5.3–62 mM total furans; Table 4). To overcome this problem, the hydrolysates were preconditioned and detoxified by passing them through a column packed with the resin Amberlite™ XAD-4. XAD-4 is a polymeric adsorbent recommended for removal of small aromatic molecules from aqueous streams and that has been found to allow for the efficient removal of furans from hydrolysates (Weil et al. 2002). Treatment with XAD-4 reduced the furans concentrations to 4.6–26.2 mM. The total hexose concentration was not significantly affected (only about 5% for most samples). We chose XAD-4 in part because it does not absorb sugars. Although it was not recycled/reused in this study, XAD-4 can be regenerated by flushing with ethanol (Weil et al. 2002).

The fermentation results for the preconditioned hydrolysate are listed in Table 3. SPORL pretreatments without dilute-acid catalyst (A0B8) led to very low hexose concentrations (7.1 and 7.7 g/L) and subsequently poor fermentation efficiencies. For the other pretreatment conditions, fermentation efficiencies were 65–94% (calculated based on the total glucose and mannose content in the detoxified hydrolysate listed in Table 3), excluding a failed fermentation for the hydrolysate with the highest furans concentration (26.2 mM). Finally, while glucose was exhausted during the fermentation, residual mannose ranged from 1.9–8.7 g/L.

Based on the results presented above, the total fermentable sugars (glucose and mannose only) recovered from both enzymatic hydrolysis and the pretreatment hydrolysate before detoxification ranged from 383 to 518 kg/ton wood for the SPORL pretreatments (Table 3), or 64% to 86% of available glucan and mannan in wood. A fermentable sugar recovery of 504 kg/ton wood or 84% of theoretical averaged over triplicate experiments (1-A2B8-1, 2-A2B8-2, 3-A2B8-3) was achieved by SPORL pretreatment at an acid and bisulfite charge of 2.21% and 8%, respectively. The 84% of fermentable sugar recovery from lodgepole pine (a softwood) is much higher than those reported in the literature using steam explosion from spruce (Soderstrom et

Table 4 Inhibitor reduction through detoxification using Amberlite™ XAD-4

	Beginning values			Final values		
Sample label	Furfural (mM)	HMF (mM)	Furans[a] (mM)	Furfural (mM)	HMF (mM)	Furans[a] (mM)
1-A2B0-1	Na	Na	Na	Na	Na	Na
1-A2B4-1	44.0	18.0	62.0	13.3	11.3	24.6
1-A2B8-1	25.9	14.2	40.1	13.8	10.4	24.1
2-A2B8-2	26.8	17.5	44.3	14.1	12.1	26.2
3-A2B8-3	18.3	11.0	29.3	12.2	8.8	21
2-A1B8-1	20.7	15.4	36.1	12.6	12.1	24.7
3-A1B8-2	13.5	8.5	22.0	9.7	7.3	17
2-A0B8-1	6.8	3.2	9.9	4.9	2.6	7.4
3-A0B8-2	3.9	1.4	5.2	3.3	1.3	4.6

[a] Furfural+HMF

Table 5 Energy efficiencies in pretreatment and ethanol production

Sample label	Wood chip milling energy[a]	Total energy input in pretreatment[a]	Total monomeric sugar yield[b]	$\eta_{Pretreatment}$[c]	Total ethanol energy[a]	η_{energy} (%)
1-A2B0-1	0.12	1.63	219.4	0.135	Na	Na
1-A2B4-1	0.12	1.63	419.9	0.257	4.43	172
1-A2B8-1	0.41	1.92	517.6	0.270	6.47	237
2-A2B8-2	0.39	1.90	496.8	0.261	5.42	185
3-A2B8-3	0.49	2.00	496.1	0.248	5.91	195
2-A1B8-1	0.59	2.10	487.9	0.233	6.23	197
3-A1B8-2	0.76	2.27	470.2	0.207	5.37	137
2-A0B8-1	1.16	2.67	392.2	0.147	3.90	46
3-A0B8-2	1.25	2.76	382.7	0.139	3.93	42

[a] In GJ/od metric ton wood

[b] Sum of mannose and glucose in kg/od metric ton wood

[c] In kg monomeric sugar/MJ

al. 2004) and organosolv pretreatment of lodgepole pine (Pan et al. 2008). The total ethanol yields ranged from 166 to 276 L/ton wood with overall fermentation efficiency between 66% and 86%. The maximum ethanol yield of 276 L/ton wood was obtained from the sample 1-A2B8-1 pretreated at acid charge 2.2% and sodium bisulfite 8%. This yield is about 72% theoretical based on glucan and mannan content of 53.5% in lodgepole pine.

Preliminary evaluation of net energy output

A preliminary energy audit for the process was used to determine the net energy production in terms of liquid fuel (Fig. 4). Sub-processes shown with dashed lines were not investigated in this study and therefore not included in the energy balance. Likewise, the SSF and hydrolysate fermentation were done at laboratory-scale as batch processes, under conditions that do not fully reflect industrial conditions, and so these were not included in the preliminary energy balance, either. The wood-chipping energy is estimated to be 50 Wh/kg (0.18 MJ/kg) based on the experience in pulp and paper industry and our laboratory practice. The thermal energy consumption in pretreatment of 1.33 MJ/kg wood was based on the enthalpy of wood pulp at 25% consistency (L/W=3) and 180°C with the consideration of thermal energy recovery of 50%. The wood size-reduction energy consumptions were measured as listed in Table 5. According to (Zhu and Pan 2010), the pretreatment energy efficiency is defined as follows:

$$\eta_{\text{pretreatment}} = \frac{\text{Total monomeric sugar yield}}{\text{Total energy consumption in pretreatment}} \quad (1)$$

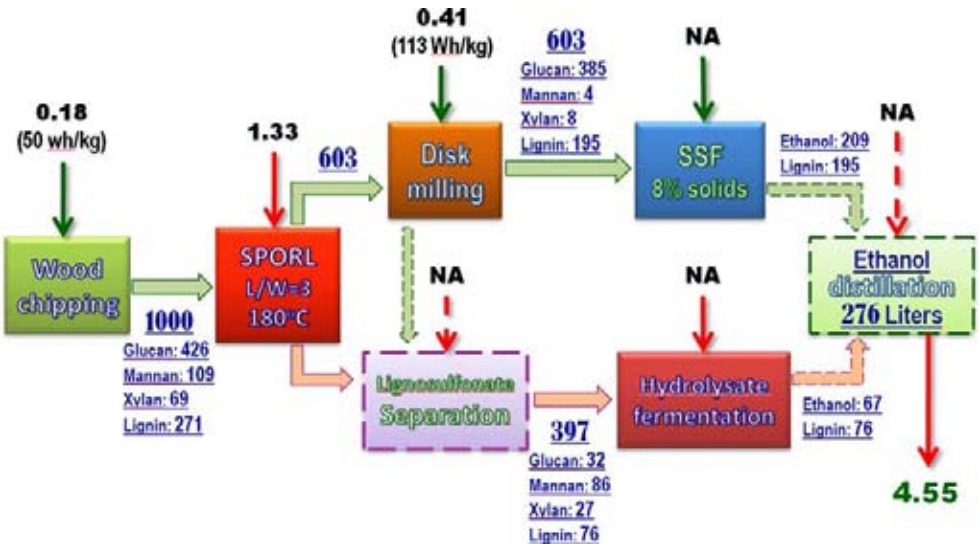

Fig. 4 A block diagram shows process mass and energy balance. Unless indicated, energy values are stated in GJ/ton wood and mass in kilograms

Similarly, the ethanol production energy efficiency or gain factor can be defined as net energy output divided by the total energy input, i.e.,

$$\eta_{\text{Energy}} = \frac{\text{Net energy ouput}}{\text{Total energy input}} \quad (2)$$

For the present study, only the ethanol energy was used in calculating the net energy output. As listed in Table 5, a pretreatment energy efficiency of 0.27 kg monomeric sugar/MJ and a maximum ethanol production energy efficiency of 237% were achieved from sample 1-A2B8-1 with an acid charge of 2.21% and sodium bisulfite charge of 8%. The average pretreatment energy efficiency over the triplicate runs (A2B8) was 0.26 kg sugar/MJ. The average ethanol production energy efficiency of the triplicate runs was 206% which includes one non-fermentable pretreatment hydrolysate (2-A2B8-2).

Based on the results listed in Tables 2, 3, and 5, the preliminary mass and energy balances for the run 1-A2B8-1, which achieved maximal ethanol yield and production energy efficiency are shown in Fig. 4. The figure provides a clear picture about component recovery and energy consumption in each subprocess and the overall ethanol yield and net energy output. A net ethanol energy yield was 4.55 GJ/ton wood (energy from lignin not included).

Discussions

This study demonstrated the effectiveness of SPORL pretreatment for converting lodgepole pine into ethanol. SPORL produced near complete recovery of glucan within the range of the treatment conditions evaluated in this study. SPORL pretreatment at zero acid charge (A0B8) produced a slightly higher glucan recovery from the pretreated solid substrate offset by a low glucose recovery in the pretreatment hydrolysate. This is most likely due to the slightly higher pH at zero acid charge that resulted in (1) the low dissolution of glucan and (2) incomplete hydrolysis of glucan to mainly oligomers that were not detected. SPORL pretreatments with low acid charges resulted in low removals of hemicelluloses (A1B8 and A0B8) that contributed to low recoveries of hemicelluloses (Table 2). Reducing pretreatment pH through reduce bisulfite charge (A2B4 and A2B0) can increase hemicelluloses removal (Table 2). However, the increased removal does not necessary translate to increased hemicelluloses sugar recovery due to degradation to other by-products such as furfural and HMF (A2B0). As a result, there is an optimal acid and bisulfite charge on od wood (2.21% and 8%, respectively, for the experiments conducted in this study) at which overall saccharide recovery was maximized (A2B8 in Table 2). SED was also maximized (Fig. 3) at these conditions (A2B8). The maximum EHGY of about 395 kg glucose/ton wood (Table 3) was achieved at acid charge between 1.40% and 2.21% and bisulfite charge of 8% (A2B8 and A1B8). Under the same pretreatment conditions except with no bisulfite charge, EHGY was only 158 kg glucose/ton. By increasing bisulfite charge to just 4%, EHGY was increased to 286 kg glucose/ton. This observation suggests that bisulfite plays an important role in removing wood recalcitrance through partial delignification and lignin sulfonation as demonstrated in our previous studies (Shuai et al. 2010; Zhu et al. 2009a).

Fermentation of the pretreated solid substrate and hydrolysate using *Saccharomyces cerevisiae* D5A achieved a maximal ethanol yield of 276 L/ton wood (Table 3), which is 72% of the theoretical ethanol yield based on a glucan and mannan content of 53.5% in lodgepole pine. The average SSF fermentation efficiency was 83% (Table 3), which is based upon 5 independent runs using acid charges between 1.4% and 2.1% for pretreatment. The average hydrolysate fermentation efficiency was 77% (Table 3) for the same set of independent runs, excluding a single failed fermentation. This further validates the effectiveness of SPORL pretreatment for cellulosic ethanol production from lignocellulose. Analysis of fermentation broth indicated that mannose was not completely consumed. This suggests that it might be worthwhile to include a *Saccharomyces* strain selected for mannose utilization in the future.

A preliminary evaluation of mass and energy balances was conducted based on the process data obtained. The average pretreatment energy efficiency was 0.26 kg monomeric sugar/MJ (Table 5), and the ethanol production energy efficiency was 206% (Table 5), based on triplicate runs conducted using a sulfuric acid and sodium bisulfite charges of 2.21% and 8%, respectively. Finally, the ethanol yield of 276 L/ton wood equates to a net energy output of 4.55 GJ/ton wood (Fig. 4). This figure will be further refined in the future to include other energy intensive processes such as high solids SSF that was not conducted in the present study. The mass of lignosulfonate will be determined experimentally in the future rather than based on balance as shown in Fig. 4.

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