Bioresource Technology 102 (2011) 8921–8929

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

High titer ethanol production from simultaneous enzymatic saccharification and fermentation of aspen at high solids: A comparison between SPORL and dilute acid pretreatments ☆

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ARTICLE INFO

Article history:
Received 16 May 2011
Received in revised form 6 July 2011
Accepted 15 July 2011
Available online 22 July 2011

Keywords:
(Woody) Biomass
Pretreatment
High solids
Simultaneous saccharification and fermentation (SSF)
Cellulosic ethanol titer

ABSTRACT

Native aspen (*Populus tremuloides*) was pretreated using sulfuric acid and sodium bisulfite (SPORL) and dilute sulfuric acid alone (DA). Simultaneous enzymatic saccharification and fermentation (SSF) was conducted at 18% solids using commercial enzymes with cellulase loadings ranging from 6 to 15 FPU/g glucan and *Saccharomyces cerevisiae* Y5. Compared with DA pretreatment, the SPORL pretreatment reduced the energy required for wood chip size-reduction, and reduced mixing energy of the resultant substrate for solid liquefaction. Approximately 60% more ethanol was produced from the solid SPORL substrate (211 L/ton wood at 59 g/L with SSF efficiency of 76%) than from the solid DA substrate (133 L/ton wood at 35 g/L with SSF efficiency 47%) at a cellulase loading of 10 FPU/g glucan after 120 h. When the cellulase loading was increased to 15 FPU/g glucan on the DA substrate, the ethanol yield still remained lower than the SPORL substrate at 10 FPU/g glucan.

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1. Introduction

Many technological barriers remain for the economical production of ethanol from lignocellulosic biomass. Efficient and high titer ethanol production through the enzymatic saccharification and fermentation of cellulose is a promising process strategy. Despite recent progress in the development of lignocellulosic pretreatments (Sun and Cheng, 2002; Zhu and Pan, 2010) and in enzymatic hydrolysis of cellulose (Mansfield et al., 1999; Zhang and Lynd, 2004), few studies have demonstrated efficient high titer (>40 g/L) ethanol production. Furthermore, there have been only limited investigations on the simultaneous enzymatic saccharification and fermentation (SSF) at high-solids loadings. Most of the reported studies resulted in low conversion efficiencies unless very high cellulase loading levels were used (Jorgensen et al., 2007; Lu et al., 2010; Zhang et al., 2010). For example, an enzyme loading of 21.5 FPU/g glucan (7 FPU/g substrate) produced an ethanol concentration of 25 g/L with an SSF efficiency of approximately 68% using steam exploded corn stover at 200 °C, at a solids loading of 20% (Zhang et al., 2010). Other examples include an SSF efficiency less than 50% using hot water pretreated wheat straw at 205 °C that was hydrolyzed and fermented at 20% solids using a cellulase loading of 11.8 FPU/g glucan (Jorgensen et al., 2007), and a saccharification efficiency of approximately 70% using a dilute acid (DA) pretreated corn stover that was enzymatically saccharified at 20% solids with a cellulase loading of 15 FPU/g glucan (Dasari and Berson, 2007). In addition, it was shown that cellulose conversion decreased rapidly with further increases in substrate solids loading (Zhang et al., 2010).

Many factors affect enzymatic saccharification kinetics and fermentation efficiency at high-solids loadings. Product (sugar) inhibition can significantly affect enzymatic saccharification at high sugar concentrations (Holtzapple et al., 1990). However, this effect can be eliminated by using SSF to maintain a low sugar concentration throughout the fermentation process. Intensive mixing at high-solids may also play an important role in the saccharification rate of cellulose. Different mixers, such as a peg mixer (Zhang et al., 2009), helical ribbon mixers (Zhang et al., 2010), and a tumbling reactor (Jorgensen et al., 2007), have been investigated. But mixing energy consumption was not reported. This makes it difficult to assess mixing efficiency and process energy requirements for enzymatic saccharification. The cellulose accessibility of the pretreated substrate is an important factor affecting enzymatic saccharification efficiency. In one study, a highly digestible organosolv pretreated poplar wood substrate hydrolyzed at 20% solids using

☆ This work was conducted on official US government time by Zhu, Gleisner, and Scott while Luo and Tian were visiting scientists at the USDA Forest Products Laboratory.

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0960-8524/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.biortech.2011.07.047

cellulase loading of 20 FPU/g glucan, achieved a relatively high enzymatic saccharification efficiency (80%) resulting from a glucose concentration of around 150 g/L even with the presence of strong product inhibition (Zhang et al., 2009). However, few studies have compared the SSF efficiencies of different pretreated substrates from the same feedstock under high solids loadings (>10%).

The objective of the present study is to examine the effectiveness of a new wood chip pretreatment process, SPORL – Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses (Zhu et al., 2009a) for high titer ethanol production through SSF of the solid fraction from natural aspen (*Populus tremuloides*). The SPORL process has been shown to be effective as a robust and efficient pretreatment in terms of enzymatic cellulose conversion and ethanol production, particularly when applied to highly recalcitrant softwood species (Tian et al., 2010; Zhu et al., 2010a). It has also been shown to be effective on less recalcitrant feedstocks such as natural aspen (*P. tremuloides*) (Tian et al., 2011; Wang et al., 2009). The low recalcitrance nature of natural aspen was evidenced from the near complete cellulose saccharification at solids loading of 2% with an enzyme dosage of 10 FPU/g glucan after a mild DA pretreatment at 170 °C for approximately 20–30 min (Tian et al., 2011). The advantage of SPORL pretreatment over DA for ethanol production from aspen through SSF is marginal at low-solids loadings (Tian et al., 2011). It would be of interest to compare SPORL and DA for high titer ethanol production under high-solids loadings. This is because high-solids loading can reduce capital cost (Wingren et al., 2003) and process water usage while increasing ethanol titer to reduce distillation energy and cost, significant to commercial productions.

In the present study, both SPORL and DA pretreatments were applied directly to commercial aspen wood chips. The selected SPORL and DA pretreatment conditions were based on a previous study using the same wood chips (Tian et al., 2011). After pretreatment, the liquor was decanted and the chips were disk-milled to prepare a substrate for high solids SSF. We then measured the ethanol produced and process energy consumed to quantify pretreatment effectiveness at high-solids loadings.

2. Methods

2.1. Raw materials and chemicals

Fresh natural aspen (*P. tremuloides*) wood logs were obtained from northern Wisconsin, USA and debarked and chipped at the Forest Products Laboratory, Madison, Wisconsin. The sizes of the wood chips used for the study ranged from 6 to 38 mm in two dimensions with a thickness variation from 1 to 5 mm. The chips were stored in a freezer at a temperature of approximately −16 °C until used.

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

2.2. Microorganism and culture

Saccharomyces cerevisiae Y5 (Strain preserved No. CGMCC2660, China General Microbiological Culture Collection Center) was from Capital Normal University of Beijing, China. Details of the inhibitor-tolerance profiles of this yeast was reported (Li et al., 2009). The strain was grown at 30 °C for 2 days on YPD-agar plates containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 20 g/L agar. A colony from the plate was then transferred by loop to a liquid YP medium supplemented with 30 g/L glucose in a flask. The *S. cerevisiae* Y5 seed was grown overnight at 35 °C with agitation at 90 rpm on a shaking bed. The harvested culture was centrifuged at 5000*g* (International Centrifuge, Boston, MA, model EXD) for 5 min at 20 °C to yield cell pellets after decanting the supernatant.

2.3. SPORL and dilute acid (DA) substrate production

SPORL and DA pretreatments were directly applied to aspen wood chips for substrate production according to the schematic flow diagram shown in Fig. 1. A laboratory wood pulping digester of capacity of 23 L was used to conduct pretreatment as described in our previous study (Zhu et al., 2009a). The digester was heated by a steam jacket and rotated at 2 rpm for mixing. The oven dry (od) weight of wood chips in each pretreatment was 2 kg. The pretreatment liquid to wood ratio (L/W) was kept at 3 (v/w) and the temperature was fixed at 170 °C for both SPORL and DA pretreatments. The temperature ramping time to 170 °C was about 10 min. Pretreatment duration at 170 °C was fixed at 25 min. The sulfuric acid charge was 1.10% (w/w) on od wood basis for both SPORL and DA pretreatments. Sodium bisulfite charges were 3.0% and 0% (w/w) for the SPORL and DA pretreatments, respectively. These pretreatment conditions were selected for two reasons: (1) the sugar yields were close to their respective optimal values for both SPORL and DA based on a previous study using a total of 51 different SPORL and DA pretreatments of 150 gram capacity utilizing a 1 L autoclave type reactor at a temperature range of

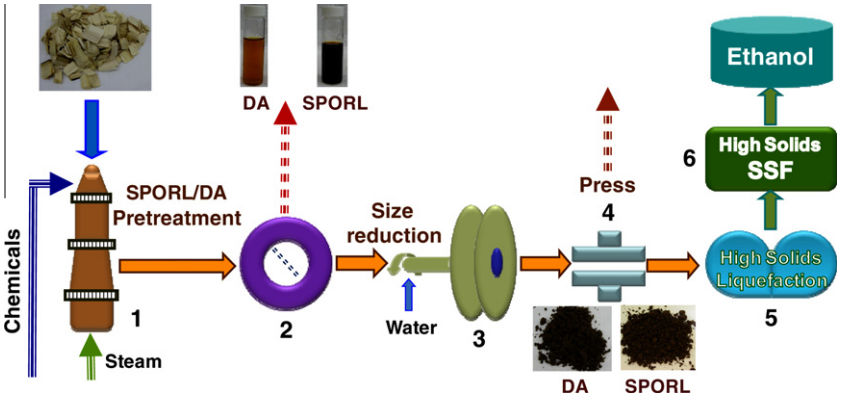

Fig. 1. Schematic experimental flow diagram showing pretreatment, substrate production, solid substrate liquefaction, and simultaneous enzymatic saccharification and fermentation. (1) Digester; (2) Screen; (3) Disk mill; (4) Vacuuming canvas bag; (50) Torque rheometer; (6) Shaking incubator.

160–180 °C, sulfuric acid and sodium bisulfite charges of 0–3.3% and 0–4.5%, respectively, and pretreatment duration of 0–30 min (Tian et al., 2011). Moreover, statistical analyses indicated that the sugar yield response surfaces were flat in the vicinity of the selected conditions for both SPORL and DA. (2) The comparisons of ethanol production between SPORL and DA can be made under the identical conditions except for the 3% sodium bisulfite applied in the SPORL run. The initial liquor pH was measured at 1.5 and 2.0 for the DA and SPORL pretreatments, respectively, prior to adding wood chips. After digestion, the hydrolysate was collected.

The pretreated wood chips remained intact and were disk milled using plates of pattern D2B-505 with a disk plate gap of 0.25 mm and adjusted to a refiner inlet consistency of 10% with dilution water. The discharging solids consistency was reduced further to approximately 2.5% to 4.0% by adding water at the refiner outlet. The energy consumption for disk milling was recorded as described elsewhere (Zhu et al., 2009b, 2010b). The size-reduced solids were directly dewatered to a solids content of about 30% by vacuum pressing in a canvas bag, without any additional washing, therefore it may contain a small amount of dissolved solids. 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 remaining substrates after the first set of SSF experiments were frozen at −16 °C. The chemical compositions of the substrates along with the spent liquors (pretreatment hydrolysates) were analyzed according to the methods described later in the text. The results are listed in Table 1.

2.4. Enzymatic saccharification and fermentation of solid substrates

A torque rheometer (Ehrhardt et al., 2010) was used to continuously mix enzymes with the wood substrates at high-solids concentrations between 12 and 18%. It has been shown that the addition of cellulase enzymes to acid-hydrolyzed corn stover could significantly reduce mixing torque (Samaniuk et al., 2011). The torque rheometer has two identical cylindrical stainless steel mixing chambers. Two chrome-plated steel impellers (non-intermeshing) counter-rotate at a 3:2 differential speed to impose shear on the fiber suspension. The torque rheometer was designed to replicate the intense shear mixing that occurs in a twin screw extruder or other similarly intense mixing reactor. The total volumetric capacity of the torque rheometer is 100 mL. In this study, cellulase enzymes were added to the pretreated wood substrate to initiate enzymatic saccharification while mixing proceeded continuously. The torque response was recorded and specific mixing energy calculated as a means to characterize the degree of liquefaction as a function of energy input.

The pretreated substrates were prepared for enzymatic hydrolysis and fermentation by adjusting the target consistency with a sodium acetate buffer solution (pH 4.8). The buffer solution was first poured into the mixing chamber of the torque rheometer before adding the substrate. The suspension was then premixed for 2 min at 55 rpm and 50 °C and then stopped for the addition of enzymes. A predetermined volume of enzymes was then added and the torque rheometer restarted. Each liquefaction experiment used 80 grams of sample (substrate + buffer + enzymes). Enzyme loading ranged from 6 to15 FPU/g glucan for Celluclast 1.5 L with Novozyme 188 at 9 to 22.5 CBU/g glucan, for both SPORL and DA substrates. Liquefaction experiments ranged from 30 min to 1 h. After liquefaction, the slurry was transferred to a 120 mL screw-top plastic cup. For sequential hydrolysis and fermentation (SHF), saccharification continued at 50 °C for 120 h on the shaking bed incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 200 rpm. Fermentation was then conducted with the addition of the harvested yeast culture, after centrifuging, to an initial cell concentration of 2 g wet cell weight per liter. For simultaneous saccharification and fermentation (SSF), an aliquot of harvested yeast culture, was added to the slurry to the same initial cell concentration of 2 g wet cell weight per liter for fermentation. No additional nutrients were added in all fermentation experiments. Fermentation was carried out by placing the plastic cup in the shaking bed incubator at 35 °C and 90 rpm. Samples of the fermentation broth were taken periodically and centrifuged at 10,000*g* for 5 min and were stored at −4 °C until analyzed for sugar and ethanol.

2.5. Sampling of fermentation broth and analytical methods

The chemical compositions of the original and pretreated biomass were analyzed by the Analytical and Microscopy Laboratory of the Forest Products Laboratory as described previously (Luo et al., 2010). All biomass samples were Wiley milled (model #2, Arthur Thomas Co., Philedelphia, PA). The milled sample of 20 mesh (~1 mm) in size was hydrolyzed in two stages using sulfuric acid of 72% (v/v) at 30 °C for 1 h and 3.6% (v/v) at 120 °C for 1 h. The hydrolysate supernatant and remaining solids are then filtered through a Gooch Crucible lined with a 21 mm Whatman filter into a volumetric flask. The supernatant was used for carbohydrate analysis using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD). Klason lignin (acid insoluble) retained on the filter paper was quantified gravimetrically after drying.

To determine glucose concentration during high-solids enzymatic hydrolysis, we developed a sampling technique that entails periodically extracting a 0.5–1.0 g sample from the torque rheometer (Kristensen et al., 2009; Samaniuk et al., 2011), and placing the sample into a 15 mL polypropylene screw-cap centrifuge tube (United Laboratory Plastic, St. Louis, MO) containing 10 mL of sodium bicarbonate buffer solution (pH 10.5) to stop hydrolysis

Table 1
Chemical compositions of untreated and pretreated aspen wood chips and pretreatment hydrolysates. Unless indicated, data are in wt% of substrate. Liquid to wood ratio (L/W) in pretreatment was 3.

	Untreated aspen	SPORL-pretreated solids	DA-pretreated solids	SPORL hydrolysate[a] (g/L)	DA hydrolysate[a] (g/L)
Solids yield (g/kg wood)	1000.0	581.5	634.5		
Glucan	45.61	66.23	61.57	3.95	3.74
Xylan	16.35	1.94	3.15	34.81	32.64
Mannan	1.41	0.30	0.35	3.19	3.23
Klason Lignin	20.20	26.10	29.95	16.74	3.99
Arabinan	0.35	0.01	0.03	1.05	0.95
Galactan	0.46	0.02	ND	1.30	1.28
Furfural				2.72	3.79
HMF				0.86	0.89
Acetic acid				17.58	15.57
Levulinic acid				0.87	3.84

[a] Sugars are expressed as hexosan or pentosan.

without boiling the sample. The tube and buffer solution were weighed before and after adding the sample. During the early stages of hydrolysis, the substrate slurry was very viscous and the sample is collected with a spatula. After considerable liquefaction, the slurry was collected by pipetting. Each sample is then briefly shaken, weighed, and placed under refrigeration. Just prior to glucose analysis, the samples were placed on a vortex shaker for 10 s and then centrifuged for 10 min at 900*g*. The supernatant was then sampled for glucose using a Biochemistry Analyzer (YSI 2700D, YSI Inc., Yellow Springs, OH). Based on the sodium bicarbonate dilution level, the glucose reading was converted to an un-diluted value which was then used to determine the percent cellulose conversion per National Renewable Energy Laboratory (NREL) Analytical Procedure – LAP-009 (Brown and Torget, 1996). The standard deviation in sugar analysis was 2.5% based on duplicate measurements.

Ethanol analysis in the cellulosic substrate fermentation broth was carried out using a gas chromatograph (GC, model 7890, Agilent Technologies, Palo Alto, CA) through direct sample injection using an external standard for calibration (Tian et al., 2011). The sample of the fermentation broth was also centrifuged and the supernatant was used for GC injection. Reported results are the average of duplicates with an average relative standard deviation of approximately 4%.

2.6. Measuring substrate cellulose accessibility to cellulase

Cellulose accessibility to cellulase was evaluated by measuring the adsorption of a commercial endoglucanase, Fibercare®, using a UV–Vis spectrophotometric method (Liu et al., 2011). The method corrected for cellulase adsorption by the leached lignin using a dual-wavelength method. Approximately 30 mL of substrate suspension containing 1 g/L glucan and 5 g/L BSA in 50 mM citrate buffer at pH 4.8 were first well mixed using a magnetic stir at a speed of 200 rpm at 25 °C. The application of BSA was to block the adsorption of cellulase by lignin on the solid substrate (Zhu et al., 2009c) based on the nonspecific binding between BSA and lignin(Yang and Wyman, 2006). After approximately 1 h mixing, 0.36 mL Fibercare®, equivalent to an endoglucanase concentration of 77.7 mg/L, was added to the substrate suspension. The suspension was continuously circulated through a flow loop into a glass cuvette of 1 mm optical path length using a peristaltic pump (Model M312, Gilson, Middleton, WI). A mesh screen was used at the inlet of flow loop and a fiber mat was developed on the screen preventing fibers entering the glass cuvette. The protein concentration in the solution was continuously monitored by a UV–Vis spectrophotometer (model 8453 Agilent, Palo Alto, CA) using absorption at 280 nm. Cellulase adsorption experiments were at least duplicates. The average data were reported and one standard deviations were used as error bars.

2.7. Definitions and calculations

The following definitions to quantify and compare the efficiencies of pretreatment and ethanol production were used. The pretreatment efficiency, $\eta_{\text{Pretreatment}}$, was defined as the sugar yield (kg/ton wood) from unit energy input (MJ/ton wood) for pretreatment including energy used for biomass size reduction (Zhu and Pan, 2010).

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

Fermentation efficiency, $\eta_{\text{Fermentation}}$, was defined as the amount of ethanol produced (from solids substrate only in this study) as a percentage of the theoretical maximal amount of ethanol based on the amount of the saccharide in the substrate (only glucan in the present study).

$$\eta_{\text{Fermentation}} = \frac{\text{Ethanol from substrate}\left(\frac{\text{kg}}{\text{kg substrate}}\right)}{\left(\frac{0.511}{0.9}\right) \times \text{glucan content of substrate}\left(\frac{\text{kg}}{\text{kg substrate}}\right)} \quad (2)$$

It should be pointed out that fermentation efficiency is different from ethanol yield that is defined as the amount of ethanol produced (from solids substrate only in this study) as a percentage of the theoretical maximal amount of ethanol based on the amount of the saccharide in the untreated wood (only glucan in the present study), i.e.,

$$y_{\text{Ethonal}}\left(\frac{\text{L}}{\text{ton}}\right) = \frac{\text{Ethanol from substrate}\left(\frac{\text{L}}{\text{kg substrate}}\right) \times \text{Solid substrate yield}\left(\frac{\text{kg substrate}}{\text{ton wood}}\right)}{(0.511/(0.9 \times 0.789)) \times \text{glucan content of wood}\ (\text{kg}/(\text{ton wood}))}$$

Left side of Eq. (3), the unit should %, not L/ton (3)

The amount of ethanol produced is determined from the measured ethanol concentration in the fermentation broth, the total volume of the fermentation broth, and the od weight of the substrate used.

For mass and energy balance analyses, component yields from the solid fraction were determined based on the measured chemical composition of the substrate and the yield of the solid fraction. Component yields in the pretreatment hydrolysates (spent liquors) are based on the measured component concentrations (Table 1) and the pretreatment liquor to wood ratio of L/W = 3. Wood chipping energy was estimated to be 0.18 GJ/ton wood based on pulp mill experience (Klugman et al., 2007). Pretreatment thermal energy was based on thermodynamic calculations of enthalpy of the pulp suspension at 25% solids consistency (L/W = 3) at the pretreatment temperature, with the consideration of 50% thermal energy recovery (Zhu et al., 2010a). Energies for size reduction of the pretreated woodchips were reported on untreated wood base by dividing the total measured milling energy by the amount of untreated wood used (2 kg). The mixing energy for SSF was estimated as will be discussed later in the text. The final ethanol production energy efficiency is defined as the net energy output from ethanol (solid fraction only in this study) divided by the total energy input (Zhu et al., 2020).

$$\eta_{\text{Ethanol}} = \frac{0.789 \times 29.7\left(\frac{\text{GJ}}{\text{ton ethanol}}\right) \times 0.001 y_{\text{Ethanol}}\left(\frac{\text{L}}{\text{ton wood}}\right) - \text{Total energy input}\left(\frac{\text{GJ}}{\text{ton wood}}\right)}{\text{Total energy input}\left(\frac{\text{GJ}}{\text{ton wood}}\right)}. \quad (4)$$

3. Results and discussions

3.1. Experimental repeatability and verification

Good experimental repeatability is required to validate experimental procedures and quantify the effects of various experimental parameters on ethanol production. Three separate groups of SSF

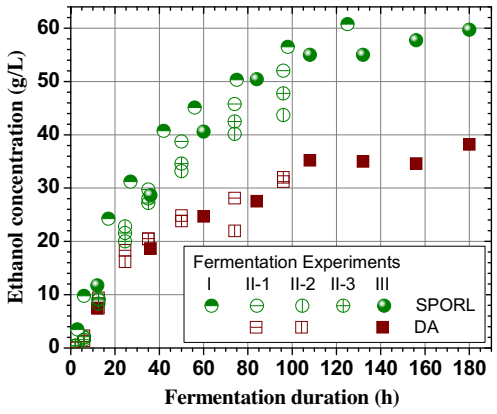

Fig. 2. Experimental repeatability and comparison of time-dependent ethanol concentrations in fermentation broths using SPORL and DA substrate. Solids loading 18% and cellulase loading 10 FPU/g glucan.

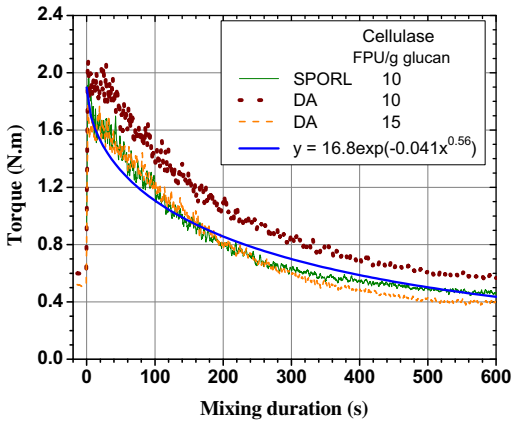

Fig. 3. Comparisons of time-dependent torque of the rheometer rotor between SPORL and DA liquefaction runs at two cellulase loadings.

experiments were conducted over a 6-month period. Three replicate SSF runs using the SPORL-pretreated substrate and two replicate runs using the DA substrate were carried out in the second (II) group experiments (September 2010). Single replicate runs were conducted in the third (III) group (December 2010) using both SPORL and DA substrate while the first (I) group (July 2010) had single run with SPORL substrate only (labels I, II and III shown in Fig. 2). Several additional runs were also conducted at varying solids levels and cellulase loadings over the three groups. The discrepancies in ethanol concentration among the 5 SPORL runs shown in Fig. 2 may be attributed to the fact that the experiments were conducted over a 6-month period and the possibility of non-representative sampling since fermentation was conducted on a shaking bed at 90 rpm without shear mixing. However, the measured time-dependent ethanol concentrations in the fermentation broth showed good repeatability for all the SSF runs conducted using both the SPORL- and DA-pretreated substrate (Fig. 2), when conducted at 18% solids and 10 FPU/g glucan cellulase loading. The mean values from all replicates are therefore used for data presentation in the discussion below. One standard deviations were used as error bars.

The effect of liquefaction duration in the torque rheometer on ethanol production in subsequent SSF treatments was verified using the SPORL-pretreated substrate at solids loading of 18% and 10 FPU/g cellulose. There was no difference in the measured time-dependent ethanol concentrations throughout the fermentation process between the two SSF runs with 30 min (data not shown) and 60 min liquefaction duration. However, all samples reported were liquefied for 1 h in the torque rheometer before being transferred to the shaking bed incubator.

3.2. Mixing energy and solid liquefaction

In torque rheometry experiments, the measured time-dependent torque response of the mixer can be related to the degree of liquefaction of the solids by enzymes. It can also be used to determine the mixing energy. Solid liquefaction took place rapidly as evidenced by the exponential decrease (although using 4th order polynomial produced a better fit) in the measured time-dependent torque of the mixer (Fig. 3). For most tests, the torque was reduced by 50% from its peak value within approximately 2 min. However, due to the limited torque sensitivity at very low torque levels and the asymptotic nature of the exponential decay function of the time-dependent torque curve, it is difficult to accurately determine the time required to achieve liquefaction. Therefore, the time required to achieve 50% reduction in torque from its peak value

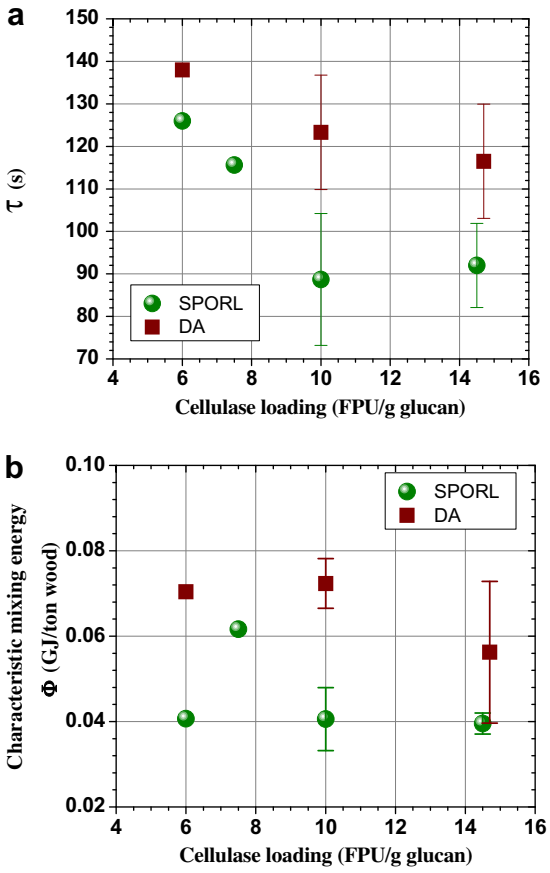

Fig. 4. Comparisons between SPORL and DA pretreatment on the (a) characteristic liquefaction time, τ, and (b) characteristic mixing energy Φ at solids loading 18% under various cellulase loadings.

measured before enzyme addition was used to represent a characteristic time scale, τ, for liquefaction. The characteristic liquefaction time τ decreased from 130 to 90 s, and 140 to 120 s for the SPORL and DA substrate, respectively, at solids loading of 18% when cellulase loading was increased from 6 to 15 FPU/g glucan (Fig. 4a). The results in Fig. 4a also suggest that the initiation of liquefaction of the SPORL substrate occurred earlier than the DA substrate at all cellulase loading levels. The measured time-dependent torques for the DA substrate were consistently greater than those

Table 2
Comparisons of yields and energy efficiencies of ethanol (from SSF of solid fraction only) production between SPORL and DA pretreatments.

Run label[a]	Wood chip milling energy (GJ/ton)	Liquefaction energy, Φ (GJ/ton)	Total energy input (GJ/ton)[b]	Ethanol titer @ 120 h (g/L)	$y_{Ethanol}$ @ 120 h (L/ton)	$\eta_{Fermentation}$ @ 20 h (%)	SSF ethanol energy (GJ/ton)	Net ethanol energy (GJ/ton) [c]	$\eta_{Ethanol}$ @120 h (%) [c]
SP-S12-C10	0.193	0.016	1.506	40.8	229.6	82.9	5.380	3.875	257
SP-S15-C10	0.193	0.030	1.562	52.5	230.6	83.2	5.404	3.842	246
SP-S18-C10	0.193	0.041	1.606	59.3	210.7	76.0	4.937	3.331	207
SP-S18-C6	0.193	0.041	1.606	37.7	129.8	46.8	3.041	1.435	89
SP-S18-C7	0.193	0.062	1.690	43.0	150.5	54.3	3.527	1.837	109
SP-S18-C15	0.193	0.040	1.601	60.0	213.3	77.0	4.999	3.398	212
DA-S18-C6	0.570	0.070	1.725	25.3	95.1	33.8	2.228	0.503	29
DA-S18-C10	0.570	0.072	2.109	35.1	133.1	47.3	3.119	1.009	48
DA-S18-C15	0.570	0.057	2.046	52.9	203.8	72.5	4.775	2.730	133

[a] SP stands for SPORL, DA stands for dilute sulfuric acid, Sxx stands for solids loadings (w/w) percentage, Cxx stands for cellulase loadings in FPU/g glucan.
[b] Including estimated wood chipping energy of 0.18 GJ/ton wood and thermal energy of 1.25 GJ/ton wood for pretreatment at 170 °C with liquid to solid ratio of 3 determined by thermal dynamic calculations with 50% thermal energy recovery. Mixing energy for SSF is estimated to be four times of the characteristic liquefaction energy Φ. Distillation energy not included.
[c] Before distillation.

for the SPORL substrate (Fig. 3), even though the torque measured before cellulase addition, averaged over a 2 min period for both substrates, were very similar, i.e., 1.90 ± 0.13 and 1.88 ± 0.10 N m for DA and SPORL, respectively, suggesting a higher rate of liquefaction for the SPORL substrate than that of the DA substrate.

The characteristic liquefaction energy, Φ, consumed in the first 10 min of mixing in the torque rheometer was used to characterize the differences in the energy for liquefaction. Again, the limited measurement resolution at low torque values prevented us from determining the total liquefaction energy consumed for the entire liquefaction period (1 h). The rapid reduction (exponential decay) in the viscosity of the slurry resulted in very low torque, i.e., low energy consumption, beyond 10 min of mixing. Therefore Φ, calculated for the first 10 min of mixing, represents approximately 50% of the energy consumed in 1 h. The greater torque data of the DA substrate as shown in Fig. 3 results in a higher characteristic liquefaction energy, Φ, than that of SPORL substrate. The characteristic liquefaction energy Φ for the DA substrate was approximately 0.070 GJ/ton wood for enzyme loadings of 6 and 10 FPU/g glucan, or approximately 50% greater than the 0.041 GJ/ton wood for the SPORL substrate under the same enzyme loadings (Fig. 4b and Table 2). Even at an enzyme loading of 15 FPU, the characteristic liquefaction energy Φ for DA substrate remains 0.057 GJ/ton wood, higher than that for the SPORL substrate at enzyme loading of 10 FPU/g glucan (Table 2).

3.3. Comparison of ethanol production between SSF and SHF of a SPORL-pretreated substrate

SSF can eliminate sugar inhibition at high solids loadings by maintaining a low glucose concentration in SSF broth and thereby enhance cellulose saccharification (Alfani et al., 2000; Olsson et al., 2006). As shown in Fig. 5a, the time-dependent glucose concentration in the fermentation broth using the SPORL-pretreated substrate is independent of solids loadings for the three solids loadings of 12%, 15%, and 18% conducted. The measured glucose concentration peaked at approximately 25 g/L in 3 h and then decreased rapidly to near zero at 15 h. Initial glucose concentration was as high as 95 g/L in SHF broth obtained using the same substrate at 18% solids loading after 120 h of saccharification. Glucose concentration did not drop to near zero until 40 h of fermentation. SHF should produce a faster initial rate of ethanol production than SSF simply because more glucose was initially available, and this can be clearly seen from the time-dependent ethanol concentration in the fermentation broth at solids loading of 12% (Fig. 5b). Ethanol concentration leveled out at approximately 40 h. It took approximately 80 h to achieve maximal ethanol concentration for the SSF at solids loading of 12%. The final ethanol concentrations were not much different at 12% solids loading, suggesting sugar inhibition on enzymatic cellulose saccharification was negligible at solids loadings below 12%. At solids loading of 18%, however, glucose availability is no longer an issue for fermentation in SSF but sugar inhibition is dominant in SHF. As a result, the initial rates of ethanol production for SHF and SSF were approximately the same at 18% solids (Fig. 5b). Sugar inhibition limits the release of glucose in SHF which may have resulted in a lower final ethanol concentration of less than 50 g/L reached at approximately 60 h. The removal of sugar inhibition in SSF enabled more sugar to be continuously released which in turn continuously increased ethanol production and resulted in a higher ethanol titer of approximately 60 g/L in 140 h.

3.4. Comparison of SPORL and DA pretreatment for high titer ethanol production through SSF

Demonstrating the advantages of SPORL over DA for cellulosic ethanol production at high solids SSF is more relevant to practical applications. Figs. 2 and 6 (averaged over replicate runs) compare the time-dependent ethanol concentrations in the fermentation broths from SPORL and DA SSF experiments at solids loading of 18%. The pretreatment conditions, i.e., temperature, acid charge, and duration, for both SPORL and DA were identical except that 3% sodium bisulfite on od wood charge was added in the SPORL pretreatment. The results show that the ethanol concentrations in the SPORL broths were significantly higher than those in the corresponding DA broths. This is especially true at low enzyme dosages, i.e., 10 FPU/g glucan (Fig. 2) and 6 FPU/g glucan (Fig. 6). The ethanol concentrations in the SPORL fermentation broths were approximately 50% higher than those in the corresponding DA broths when fermentation duration was extended to 120 h and beyond at these two enzyme dosages. To account for the effect of the slightly higher glucan content of the SPORL substrate (66.2%, Table 1) than that of the DA substrate (61.6%, Table 1) on ethanol concentration shown in Figs. 2 and 6, we determined the SSF efficiencies for the experiments conducted. The results clearly show that the SSF efficiencies of the SPORL substrate are approximately 40% higher than those of the DA substrate in 96 h fermentation at both enzyme loadings of 6 and 10 FPU/g glucan (Fig. 7). The fermentation efficiency of the SPORL substrate was 76.0% compared to 42.4% for the DA substrate when SSF was extended beyond 120 h at an enzyme loading of 10 FPU/g substrate (Table 2). This clearly indicates the advantages of SPORL over DA in terms

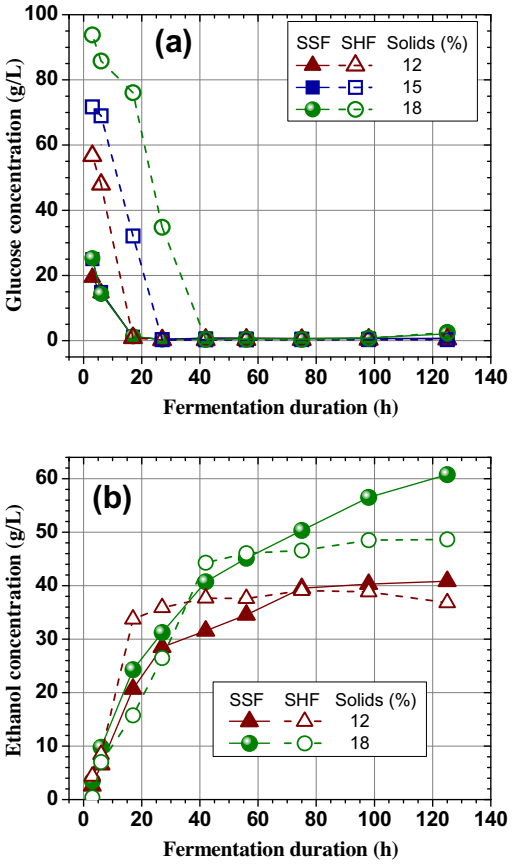

Fig. 5. Comparisons between SSF and SHF on time-dependent (a) glucose and (b) ethanol concentrations in the fermentation broths using the SPORL substrate at various solids loadings.

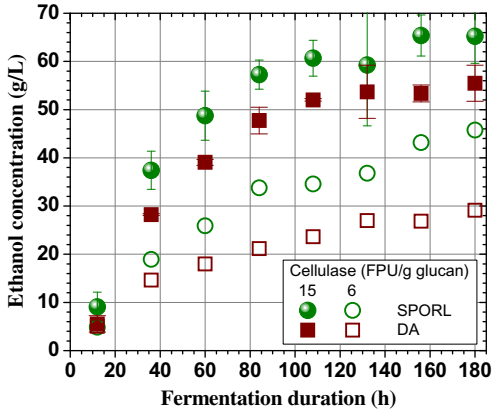

Fig. 6. Comparison of time-dependent ethanol concentrations in fermentation broths using SPORL and DA substrate at low (6 FPU/g glucan) and high (15 FPU/g glucan) cellulase loadings at 18% substrate solids.

of ethanol productivity at high solids SSF even when applied to a very low recalcitrant feedstock, namely aspen.

The cellulose accessibility of the SPORL and DA substrate was found to be 29 ± 11 and 28 ± 8 mg protein/g glucan, respectively, based on adsorption measurements using a commercial endoglucanase, Fibercare® of Novozyme. The difference is well within the measurement standard deviations. This suggests that both SPORL and DA pretreatments were effective in terms of opening the physical structure to increase cellulose accessibility to cellulase.

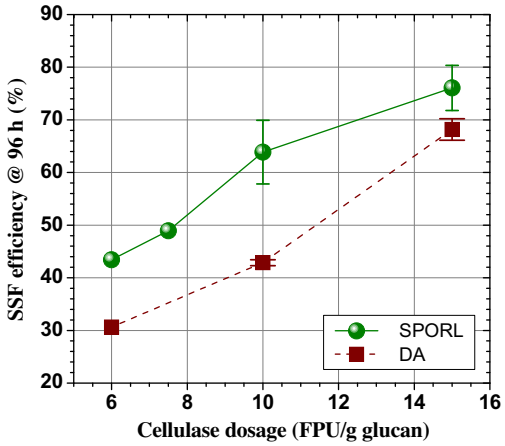

Fig. 7. Comparisons of SSF fermentation efficiencies between SPORL and DA runs in 96 h at 18% solids and various cellulase loadings.

However, the remaining xylan and lignin (insoluble or soluble) on a solid substrate can reduce cellulase activity through nonproductive adsorption (Qing et al., 2010; Sewalt et al., 1997; Yang and Wyman, 2006). As described earlier in substrate production, separate washing of the two substrates were not applied. Therefore some fractions of the measured xylan and lignin can be soluble solids that are more inhibitive. Furthermore, inhibitory components, especially lignin, can be leached or released from the substrate into the hydrolysate as saccharification proceeds. Lingin leaching in fiber suspension is well known (Chai et al., 2001; Liu et al., 2011). Some small solid residual particles resulting from hydrolysis contained mainly lignin and can also affect cellulase activity. At high solids loadings, the concentrations of these inhibitory components can be high in the enzymatic hydrolysates. The xylan and lignin contents in the DA substrate were higher than those in the SPORL substrate (Table 1), suggesting more severe cellulase inhibition could take place in enzymatic hydrolysis of the DA substrate. Furthermore, the differences in the chemical structures of the lignin and xylan in the SPORL and DA substrates may also affect cellulase activity differently. These arguments are supported by the fact that when the cellulase loading was increased to approximately 14 FPU/g glucan (to compensate non-productive adsorption), the SSF fermentation efficiency of the DA substrate matched that of the SPORL substrate achieved at cellulase 10 FPU/g glucan (Fig. 7).

3.5. Comparisons of mass and energy balances for SPORL and DA pretreatments

Mass and energy balances between SPORL and DA pretreatment were compared using the SSF runs conducted at 18% solids with cellulase loading of 10 FPU/g glucan to provide information about these two processes. All mass data (in kg) are underlined and energy data (in GJ/ton wood) are shown with the vertical arrows and bold face font in Fig. 8. The sugar yields from the SPORL pretreatment hydrolysate were slightly higher than those from DA pretreatment, in agreement with our previous study using a smaller reactor (Tian et al., 2011). The substrate SSF mixing energy for a period of 100 h is conservatively estimated to be double the mixing energy in the first hour, because the average mixing energy after 1 h was reduced to approximately 1% of the average mixing energy in the first hour. Recall Φ approximately represents half of the mixing energy in the first hour. Therefore, the total mixing energy is approximately four times of the characteristic liquefaction energy, Φ, determined from the first 10 min of torque data. The ethanol energy data were determined using the measured ethanol

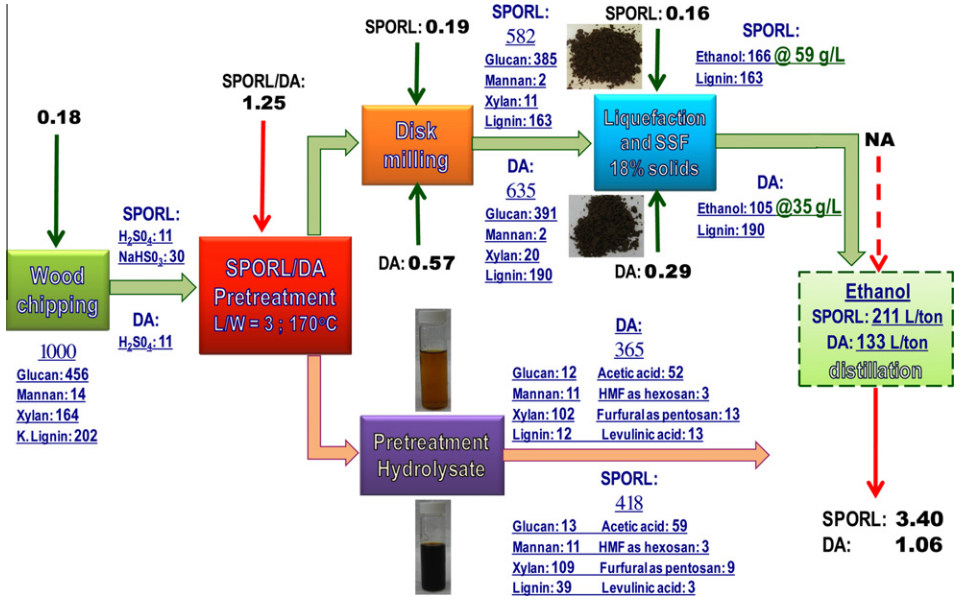

Fig. 8. Mass and energy balance comparisons between SPORL and DA pretreatment of aspen and subsequent disk milling, liquefaction, and SSF at 18% solids and 10 FPU/g glucan cellulase loading. Unless indicated, energy data (bold italic font with vertical arrows) are in GJ/ton wood and mass data (underlined) are in kg.

concentration at 120 h SSF. The results indicate that the SPORL pretreatment reduced energy consumption in post-pretreatment size reduction, as well as energy for mixing the resultant solid substrate during liquefaction and SSF (Table 2). More importantly the SPORL pretreatment produced higher SSF efficiency of 76% and titer of 59 g/L than the corresponding values of 42% and 35 g/L from the DA pretreatment (Table 2). This represents approximately an 80% and 70% increase in fermentation efficiency and ethanol titer, respectively, by SPORL (with the addition of only 3% sodium bisulfite on wood) over DA. As a result, a higher ethanol yield of 211 L/ton wood was achieved through SSF of the solid substrate from SPORL pretreatment compared to 133 L/ton wood from DA (Fig. 8 or Table 1), or an increase in ethanol yield of approximately 60% by SPORL over DA. This is despite the fact that the glucan yield (retained) on the SPORL solid substrate (385 kg/ton wood) was slightly lower than that on the DA substrate (391 kg/ton wood) as shown in Fig. 8. The net energy (from SSF ethanol only excluding energy from lignin and pretreatment hydrolysate sugars) output from the SPORL run was 3.33 GJ/ton wood ($\eta_{Ethanol}$ = 207%) before distillation. Only 1.01 GJ/ton wood net energy ($\eta_{Ethanol}$ = 48%) was produce from the DA run before distillation. Using an estimated distillation energy of 5.6 GJ/ton ethanol or approximately 1 GJ/ton wood by assuming ethanol yield is 225 L/ton wood (Stampe et al., 1983), the net energy output after distillation was over 2.0 GJ/ton wood from the SPORL run compared with approximately zero from the DA run. Based on the results presented in Figs. 2, 6 and 7, a cellulase dosage of 15 FPU/g glucan is required for the DA substrate to achieve sensible net energy output of 2.73 GJ/ton wood which is still less than 3.33 GJ/ton wood from the SPORL run at 10 FPU/g glucan (Table 2). The results in Table 2 also indicate that a high cellulase loading of 15 FPU/g glucan applied to the SPORL substrate produced little benefit in terms of ethanol titer and net energy output.

The monomeric sugar yields from the SPORL and DA pretreatment hydrolysates were analyzed to further investigate the difference between the SPORL and DA pretreatments in terms of ethanol/sugar yield and net energy output as discussed above. SPORL resulted in slightly higher yields of glucose (13 kg/ton wood) and xylose (109 kg/ton wood) than the corresponding values of 12 and 102 kg/ton wood from DA (Fig. 8). Mannose yields were approximately the same for both pretreatments (11 kg/ton wood). SPORL produced significantly less furfural (9 kg/ton wood) than DA (13 kg/ton wood) (Fig. 8). This outcome came about because the DA pretreatment is much more acidic (initial = pH 1.5) than the SPORL run (initial pH = 2.0). The SPORL also produced a lower amount of levulinic acid for the same reason, in agreement with the SPORL and DA comparison using a softwood (Shuai et al., 2010). The potential effects of dissolved lignosulfonate as well as low furfural formation in the SPORL pretreatment hydrolysate on fermentation of the hemicellulosic sugar stream will be evaluated in the future using a xylose fermenting strain.

4. Conclusions

High titer ethanol production from SPORL-pretreated aspen was achieved through simultaneous enzymatic saccharification and fermentation (SSF) at solids loading of 18% with low to moderate cellulase loadings between 6 and 10 FPU/g glucan. When compared to DA pretreatment, SPORL not only reduced energy consumption for post pretreatment wood chip size-reduction, but also reduced mixing energy of the resultant substrate for solid liquefaction. Furthermore, approximately 60% more ethanol was produced (211 L/ton wood at 59 g/L) from the SPORL substrate than that from the DA substrate (133 L/ton wood at 35 g/L) at cellulase loading of 10 FPU/g glucan after 120 h SSF of the solid substrate. As a result, net energy from ethanol of 3.33 GJ/ton wood was produced from the SPORL run comparing to 1.01 GJ/ton wood from the DA run, before distillation. Even when the cellulase loadings was increased to 15 FPU/g glucan (or 50% more), the DA substrate still produced lower ethanol yield and titer than those from the SPORL substrate at 10 FPU/g glucan.

Acknowledgements

We acknowledge Diane Dietrich of US Forest Service, Forest Products Laboratory (USFS-FPL) for carrying out the many careful analyses of ethanol in fermentation broth samples, and Fred Matt (USFS-FPL) for carbohydrate composition analysis of the solid substrates. The USFS Program of Woody Biomass, Bioenergy, and Bio-

products (WBBB, 2009) provided financial support to Luo for his visiting appointments at the University of Wisconsin–Madison and USFS-FPL. The Ministry of Science and Technology (MOST) of China and USFS provided financial support to Tian for her visiting appointment at USFS-FPL.

References

Alfani, F., Gallifuoco, A., Saporosi, A., Spera, A., Cantarella, M., 2000. Comparison of SHF and SSF processes for the bioconversion of steam-exploded wheat straw. Journal of Industrial Microbiology and Biotechnology 25 (4), 184–192.

Brown, L., Torget, R. 1996. Enzymatic saccharification of lignocellulosic biomass, National Renewable Energy Laboratory, LAP-009.

Chai, X.-S., Zhu, J.Y., Li, J., 2001. A simple and rapid method to determine hexeneuronic acid groups in chemical pulps. Journal of Pulp and Paper Science 27 (5), 165–170.

Dasari, R.K., Berson, R.E., 2007. The effect of particle size on hydrolysis reaction rates and rheological properties in cellulosic slurries. Applied Biochemistry and Biotechnology 137, 289–299.

Ehrhardt, M.R., Monz, T.O., Root, T.W., Connelly, R.K., Scott, C.T., Klingenberg, D.J., 2010. Rheology of dilute acid hydrolyzed corn stover at high solids concentration. Applied Biochemistry and Biotechnology 160 (4), 1102–1115.

Holtzapple, M., Cognata, M., Shu, Y., Hendrickson, C., 1990. Inhibition of *Trichoderma reesei* cellulase by sugars and solvents *Biotechnology and Bioengineering* 36, 275–287.

Jorgensen, H., Vibe-Pedersen, J., Larsen, J., Felby, C., 2007. Liquefaction of lignocellulose at high-solids concentrations. Biotechnology and Bioengineering 96 (5), 862–870.

Klugman, S., Karlsson, M., Moshfegh, B., 2007. A Scandinavian chemical wood pulp mill. Part 1. Energy audit aiming at efficiency measures. Applied Energy 84 (3), 326–339.

Kristensen, J.B., Felby, C., Jørgensen, H., 2009. Determining yields in high solids enzymatic hydrolysis of biomass. Applied Biochemistry and Biotechnology 156, 557–562.

Li, T.F., Tian, S., Yu, Y., Zhou, X.S., Yang, X.S., 2009. Ethanol production from dilute-acid hydrolysate in situ detoxification by co-culture of yeasts, Acta Energiae Solaris Sinica. Acta Energiae Solaris Sinica 31 (11), 1582–1586.

Liu, H., Zhu, J.Y., Chai, X.S., 2011. In situ, rapid, and temporally resolved measurements of cellulase adsorption onto lignocellulosic substrates by UV–vis spectrophotometry. Langmuir 27 (1), 272–278.

Lu, Y., Wang, Y., Xu, G., Chu, J., Zhuang, Y., Zhang, S., 2010. Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass. Applied Biochemistry and Biotechnology 160, 360–369.

Luo, X., Gleisner, R., Tian, S., Negron, J., Horn, E., Pan, X.J., Zhu, J.Y., 2010. Evaluation of mountain beetle infested lodgepole pine for cellulosic ethanol production by SPORL pretreatment. Industrial and Engineering Chemistry Research 49 (17), 8258–8266.

Mansfield, S.D., Mooney, C., Saddler, J.N., 1999. Substrate and enzyme characteristics that limit cellulose hydrolysis. Biotechnology Progress 15, 804–816.

Olsson, L., Soerensen, H.R., Dam, B.P., Christensen, H., Krogh, K.M., Meyer, A.S., 2006. Separate and simultaneous enzymatic hydrolysis and fermentation of wheat hemicellulose with recombinant xylose utilizing *Saccharomyces cerevisiae*. Applied Biochemistry and Biotechnology 129, 117–129.

Qing, Q., Yang, B., Wyman, C.E., 2010. Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. Bioresource Technology 101 (24), 9624–9630.

Samaniuk, J.R., Tim Scott, C., Root, T.W., Klingenberg, D.J., 2011. The effect of high intensity mixing on the enzymatic hydrolysis of concentrated cellulose fiber suspensions. Bioresource Technology 102, 4489–4494.

Sewalt, V.J.H., Glasser, W.G., Beauchemin, K.A., 1997. Lignin impact on fiber degradation. 3. Reversal of inhibition of enzymatic hydrolysis by chemical modification of lignin and by additives. Journal of Agricultural and Food Chemistry 45 (5), 1823–1828.

Shuai, L., Yang, Q., Zhu, J.Y., Lu, F., Weimer, P., Ralph, J., Pan, X.J., 2010. Comparative study of SPORL and dilute acid pretreatments of softwood spruce for cellulose ethanol production. Bioresource Technology 101, 3106–3114.

Stampe, S., Alcock, R., Westby, C., Chisholm, T., 1983. Energy consumption of a farm-scale ethanol distillation system. Energy in Agriculture 2, 355–368.

Sun, Y., Cheng, J.Y., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology 83 (1), 1–11.

Tian, S., Luo, X.L., Yang, X.S., Zhu, J.Y., 2010. Robust cellulosic ethanol production from SPORL-pretreated lodgepolep pine using an adapted strain *S. Cerevisiae* without detoxification. Bioresource Technology 101, 8678–8685.

Tian, S., Zhu, W., Gleisner, R., Pan, X.J., Zhu, J.Y., 2011. Comparisons of SPORL and dilute acid pretreatments for sugar and ethanol productions from aspen. Biotechnology Progress 27 (2), 419–427.

Wang, G.S., Pan, X.J., Zhu, J.Y., Gleisner, R., 2009. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. Biotechnology Progress 25 (4), 1086–1093.

Wingren, A., Galbe, M., Zacchi, G., 2003. Techno-economic evaluation of producing ethanol from softwood: comparison of SSF and SHF and identification of bottlenecks. Biotechnology Progress 19 (4), 1109–1117.

Yang, B., Wyman, C.E., 2006. BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. Biotechnology and Bioengineering 94 (4), 611–617.

Zhang, J., Chu, D., Huang, J., Yu, Z., Dai, G., Bao, J., 2010. Simultaneous saccharification and ethanol fermentation at high corn stover solids loading in a helical stirring bioreactor. Biotechnology and Bioengineering 105, 718–728.

Zhang, X., Qin, W., Paice, M.G., Saddler, J.N., 2009. High consistency enzymatic hydrolysis of hardwood substrates. Bioresource Technology 100, 5890–5897.

Zhang, Y.H.P., Lynd, L.R., 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnology and Bioengineering 88 (7), 797–824.

Zhu, J.Y., Pan, X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. Bioresource Technology 101, 4992–5002.

Zhu, J.Y., Pan, X.J., Wang, G.S., Gleisner, R., 2009a. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. Bioresource Technology 100 (8), 2411–2418.

Zhu, J.Y., Wang, G.S., Pan, X.J., Gleisner, R., 2009b. Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. Chemical Engineering Science 64 (3), 474–485.

Zhu, J.Y., Zhu, W., OBryan, P., Dien, B.S., Tian, S., Gleisner, R., Pan, X.J., 2010a. Ethanol production from SPORL-pretreated lodgepole pine: preliminary evaluation of mass balance and process energy efficiency. Applied Microbiology and Biotechnology 86 (5), 1355–1365.

Zhu, W., Zhu, J.Y., Gleisner, R., Pan, X.J., 2010b. On energy consumption for size-reduction and yield from subsequent enzymatic saccharification of pretreated lodgepole pine. Bioresource Technology 101 (8), 2782–2792.

Zhu, Z., Sathitsuksanoh, N., Vinzant, T., Schell, D.J., McMillan, J.D., Zhang, Y.-H.P., 2009c. Comparative study of corn stover pretreated by dilute acid and cellulose solvent-based lignocellulose fractionation: Enzymatic hydrolysis, supramolecular structure, and substrate accessibility. Biotechnology and Bioengineering 103 (4), 715–724.