

## ***Using sulfite chemistry for robust bioconversion of Douglas-fir forest residue to bioethanol at high titer and lignosulfonate: A pilot-scale evaluation***

The Faculty of Oregon State University has made this article openly available.  
Please share how this access benefits you. Your story matters.

<b>Citation</b>	Zhu, J. Y., Chandra, M. S., Gu, F., Gleisner, R., Reiner, R., Sessions, J., ... & Anderson, D. (2015). Using sulfite chemistry for robust bioconversion of Douglas-fir forest residue to bioethanol at high titer and lignosulfonate: A pilot-scale evaluation. <i>Bioresource Technology</i> , 179, 390-397. doi:10.1016/j.biortech.2014.12.052
<b>DOI</b>	10.1016/j.biortech.2014.12.052
<b>Publisher</b>	Elsevier
<b>Version</b>	Version of Record
<b>Terms of Use</b>	<a href="http://cdss.library.oregonstate.edu/sa-termsfuse">http://cdss.library.oregonstate.edu/sa-termsfuse</a>



Contents lists available at ScienceDirect

# Bioresource Technology

journal homepage: [www.elsevier.com/locate/biortech](http://www.elsevier.com/locate/biortech)



## Using sulfite chemistry for robust bioconversion of Douglas-fir forest residue to bioethanol at high titer and lignosulfonate: A pilot-scale evaluation <sup>☆</sup>



J.Y. Zhu <sup>a,\*</sup>, M. Subhosh Chandra <sup>a,b</sup>, Feng Gu <sup>c,a</sup>, Roland Gleisner <sup>a</sup>, Rick Reiner <sup>a</sup>, John Sessions <sup>d</sup>, Gevan Marrs <sup>e</sup>, Johnway Gao <sup>e</sup>, Dwight Anderson <sup>e</sup>

<sup>a</sup>USDA Forest Service, Forest Products Lab, Madison, WI, USA

<sup>b</sup>Dept. of Microbiology, Yogi Vemana University, Kadapa, India

<sup>c</sup>Jiangsu Provincial Key and Pulp and Paper Science and Technology, Nanjing Forestry University, Nanjing, China

<sup>d</sup>College of Forestry, Oregon State University, Corvallis, OR, USA

<sup>e</sup>Weyerhaeuser Company, Federal Way, WA, USA

### HIGHLIGHTS

- High ethanol yield of 284 L/tonne from forest residue at 42 g/L by SPORL.
- Enzymatic hydrolysis and fermentation of non-detoxified pretreated whole slurry.
- Low cellulase loading of 30 mL/kg forest residue.
- Lignosulfonate of equivalent properties of commercial product directly marketable.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 25 November 2014

Received in revised form 12 December 2014

Accepted 13 December 2014

Available online 18 December 2014

#### Keywords:

Sulfite pulping  
Pretreatment  
High titer biofuel  
Forest residues  
Lignosulfonate

### ABSTRACT

This study demonstrated at the pilot-scale (50 kg) use of Douglas-fir forest harvest residue, an underutilized forest biomass, for the production of high titer and high yield bioethanol using sulfite chemistry without solid–liquid separation and detoxification. Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL) was directly applied to the ground forest harvest residue with no further mechanical size reduction, at a low temperature of 145 °C and calcium bisulfite or total SO<sub>2</sub> loadings of only 6.5 or 6.6 wt% on oven dry forest residue, respectively. The low temperature pretreatment facilitated high solids fermentation of the un-detoxified pretreated whole slurry. An ethanol yield of 282 L/tonne, equivalent to 70% theoretical, with a titer of 42 g/L was achieved. SPORL solubilized approximately 45% of the wood lignin as directly marketable lignosulfonate with properties equivalent to or better than a commercial lignosulfonate, important to improve the economics of biofuel production.

Published by Elsevier Ltd.

## 1. Introduction

Forest residues from logging operations are underutilized and have significant advantages over herbaceous biomass for producing biofuel and bioproducts. Their relatively high bulk density reduces transportation cost, and flexible harvesting schedules

<sup>☆</sup> This work was conducted when Chandra and Gu were visiting scientists at the USDA Forest Products Laboratory and on official government time of Zhu, Gleisner, and Reiner.

\* Corresponding author.

E-mail address: [jzhu@fs.fed.us](mailto:jzhu@fs.fed.us) (J.Y. Zhu).

eliminate long term storage needs (Zhu and Pan, 2010). Forest residues are one of the most affordable feedstocks (National Research Council, 2011) and can be sustainably produced in large quantities in various regions of the world (Gan and Smith, 2006; Perlack and Stokes, 2011). However, forest residues tend to have high lignin content due to the presence of bark and juvenile wood from the tree tops and branches (Yamamoto et al., 2014; Zhang et al., 2012). As a result, they have very strong recalcitrance to bioconversion to sugars (Leu et al., 2013; Yamamoto et al., 2014), especially those from softwoods such as the Douglas-fir (*Pseudotsuga menziesii*) forest harvest residue, as is studied here. Few processes are capable of removing this strong recalcitrance for efficient sugar/biofuel production despite that many research efforts have been devoted to woody biomass pretreatment (Iakovlev & van Heiningen, 2012; Monavari et al., 2010; Pan et al., 2007).

Sulfite chemistry is known for efficient depolymerization of carbohydrates and excellent delignification even under acidic conditions which further facilitates dissolution of hemicelluloses (Bryce, 1980). Both characteristics are very suitable for deconstruction of lignocelluloses (Leu and Zhu, 2013). The dissolved lignin can serve as a surfactant to substantially enhance enzymatic saccharification (Lou et al., 2014; Wang et al., 2013; Zhou et al., 2013a). Furthermore, the retained lignin on the solid substrate is sulfonated lowering its affinity to cellulase and substantially reducing nonproductive cellulase binding (Lou et al., 2013). Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL) (Zhu et al., 2009) utilizes these advantages of sulfite chemistry and has achieved robust performance for woody biomass bioconversion to sugar and bioethanol (Wang et al., 2009; Zhou et al., 2013b; Zhu et al., 2011). Specifically, the elevated temperatures used in SPORL (Fig. 1), as compared to those used in sulfite pulping with nearly complete delignification, enhances hemicellulose dissolution, maintains a certain degree of lignin sulfonation without complete delignification, and requires a shorter reaction time to reduce cost. Building upon our previous success in efficient ethanol production from softwoods using SPORL, we demonstrated here high titer ethanol production from the un-detoxified whole slurry of Douglas-fir forest harvest residue pretreated in a pilot-scale wood pulping digester to pave the way for commercial-scale trial runs in a wood pulp mill. The technical issues addressed in this study are: (1) demonstrating SPORL process using commercial pulp mill chemistry (Gao et al., 2013), i.e., bubbling  $\text{SO}_2$  into a hydroxide solution to produce the sulfite solution, rather than using  $\text{H}_2\text{SO}_4$  and sodium bisulfite as practiced in all our previous studies for ease of pretreatment experiments in the laboratory (Luo et al., 2010; Wang et al., 2009; Zhou et al., 2013b; Zhu et al., 2009, 2011); (2) using a low pretreatment temperature of 145 °C to

accommodate facility limitations at pulp mills without reducing cellulose saccharification efficiency; (3) direct enzymatic saccharification and fermentation of the pretreated whole slurry at high solids without solids washing or slurry detoxification to simplify process integration.

Optimal pretreatment conditions derived from conventional optimization studies based on sugar yield from very recalcitrant feedstock such as forest residue (Leu et al., 2013) may not be optimal for ethanol production due to elevated sugar degradation to inhibitors to cause difficulties in fermentation without detoxification. Furthermore, the maximal allowable operating temperature for commercial sulfite pulp mill digesters is well below the optimal temperature from previous optimization studies (Tian et al., 2011; Zhou et al., 2013b), which creates difficulties for process scale-up. Low temperature pretreatment has been used to reduce inhibitor formation but at the expense of additional processing steps to maintain enzymatic saccharification efficiency (Chen et al., 2012). To overcome these difficulties and balance sugar yield and sugar degradation, we used a combined hydrolysis factor (CHF) (Zhu et al., 2012) as a pretreatment severity measure to scale-up SPORL to a pilot scale of 50 kg at a low temperature of 145 °C used in this study. Utilizing a highly recalcitrant but low cost feedstock, forest harvest residue, avoids biofuel production competing for raw materials with the existing solid wood and pulp and paper industries. Therefore, this study has vital commercial importance.

## 2. Methods

### 2.1. The Douglas-fir forest residue

Douglas-fir forest residue was collected from a regeneration harvest in a primarily Douglas-fir stand on Mosby Creek southeast of Cottage Grove in Lane County, OR and owned by Weyerhaeuser Company. A horizontal drum fixed-hammer grinder (Model 4710B, Peterson Pacific Corporation, Eugene, OR) equipped with a combination of 76 and 102 mm grates was used to grind road piles of the residue. The ground residue was shipped to Weyerhaeuser Company at Federal Way, WA, by truck. The moisture content of the residue measured at arrival was 43.9%. A gyratory screen (Black-Clawson) equipped with a 44.5 mm (1.75 in.) diameter round-hole punched-plate top deck was used to remove oversized particles and a 3.2 mm (1/8 in.) clear-opening woven wire bottom screen (6 wires/inch mesh) to remove fines. The screening resulted in 7.6% fines and 9.8% oversize. The oversize fraction was further hammer milled, which resulted in near zero oversized particles and 14.9% fines from the 9.8% original screen oversize fractions. The total rejection of fines was 9.0%. The accept forest residue labeled as FS-10 was then air-dried to a moisture content of 15% before being shipped to the USDA Forest Products Laboratory, Madison, WI.

### 2.2. Enzymes, chemicals and yeast

Multiplex cellulase enzymes Cellic<sup>®</sup> CTec3 (abbreviated CTec3) was complementarily provided by Novozymes North America (Franklinton, NC). ACS reagent grade sodium acetate, acetic acid, and sulfuric acid were used as received from Sigma-Aldrich (St. Louis, MO). Calcium hydroxide (95% purity) was purchased from Alfa Aesar (Ward Hill, MA). Sulfur dioxide anhydrous was from Airgas USA (Madison, WI). Calcium bisulfite was produced onsite by bubbling sulfur dioxide to a calcium oxide slurry at the Forest Products Lab.

An engineered yeast strain of *Saccharomyces cerevisiae* YRH-400 was obtained from USDA Agriculture Research Service (Hector et al., 2011). Yeast extract peptone dextrose (YPD) agar plates

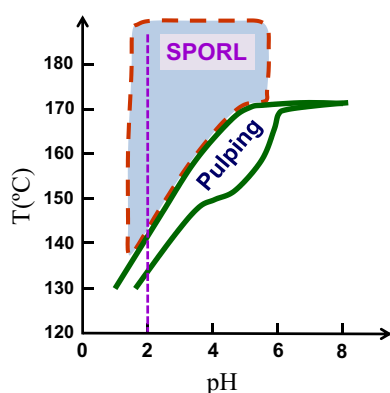


Fig. 1. Comparison of operating conditions between SPORL and sulfite pulping in the Temperature-pH diagram.

containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 20 g/L agar were used to grow the strain at 30 °C for 2 days. A colony from a plate was transferred by an inoculum loop to liquid YPD medium in a flask and cultured overnight at 30 °C with agitation at 90 rpm on a shaking bed incubator (Thermo Fisher Scientific, model 4450, Waltham, MA). After the yeast seed culture being grown for 24 h, the centrifuged yeast seed was used to inoculate the fermentation medium.

### 2.3. Determination of pretreatment duration for pilot-scale experiment

A combined hydrolysis factor (CHF) as defined in Eq. (1) was derived from hemicellulose dissolution kinetics (Zhu et al., 2012) and can be used to accurately predict hemicellulose dissolution for both hardwoods and softwoods (Zhang et al., 2014; Zhou et al., 2013b):

$$CHF = e^{(\alpha - \frac{E}{RT} + \beta C_A + \gamma C_B)} (C_A + C_B)t \quad (1)$$

where  $C_A$  and  $C_B$  are the concentrations of chemical A ( $\text{SO}_2$ ) and chemical B (hydroxide) used in pretreatment, respectively;  $\alpha$ ,  $\beta$  and  $\gamma$  are adjustable parameters,  $E = 100,000$  J/mole is the apparent activation energy for softwoods (Zhou et al., 2013b),  $R$  is the universal gas constant of 8.314 J/mole/K,  $t$  is the time in min, and  $T$  is the absolute pretreatment temperature in degrees Kelvin.

CHF is a pretreatment severity measure that can be used to adjust pretreatment temperature in the pilot-scale while maintaining the extent of hemicellulose dissolution. Using the optimal chemical loading for softwoods with a 25–30 min pretreatment at 180 °C determined at the 0.15 kg lab scale (Zhou et al., 2013b), Eq. (2) indicates the required reaction time,  $t^{T145}$ , for the pilot-scale pretreatment at 145 °C to be 230–275 min. This method was shown to perform well for scale-up from 0.15 kg to 2 kg in a narrow temperature range of 165–180 °C in a previous laboratory study (Zhou et al., 2013b):

$$t^{T145} = \exp \left[ \frac{E}{R} \left( \frac{1}{T_{145}} - \frac{1}{T_{180}} \right) \right] \cdot t^{T180} \quad (2)$$

Using a temperature of 145 °C not only accommodated the capability of the commercial pulp mill digester, but also significantly reduced sugar degradation into inhibitors which allows high solids fermentation without detoxification. Using first reaction kinetics, furan formation can be predicted using the following equation with the assumption of no further degradation of furan to other compounds (Zhang et al., 2014):

$$D = k_d \cdot t \left[ 1 - \frac{1 - \theta}{CHF} (1 - e^{-CHF}) - \frac{\theta}{f \cdot CHF} (1 - e^{-f \cdot CHF}) \right] \quad (3)$$

where  $D$  is the furan formation from sugar degradation reactions,  $k_d$  is the rate constant of sugar degradation reactions.  $\theta$  is the fraction of slow hemicelluloses and  $f$  is the ratio of the dissolution reaction rate between the slow and fast hemicelluloses. The ratio of furan formation between two pretreatments with identical severity CHFs and chemical loadings but at different temperatures,  $T_1 > T_2$ , can be determined from Eq. (4) as follows:

$$\frac{D_{T1}}{D_{T2}} = \exp \left[ \frac{E - E_d}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right) \right] \quad (4)$$

Sugar degradation reactions have higher activation energies ( $E_d = 160, 930$ ) than hemicellulose dissolution ( $E = 100,000$  J/mole) (Kamireddy et al., 2014; Zhang et al., 2014), i.e.,  $E_d > E$ , and therefore, sugar degradation is lower at a low temperature.

### 2.4. Pilot-scale production of forest residue whole slurry

A dilute sulfite solution, at approximately pH 2.0 and containing 2.28 wt%  $\text{Ca}(\text{HSO}_3)_2$  and 0.87 wt% true free  $\text{SO}_2$  was produced in a stirred barrel by bubbling  $\text{SO}_2$  regulated at a gauge pressure of 34.5 kPa and a flow rate of 90 g/min into a hydroxide solution of 139 L containing 1.25 kg (95% purity) of  $\text{Ca}(\text{OH})_2$ . After 37 min bubbling, a solution weight gain of 3.3 kg was achieved while a small amount of calcium hydroxide remained at the outer edge of the barrel. Complete reaction of  $\text{Ca}(\text{OH})_2$  was achieved by manually stirring the solution 3 times. A cover was clamped and sealed with tape to the barrel and then stored at 4 °C overnight.

Pilot-scale SPORL pretreatment of FS-10 Douglas-fir forest harvest residue was conducted in a 390 L rotating wood pulping digester using the prepared dilute sulfite solution. As schematically show in Fig. 2, the digester was heated by a steam jacket and rotated at 2 rpm during pretreatment for mixing of chemicals with woody materials. The digester was first loaded with 61.75 kg FS-10 with solid content of 81.4% (50.26 kg in oven dry (OD) weight). These conditions resulted in a total liquor volume of 150 L, total  $\text{SO}_2$  mass concentration in the sulfite solution of 2.3 wt%, and free  $\text{SO}_2$  and  $\text{Ca}(\text{HSO}_3)_2$  charge on wood 2.48 and 6.46 wt%, respectively. This translates to a total  $\text{SO}_2$  loading of 6.6 wt% on oven dry wood residue. Before calcium sulfite was added, the upper lid was lightly closed with the discharge valve open and low-pressure steam was injected into the top of the digester. This steaming was continued for 5 min after steam flow was observed at the discharge valve. The steam was stopped, the discharge valve closed and the lid quickly opened to obtain a sample. The amount of steam injected was determined to be 27.85 kg based on the moisture content of 44.18% of the steamed FS-10 in the digester. The actual liquor to wood ratio (L/W) for the pretreatment was therefore 3.55 (L/kg). The lid was then quickly sealed and a vacuum applied to the digester. The vacuum was applied for approximately 20 min at which time the vacuum valve was closed and a hose connection was made between the bottom of the digester and a centrifugal chemical transfer pump. The pump was used in case the vacuum was inadequate to pull all of the sulfite solution into the digester. After approximately 6 min all the sulfite solution was pulled into the digester. Two 50 mL samples of the liquor were collected just prior to injection for verification of sulfite concentration. The measured concentrations of  $\text{Ca}(\text{SHO}_3)_2$  was 2.01 wt% and true free  $\text{SO}_2$  was 0.43 wt% compared with calculated values of 1.93 wt% and 0.74 wt%, respectively, based on the amounts of  $\text{Ca}(\text{OH})_2$ ,  $\text{SO}_2$ , and steam applied. The lower measured  $\text{SO}_2$  concentration could be due to losses during transit to Weyerhaeuser Company (Federal Way, WA). Rotation of the digester was started immediately. It took approximately 37 min to heat the digester from 30 °C to a terminal temperature of 145 °C. The temperature was maintained for 240 min. The digester contents were discharged into a blow tank (Fig. 2) through a stainless steel pipe. An additional air blow was applied to ensure all contents were discharged. Volatiles including  $\text{SO}_2$  were vented to a wet scrubber (Fig. 2). The freely drainable portion of the pretreatment spent liquor of 42 kg was collected from the blow tank shortly after discharging from the digester. The remaining liquor stayed with the pretreated solids. After venting in the blow tank for two days to let the remaining small amount of  $\text{SO}_2$  escape, the solids were collected and weighed.

The freely drainable spent liquor was neutralized and then proportionally fed with the pretreated solids to a laboratory disk refiner (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH) to produce pretreated whole slurry of FS-10 (Fig. 2). The whole slurry had a solids content of 24.49% (including the dissolved solids from pretreatment) and was directly used for subsequent saccharification and fermentation. A small sample of the whole slurry was



**Fig. 2.** A schematic process flow diagram shows Douglas-fir forest residue grinding, transportation, pretreatment and downstream saccharification and fermentation. Process boxed by dashed lines was partially conducted.

separated into wet solids and liquor by pressing in a screen box. The moisture of the solid fraction was determined gravimetrically by oven drying the collected wet sample. An aliquot of the wet solid sample was thoroughly washed to remove soluble solids. Both the washed and unwashed solids were used to conduct enzymatic hydrolysis after neutralization using lime.

### 2.5. Enzymatic hydrolysis of washed solids and quasi-simultaneous saccharification and fermentation (Q-SSF) of whole slurry

Enzymatic hydrolyses of the washed solids were conducted at 10% and 15% (w/v) in 50 mL of 50 mM acetate buffer (pH 5.5) on a shake/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 50 °C and 200 rpm. An elevated pH of 5.5, higher than the commonly used pH 4.8–5.0, can significantly reduce nonproductive cellulase binding to lignin leading to enhanced lignocellulose saccharification (Lan et al., 2013; Lou et al., 2013; Wang et al., 2013). Buffer application could be eliminated to reduce cost based on previous time-dependent pH measurements in enzymatic hydrolysate (Lan et al., 2013). The CTec3 loading was 15 FPU/g glucan (based on glucan in the washed solids). Aliquots of 1 mL enzymatic hydrolysate were taken periodically for glucose analysis after centrifugation at 13,000g for 5 min. Each data point is the average of two analyses. The data from replicate runs were used to calculate the mean values and standard deviations that were used as error bars in plots.

The un-detoxified FS-10 whole slurry had a pH 6.2 after neutralization and was directly used for Q-SSF (Fig. 2) at 16.7 wt% total solids loading with the addition of sodium acetate buffer (50 mM, pH 5.5). Q-SSF was conducted in 250 mL Erlenmeyer flasks on a shaking incubator (Thermo Fisher Scientific, model 4450, Waltham, MA) at 50 °C and 200 rpm. Inadequate mechanical mixing on the shaking bed prevented us from conducting Q-SSF at higher solids loadings. An elevated pH 5.5 was again used to reduce nonproductive cellulase binding to lignin and enhance lignocellulose saccharification (Wang et al., 2013; Lou et al., 2013; Lan et al., 2013). CTec3 loadings of 26 and 35 mL/kg untreated FS-10 were used, equivalent to 15 and 20 FPU/g glucan (based on glucan in the whole slurry). Liquefaction of solids was achieved in approximately 24 h. The mixture was then cooled to 35 °C, and the shaker speed was reduced to 90 rpm. The cultured yeast seed of 25 mL

with optical density at 600 nm of approximately 30 was first centrifuged at 4600 rpm for 10 min to result in a volume of 0.4 mL after the removal of supernatant. The supernatant removed yeast seed of 0.4 mL (equivalent to 0.4 mg dry cell/g OD whole slurry) was applied for fermentation. No nutrients were applied during fermentation. Each flask was covered by aluminum foil. Samples were taken periodically for analyses of monosaccharides, inhibitors, and ethanol. Duplicate Q-SSF runs were conducted under each set of conditions to ensure experimental repeatability. Reported results were the average of duplicate analyses of replicate fermentation runs.

### 2.6. Analytical methods

The chemical compositions of the untreated and pretreated FS-10 were analyzed as described by Luo et al. (2010). All solid substrates were Wiley milled (model No. 2, Arthur Thomas Co, Philadelphia, PA, USA) to 20 mesh (~1 mm) and then hydrolyzed in two steps using sulfuric acid of 72% (v/v) at 30 °C for 1 h and 3.6% (v/v) at 120 °C for 1 h. Carbohydrates of the hydrolysates were analyzed by high performance anion exchange chromatography (HPLC) with pulsed amperometric detection (ICS-5000, Dionex). Klason lignin (acid insoluble) was quantified gravimetrically (Dence, 1992). For fast analysis, glucose in the enzymatic hydrolysates was measured using a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH, USA).

The total sulfur content in the calcium bisulfite liquor was analyzed in petroleum products (high temperature method) in Leco S632 Sulfur Determinator (St. Joseph, Michigan) using a standard ASTM D 1552-90 method for sulfur analysis. The calcium in the liquor was analyzed in an inductively coupled argon plasma mass spectrometry (Thermo Elemental X Series II, Waltham, MA) using a standard method equivalent to EPA Method 200.8, revision 5.4, of EPA/600/R-94/111. The sulfur result was used to calculate the total equivalent sulfur dioxide. The bound sulfur dioxide in the calcium bisulfite was determined by the total amount of soluble calcium in the liquor, and the free sulfur dioxide was determined by subtracting the bound sulfur dioxide from the total sulfur dioxide.

All liquid samples including the pretreatment spent liquor, enzymatic hydrolysates and fermentation broths were analyzed for monosaccharides (glucose, xylose, galactose, arabinose and

mannose) using a Dionex HPLC system (Ultimate 3000) equipped with an RI (RI-101) and UV (VWD-3400RS) detector and a BioRad Aminex HPX-87P column (300 mm × 7.8 mm) operated at 80 °C. Double distilled water (d.d.w.) was used as eluent at a flow rate of 0.6 mL/min. The fermentation broths were also analyzed for furans, ethanol, and organic acids using the same HPLC system equipped with a BioRad Aminex HPX-87H column (300 mm × 7.8 mm) operated at 60 °C. A 5 mM sulfuric acid solution was used as eluent at a flow rate of 0.6 mL/min. All sample injection volumes were 20 µL. Samples were diluted in deionized water and filtered by a 0.22 µm syringe filter prior to injection.

### 2.7. Characterization of lignosulfonate

The calcium lignosulfonate (Ca-LS) was purified from the spent liquor of the SPORL process using an in-house built pilot plant ultrafiltration (UF) system equipped with single-tube modules, each with a separate permeate outlet. Two membranes, ES404 and FP200 (Xylem PCI Membranes, Kostrzyn, Poland) with cut-off molecular weight of 4 kDa and 200 kDa, respectively, were used in the experiment. Both of the 4 kDa and 200 kDa membranes had 18 parallel flow channels in series with each channel having 1.2 m length and 12.5 mm inner diameter. A Grundfos® pumping system (CRN1-25, Lenntech BV, Delft, Netherlands) provided the desired cross flow velocity and feed pressure. The transmembrane feeding pressures of the pump were 1792 pa (260 psi) and 1310 pa (190 psi) for the 4 kDa and 200 kDa membranes, respectively.

The purified Ca-LS solution was analyzed for molecular weight and distribution with a HPLC system (Agilent 1100 series, Agilent Technologies, Palo Alto, CA, USA). The HPLC system was equipped with three detectors, i.e., Multi-Angle Light Scattering (MALS) (Dawn Heleos® II, Wyatt Technology Corporation, Santa Barbara, CA, USA), Infrared (IR) (Optilab® T-REX, Wyatt Technology Corporation) and Ultra-violet (UV) (Agilent 1100 series UV detector, Agilent Technologies), and a GPC column (Ultrasphere™ 250, 7.8 mm × 300 mm, Water, Milford, MA, USA) together with an Ultrasphere™ guard column (6 mm × 40 mm). Sodium nitrate at 0.1 mol/L was used as eluent with a flow rate of 0.5 mL/min at 25 °C. Sodium polystyrene sulfonates of different molecular weights were used as standards for calibration.

The sulfur content of Ca-LS was determined using an ICP-AES (Ultima II, Horiba Jobin-Yvon, Edison, NJ). A 1 mL aliquot of purified Ca-LS solution was transferred to a Teflon digestion flask with 5 ml of 70% nitric acid and digested at 165 °C for 30 min in a microwave oven (MDS-2000, CEM Corp., Matthews, North Carolina, USA). The ramp time to 165 °C was approximately 40 min. The reported sulfur content was the average of triplicate analyses.

## 3. Results and discussion

### 3.1. Recovery of FS-10 wood component from pilot scale SPORL

Rejecting the fines (3.2 mm or smaller) of the present Douglas-fir forest residue removed 9% of the mass but improved carbohydrate content by 6% (Cheng et al., 2015), similar to that found in a previous study using chipped Douglas-fir forest harvest residue (Zhang et al., 2012). The chemical composition of the screened accepted Douglas-fir forest residue FS-10 is listed in Table 1. The pretreated FS-10 was recovered from two streams (Fig. 2), i.e., the unwashed wet solids that contain approximately two-thirds of the pretreatment spent liquor and the freely drainable spent liquor (Table 1). Overall solids recovery was 96.7%. Component data shows glucan recovery was 97.2%. Recoveries of major hemicelluloses varied from approximately 47% for xylan, 63% for mannan to 78% for galactan. These data were similar to those for

lodgepole pine (*Pinus contorta*) wood without bark (Zhou et al., 2013b). Quantification of the composition and yield of washed solids revealed the modification of cell wall by SPORL. As indicated in Table 1, approximately 45% of the cell wall lignin was solubilized into the spent liquor as lignosulfonate. Over 90% of the hemicelluloses were also solubilized. Glucan solubilization was approximately 20%, slightly higher than expected. The solubilized glucan, however, was mostly recovered as glucose as can be seen from the total glucan recovery of over 97%. Sugar degradation to furans was very low at only approximately 1.0% to HMF and 4.6% to furfural (Table 1) which was expected due to the low pretreatment temperature (Zhang et al., 2014). The HMF and furfural concentrations in the spent liquor were both less than 1.0 g/L. The low furan concentrations can facilitate downstream fermentation by not requiring liquor detoxification.

### 3.2. Substrate digestibility of SPORL pretreated FS-10 at pilot scale

Enzymatic saccharification of the washed solids was conducted to evaluate the effectiveness of SPORL pretreatment in removing the recalcitrance of FS-10. Substrate enzymatic digestibility (SED), defined as the percentage of washed solids glucan enzymatically saccharified to glucose, reached 84% in 48 h with titer of 80 g/L at a washed solids loading of 15% (Fig. 3). Saccharification efficiency increased to over 90% at 10% solids loading in 24 h. The dissolved lignin or lignosulfonate from sulfite spent liquor was found to have less affinity to cellulase (Lou et al., 2013, 2014) and can act as a surfactant to enhance enzymatic saccharification (Wang et al., 2013; Zhou et al., 2013a). When enzymatic saccharification was conducted using unwashed solid substrates that containing dissolved lignin at 18.5% solids, SED reached 92% with a glucose titer of 97 g/L. This indicates more glucose will be available in Q-SSF of the SPORL pretreated whole slurry that contains lignosulfonate than using washed solids alone.

### 3.3. Sugar consumption and ethanol production during Q-SSF

The low fermentation inhibitors from the SPORL pilot scale pretreatment facilitated fermentation using *S. cerevisiae* YRH-400. At CTec3 loading of 35 mL/kg untreated FS-10, glucose was almost consumed in the first 24 h (Fig. 4a). A logarithmic scale was used in plotting the time (*x*-coordinate) to show early stage fermentation in Fig. 4a and b. The average glucose consumption rate in the first 24 h was -3.6 g/L/h (Table 2), which resulted in a rapid ethanol production with concentration of approximately 39 g/L (Fig. 4a). Mannose was also almost completely consumed in the first 24 h. YRH-400 was also able to consume a small amount of xylose despite the low xylose concentration (Fig. 4b). The time-dependent fermentation data showed excellent repeatability for the duplicate runs at CTec3 dosage of 35 mL/kg FS-10. Terminal maximal ethanol concentration of 41.9 g/L was achieved in just 72 h of fermentation.

To further evaluate SPORL pretreatment, CTec3 dosage was reduced by 25% to 26 mL/kg untreated FS-10 in the second set of fermentation runs. Very large standard deviations in the performance data between the duplicate runs were observed (Fig. 4a and b). Run I had similar sugar consumption, ethanol production, terminal maximal ethanol concentration, and ethanol yield as those at the higher CTec3 loading of 35 mL/kg FS-10 (Table 2). The large variations between Run I and Run II were mainly due to the poor mixing during liquefaction by the shaking bed at high solids loading, which caused variation in the amount of time needed for complete liquefaction for the two runs. As a result, the amounts of available sugars varied significantly while YRH-400 was applied at the same liquefaction time. This suggests fermentation was limited by sugar production. Improved mixing

**Table 1**

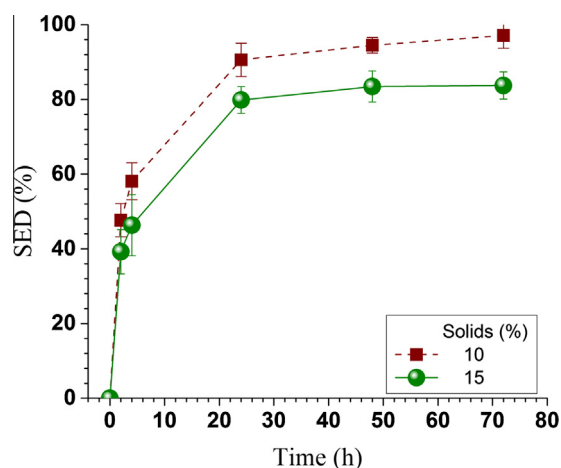
Component recovery of FS-10 Douglas-fir forest residue from SPORL pretreatment at 145 °C for 240 min with free SO<sub>2</sub> and calcium bisulfite charges on FS-10 2.49% and 6.49%, respectively.

	Untreated FS-10	Unwashed solids <sup>a</sup>	Collected spent liquor <sup>a</sup>	Total recovery	Washed solids <sup>a</sup>
Wet weight (kg)	61.75	156.40	42.00		
Solids content (%)	81.40	28.53	11.5%		
Solids (kg) <sup>b</sup>	50.26	44.62; 88.8%	4.83; 9.6	98.4%	28.90; 57.5%
Klason lignin (%)	29.30	22.15; 75.6	3.59; 12.3	87.9	16.16; 55.1
Arabinan (%)	1.04	0.32; 30.7	0.11; 10.4	41.1	0; 0
Galactan (%)	2.00	1.12; 55.9	0.44; 22.1	78.0	0; 0
Glucan (%)	40.97	39.12; 95.5	0.71; 1.7	97.2	32.97; 80.5
Mannan (%)	9.67	4.56; 47.2	1.55; 16.1	63.2	0.72; 7.5
Xylan (%)	5.70	2.08; 36.5	0.63; 11.0	47.5	0.46; 8.1
HMF (%) <sup>c</sup>		0.07; 0.8	0.02 (0.3); 0.2	1.0	
Furfural (%) <sup>c</sup>		0.21; 3.7	0.05 (0.7); 0.9	4.6	
Acetic acid (%)		0.71;	0.27		

<sup>a</sup> The numbers before “;” are based on the amount of untreated oven dry FS-10. The numbers after “;” are in wt% of theoretical based on untreated wood component.

<sup>b</sup> In oven dry (od) weight.

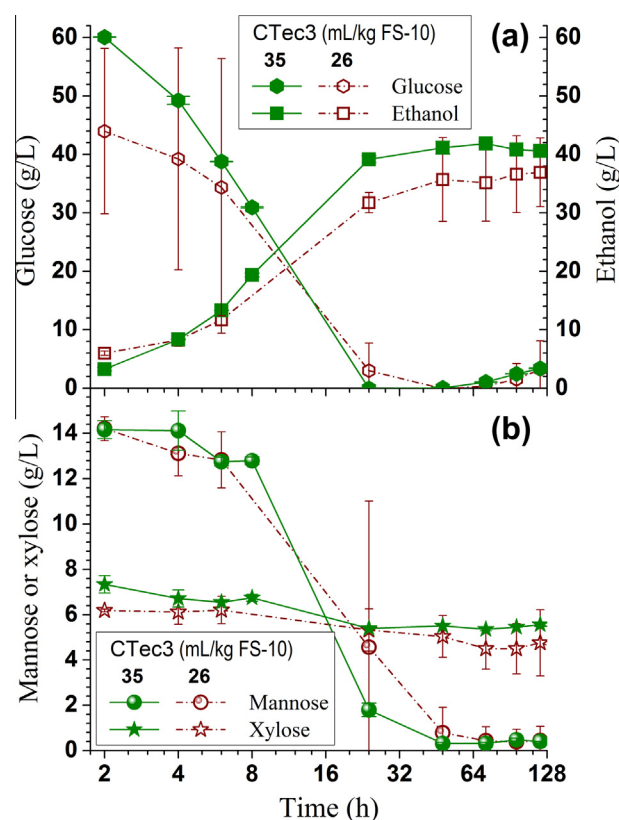
<sup>c</sup> Reported as mannan and xylan for HMF and furfural, respectively, represent percent of mannan and xylan degraded to HMF and furfural, respectively. The numbers in the parenthesis are concentration measured in the collected spent liquor in g/L.



**Fig. 3.** Time-dependent enzymatic saccharification efficiency of washed SPORL pretreated FS-10 at pilot-scale at two solids loadings with CTec3 dosage of 15 FPU/g glucan.

using mechanical devices should improve fermentation performance even at a low CTec3 dosage of 26 mL/kg FS-10. The data in Table 2 indicate that the terminal maximal ethanol concentration and yield from each fermentation run at the low CTec3 dosage, Run I and Run II, was higher than their average values. This was because the time to achieve maximal ethanol concentration for Run I and Run II were different due to the variation in solids liquefaction and rate of enzymatic saccharification. The mismatch of ethanol and sugar concentration profiles substantially contributed to large standard deviations shown in Fig. 4a and b.

When fermentation time (*x* coordinate) was plotted in linear scale for the data in Fig. 4a and b, the results clearly indicate that glucose consumption has two rates at the high CTec3 dosage of 35 mL/kg FS-10, the initial rate in the first 8 h was 25% higher than that in the 24 h (Table 2), while mannose consumption (Fig. 4b) rate was almost constant in the first 24 h. At the low CTec3 dosage of 26 mL/kg FS-10, the difference in glucose consumption rate between those in the first 6 h and in the first 24 h was 20% (Table 2), while mannose consumption rate remained a constant in the first 24 h. These observations clearly indicate that the entire fermentation was sugar production limited. Typically, mannose consumption has an initiation period with a slow rate when fermentation inhibition dominates (Zhou et al., 2013b). Fast consumption of mannose begins only when level of furan is



**Fig. 4.** Time-dependent sugar and ethanol concentrations in the fermentation broth of the SPORL pretreated FS-10 whole slurry at 16.7% unwashed solids loading. (a) Glucose and ethanol; (b) mannose and xylose.

metabolized to a low level by the yeast that relies mainly on glucose first. The furan concentrations in the fermentation broth were near the analysis detection limit and therefore were not reported here.

### 3.4. Overall process mass balance

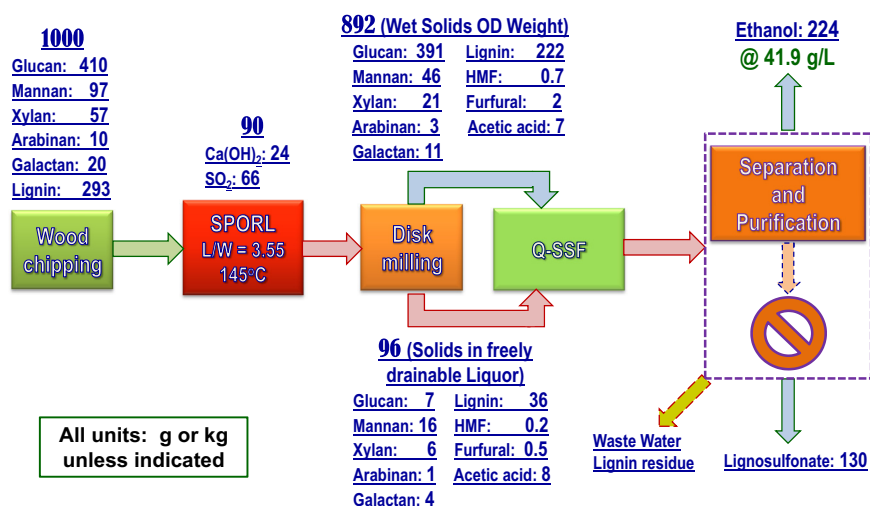
The process data under CTec3 loading of 35 mL/kg untreated FS-10 were plotted in Fig. 5 to reveal the overall mass balance of the experiment. Ethanol yield was  $224 \pm 1.6$  kg ( $284 \pm 2$  L) per tonne FS-10 at approximately 42 g/L, equivalent to 70% theoretical based on FS-10 glucan, mannan, and xylan content (Table 2). The ethanol

**Table 2**  
Fermentation of un-detoxified SPORL pretreated whole slurry of FS-10 at pilot scale: average ethanol productivity and rates of sugar consumption in the first 24 h (unless indicated), along with terminal maximal ethanol production.

CTec3 dosage	35 mL/kg FS-10	26 mL/kg FS-10		
	Average	Run I	Run II	Average
<i>Fermentation performance (g/L/h)</i>				
Ethanol productivity	1.523; 2.681 (8 h)	1.310	1.168	1.145; 1.523 (6 h)
Glucose consumption	−3.634; −4.529 (8 h)	−2.242	−1.460	−1.896; −2.273 (6 h)
Mannose consumption	−0.572	−0.606	−0.214	−0.428
Xylose consumption	−0.096	−0.059	−0.019	−0.041
<i>Terminal maximal ethanol production</i>				
Time to reach maxima (h)	72	48	144	120
Ethanol concentration (g/L)	41.9 ± 0.3	40.8	38.2	36.9 ± 5.9
Ethanol yield (g/g sugar) <sup>a</sup>	0.412 ± 0.003	0.402	0.376	0.363 ± 0.058
Ethanol yield (L/tonne wood)	284 ± 2	277	259	250 ± 40
Ethanol yield (% theoretical) <sup>b</sup>	70.0 ± 0.5	68.1	63.8	61.6 ± 9.9

<sup>a</sup> Based on the total of glucan, mannan, xylan in the pretreated FS-10 solids and glucose, mannose, and xylose in the pretreatment spent liquor.

<sup>b</sup> Theoretical yield (406 L/tonne wood) based on total glucan, mannan, xylan in the untreated FS-10.



**Fig. 5.** A block diagram shows process mass balance for the pilot-scale pretreatment of Douglas-fir forest residue FS-10 with subsequent saccharification and fermentation.

titer can be improved by increasing solids loading when a mechanical mixing device is used. This should not cause fermentation inhibition because the inhibitors level in the spent liquor was very low. Liginosulfonate yield was 130 kg/tonne FS-10 as Klason lignin or approximately 45% based on FS-10 Klason lignin content. Actual yield of liginosulfonate was higher as calcium and sulfur on the liginosulfonate were not accounted for in the analysis shown in Fig. 5.

### 3.5. Sulfur content and molecular weight of liginosulfonate from SPORL

The solid content of original collected spent liquor was 11.5 wt%, the pH was 1.8 and the ash content was 8.4 wt% (based on oven dried liquor, measured at 575 °C for 6 h). The spent liquor was first centrifuged to remove particulates. Any remaining particulates were further separated by passing through the 200 kDa membrane. The liquor was then sent to a 4 kDa membrane to remove small molecular impurities such as sugars. Each fraction was collected and weighed to determine spent liquor mass distribution and for mass balance analysis. After UF through the two membranes, 69.6% of Ca-LS and 7.6% of total sugar were retained in the purified sample (fraction of 4 k–200 kDa), as shown in Table 3. The lignin purity was only 44.5% in the original spent liquor and was increased to 86.8% after UF in the purified sample. Table 3 indicated that Ca-LS could be extracted with high recovery and purity by UF. The purified Ca-LS had a similar molecular

**Table 3**

Characterization of purified calcium liginosulfonate solution.

<i>Ultrafiltration experiment</i>				
Sample	LS-Ca (%)	Sugar (%)	Mass (%)	Lignin purity (%)
Original	100	100	100	44.5
>200 kDa	3.9	n/a	2.4	89.1
4–200 kDa <sup>a</sup>	69.6	7.6	43.2	86.8
<4 kDa	24.9	84.3	41.5	19.3
<i>GPC (MALS) analysis</i>				
Sample	Mw <sup>b</sup>	Mn <sup>c</sup>	Mw/Mn <sup>d</sup>	
4–200 kDa	23430	12910	1.8	
D748	24660	14190	1.7	
<i>GPC (UV) analysis</i>				
Sample	Mw <sup>b</sup>	Mn <sup>c</sup>	Mw/Mn <sup>d</sup>	
4–200 kDa	2704	1092	2.5	
D-748	13113	3293	4.0	

<sup>a</sup> The fraction of 4–200 kDa is the purified calcium liginosulfonate sample.

<sup>b</sup> Number-average molecular weight.

<sup>c</sup> Weight-average molecular weight.

<sup>d</sup> Polydispersity.

weight and polydispersity to the commercial liginosulfonate D748 (Borregaard LignoTech, Rothschild, WI, USA) based on GPC MALS measurements. However, GPC UV measurements indicated that the purified Ca-LS had a smaller molecular weight with lower polydispersity than D-748 (Table 3).



The Ca-LS is highly sulfonated with sulfur content of  $69.2 \pm 0.9$  mg/g which is higher than the commercial LS D-748 of  $60.1 \pm 3.9$  mg/g measured previously (Zhou et al., 2013b). The sulfur content along with the molecular weight information suggests that the Ca-LS from SPORL can be directly marketed with comparable properties of commercial lignosulfonate.

#### 4. Conclusions

Robust performance of sulfite chemistry was demonstrated to effectively remove the strong recalcitrance of a Douglas-fir forest residue for high titer (42 g/L) bioethanol production without detoxification with high yield of 282 L/tonne. Lignin sulfonation facilitated the processing of the pretreated whole slurry without solid and liquor separation or solids washing. Approximately 45% of the wood lignin was solubilized as a valuable and directly marketable co-product – lignosulfonate – to improve the ethanol production economics. This study demonstrated that underutilized woody biomass such as forest residues can be efficiently converted to biofuel and bioproducts using mature pulping technologies with proven commercial scalability.

#### Acknowledgements

The authors would like to acknowledge the financial support of the Agriculture and Food Research Initiative (AFRI) Competitive grant (No. 2011-68005-30416), USDA National Institute of Food and Agriculture (NIFA) through the Northwest Advanced Renewables Alliance (NARA). The authors also thank Novozymes North America for providing the CTec3 enzymes; Dr. Jerry Gargulak of LignoTech for providing the D-748 Lignosulfonate; Fred Matt of USFS-FPL for conducting the chemical composition analysis; Linda Lorenz for assisting measurements of lignosulfonate molecular weight using GPC; Kolby Hirth for analyzing lignosulfonate sulfur content using ICP. Drs. Bruce Dien and Ron Hector of USDA-ARS for providing the YRH-400 strain. Funding was also received from University Grants Commission (UGC), New Delhi, India for the visiting appointment of Dr. Chandra at the USDA Forest Products Lab.

#### References

- Bryce, J.R.G., 1980. Sulfite pulping, Third ed., in: Casey J. (Ed.), *Pulp and Paper: Chemistry and Chemical Technology*, Vol. I, John Wiley & Sons, New York, pp. 291–376.
- Chen, X., Shekiri, J., Franden, M.A., Wang, W., Zhang, M., Kuhn, E., Johnson, D.K., Tucker, M.P., 2012. The impacts of deacetylation prior to dilute acid pretreatment on the bioethanol process. *Biotechnol. Biofuels* 5, 8.
- Cheng, J., Leu, S.-Y., Zhu, J.Y., Gleisner, R., 2015. High titer and yield ethanol production from undetoxified whole slurry of Douglas-fir forest residue using pH-profiling in SPORL. *Biotechnol. Biofuels* 8 (in press).
- Dence, C.W., 1992. The determination of lignin. In: Lin, S.Y., Dence, C.W. (Eds.), *Methods in Lignin Chemistry*. Springer-Verlag, Berlin, pp. 33–61.
- Gan, J., Smith, C.T., 2006. Availability of logging residues and potential for electricity production and carbon displacement in the USA. *Biomass Bioenergy* 30, 1011–1020.
- Gao, J., Anderson, D., Levie, B., 2013. Saccharification of recalcitrant biomass and integration options for lignocellulosic sugars from Catchlight Energy's sugar process (CLE sugar). *Biotechnol. Biofuels* 6, 10.
- Hector, R.E., Dien, B.S., Cotta, M.A., Qureshi, N., 2011. Engineering industrial *Saccharomyces cerevisiae* strains for xylose fermentation and comparison for switchgrass conversion. *J. Ind. Microbiol. Biotechnol.* 38, 1193–1202.
- Iakovlev, M., VanHeiningen, A., 2012. Efficient fractionation of spruce by SO<sub>2</sub>-ethanol-water treatment: closed mass balances for carbohydrates and sulfur. *ChemSusChem* 5, 1625–1637.
- Kamireddy, S.R., Kozliak, E.L., Tucker, M., Ji, Y., 2014. Kinetic features of xylan depolymerization in production of xylose monomer and furfural during acid pretreatment for kenaf, forage sorghums and sunn hemp feedstocks. *Int. J. Agric. Biol. Eng.* 7, 86–98.
- Lan, T.Q., Lou, H., Zhu, J.Y., 2013. Enzymatic saccharification of lignocelluloses should be conducted at elevated pH 5.2–6.2. *Bioenergy Res.* 6, 476–485.
- Leu, S.-Y., Gleisner, R., Zhu, J.Y., Sessions, J., Marrs, G., 2013. Robust enzymatic saccharification of a Douglas-fir forest harvest residue by SPORL. *Biomass Bioenergy* 59, 393–401.
- Leu, S.Y., Zhu, J.Y., 2013. Substrate-related factors affecting enzymatic saccharification of lignocelluloses: our recent understanding. *Bioenergy Res.* 6, 405–415.
- Lou, H., Zhou, H., Li, X., Wang, M., Zhu, J.Y., Qiu, X., 2014. Understanding the effects of lignosulfonate on enzymatic saccharification of pure cellulose. *Cellulose* 21, 1351–1359.
- Lou, H., Zhu, J.Y., Lan, T.Q., Lai, H., Qiu, X., 2013. PH-induced lignin surface modification to reduce nonspecific cellulase binding and enhance enzymatic saccharification of lignocelluloses. *ChemSusChem* 6, 919–927.
- 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. *Ind. Eng. Chem. Res.* 49, 8258–8266.
- Monavari, S., Bennato, A., Galbe, M., Zacchi, G., 2010. Improved one-step steam pretreatment of SO<sub>2</sub>-impregnated softwood with time-dependent temperature profile for ethanol production. *Biotechnol. Prog.* 26, 1054–1060.
- National Research Council. 2011. *Renewable Fuel Standard: Potential Economic and Environmental Effects of U.S. Biofuel Policy*. <[http://www.nap.edu/catalog.php?record\\_id=13105](http://www.nap.edu/catalog.php?record_id=13105)>, ISBN 978-0-309-18751-0.
- Pan, X.J., Xie, D., Yu, R.W., Lam, D., Saddler, J.N., 2007. Pretreatment of lodgepole pine killed by mountain pine beetle using the ethanol organosolv process: fractionation and process optimization. *Ind. Eng. Chem. Res.* 46, 2609–2617.
- Perlack, R.D., Stokes, B.J., 2011. DOE 2011. U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry. Oakridge National Laboratory. DE-AC05-00R22725.
- 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. *Biotechnol. Prog.* 27, 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. *Biotechnol. Prog.* 25, 1086–1093.
- Wang, Z.J., Lan, T.Q., Zhu, J.Y., 2013. Lignosulfonate and elevated pH can enhance enzymatic saccharification of lignocelluloses. *Biotechnol. Biofuels* 6, 9.
- Yamamoto, M., Niskanen, T., Iakovlev, M., Ojamo, H., van Heiningen, A., 2014. The effect of bark on sulfur dioxide-ethanol-water fractionation and enzymatic hydrolysis of forest biomass. *Bioresour. Technol.* 167, 390–397.
- Zhang, C., Houtman, C.J., Zhu, J.Y., 2014. Using low temperature to balance enzymatic saccharification and furan formation in SPORL pretreatment of Douglas-fir. *Process Biochem.* 49, 466–473.
- Zhang, C., Zhu, J.Y., Gleisner, R., Sessions, J., 2012. Fractionation of forest residues of Douglas-fir for fermentable sugar production by SPORL pretreatment. *Bioenergy Res.* 5, 978–988.
- Zhou, H., Lou, H., Yang, D., Zhu, J.Y., Qiu, X., 2013a. Lignosulfonate to enhance enzymatic saccharification of lignocelluloses: role of molecular weight and substrate lignin. *Ind. Eng. Chem. Res.* 52, 8464–8470.
- Zhou, H., Zhu, J.Y., Luo, X., Leu, S.-Y., Wu, X., Gleisner, R., Dien, B.S., Hector, R.E., Yang, D., Qiu, X., Horn, E., Negron, J., 2013b. Bioconversion of beetle-killed lodgepole pine using SPORL: process scale-up design, lignin coproduct, and high solids fermentation without detoxification. *Ind. Eng. Chem. Res.* 52, 16057–16065.
- Zhu, J.Y., Gleisner, R., Scott, C.T., Luo, X.L., Tian, S., 2011. High titer ethanol production from simultaneous enzymatic saccharification and fermentation of aspen at high solids: a comparison between SPORL and dilute acid pretreatments. *Bioresour. Technol.* 102, 8921–8929.
- Zhu, J.Y., Pan, X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. *Bioresour. Technol.* 101, 4992–5002.
- Zhu, J.Y., Pan, X.J., Wang, G.S., Gleisner, R., 2009. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour. Technol.* 100, 2411–2418.
- Zhu, W., Houtman, C.J., Zhu, J.Y., Gleisner, R., Chen, K.F., 2012. Quantitative predictions of bioconversion of aspen by dilute acid and SPORL pretreatments using a unified combined hydrolysis factor (CHF). *Process Biochem.* 47, 785–791.