

# Comparisons of high titer ethanol production and lignosulfonate properties by SPORL pretreatment of lodgepole pine at two temperatures

 Cite this: *RSC Adv.*, 2014, 4, 27030

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Mountain pine beetle killed lodgepole pine wood chips were pretreated by SPORL (Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses) at 180 °C for 25 min and 165 °C for 75 min using the same chemical loadings, which represent the same pretreatment severity. The pretreated whole slurries were used to produce lignosulfonate and ethanol through simultaneous enzymatic saccharification and combined fermentation (SSCombF) up to solid loadings of 18% without detoxification. Low temperature pretreatment reduced furan formation, which facilitated ethanol production as measured by ethanol productivity and sugar consumption. The improved carbohydrate yields at 165 °C also produced high ethanol yields (liter per tonne wood) at all SSCombF solids loadings. An ethanol yield and titer of 306 L per tonne wood, or approximately 72% theoretical, and 47.1 g L<sup>-1</sup>, respectively, were achieved without detoxification at 165 °C. Lignosulfonates (LS) produced from the two SPORL runs are highly sulfonated but have lower molecular weight than a commercial high purity softwood LS. Both infrared and NMR spectra of LS from SPORL treated wood chips were compared with those of the commercial LS. The LSs from SPORL treated wood chips were found to have better dispersion properties than the commercial LS.

Received 21st April 2014

Accepted 9th June 2014

DOI: 10.1039/c4ra03608e

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## 1. Introduction

High titer biofuel production from lignocelluloses is critically important to process energy efficiency and economics.<sup>1,2</sup> While microbial fermentation is a proven route for biofuel production from lignocelluloses using the sugar platform, microbial inhibition arising from degradation products produced during the lignocellulose pretreatment step limits the final product titer.<sup>3,4</sup> Studies reporting high titer ethanol production are limited to either low yields because mild pretreatment conditions were used to reduce inhibitor formation,<sup>5</sup> or using a feedstock with very low recalcitrance such as corncob,<sup>6</sup> or just using the washed solids without including inhibitor containing pretreatment spent liquor (hemicellulosic sugar stream).<sup>7</sup> Washing is not practical because of water consumption is an environmental concern. Without washing the solids, a detoxification step is often necessary for fermentation.<sup>4,8</sup> The concept of low temperature pretreatment was developed to avoid detoxification for high solids fermentation; however pretreatment duration was arbitrary determined and severity was reduced. As a result, sugar yield was maintained at the expense of increased energy input

through additional processing steps such as alkali extraction and mechanical refining, and xylanase supplementation.<sup>9</sup>

We proposed the concept of applying a lower pretreatment temperature for longer times than is typical so as to maintain pretreatment severity and therefore the resultant substrate enzymatic digestibility in a previous study.<sup>10</sup> This is based on the fact that the activation energy of sugar degradation to microbial inhibitors is greater than that for hemicellulose dissolution; therefore hemicellulose dissolution can be favored over sugar degradation by designing a low temperature pretreatment with longer reaction time. Furan formation was found significantly reduced at a low temperature compared with a high temperature pretreatment at the same severity.<sup>10</sup> Furthermore, by maintaining the same pretreatment severity, the substrate enzymatic saccharification efficiency was not negatively affected.<sup>10</sup>

The objective of this study is to demonstrate the advantage of this low temperature pretreatment with longer reaction time for high titer ethanol production through fermentation using a *Saccharomyces cerevisiae*. Two pretreatments with the same severities but at different temperatures were compared in terms of ethanol productivity, yield, and the quality of the lignin co-product. Lodgepole pine wood chips were pretreated by SPORL (Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses)<sup>11</sup> at 180 °C for 25 min and 165 °C for 75 min using identical liquid to wood ratio and chemical loadings. SPORL was chosen because of its robust performance in removing the strong

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recalcitrance of softwoods and softwood forest residues.<sup>10,12,13</sup> The pretreated whole slurry were first liquefied and then fermented without detoxification at three unwashed solids loadings of 12, 15, and 18%. Results from this study expand upon a prior study and confirmed that lodgepole chips pretreated at the lower temperature (165 °C, 75 min) could be converted to ethanol without conditioning the whole slurry beforehand.<sup>14</sup> An additional benefit of the SPORL process is the co-production of lignosulfonate (LS), which has an existing commercial market. Therefore, the properties of lignosulfonate (LS) dissolved in the SPORL pretreatment spent liquor were also compared.

## 2. Materials and methods

### 2.1 Materials

A mountain pine beetle-killed tree (labeled as BD4) was harvested from the Canyon Lakes Ranger District of the Arapaho–Roosevelt National Forest, Colorado, USA, and debarked on site. Logs were wrapped in plastic bags before shipping to the USDA Forest Products Laboratory (FPL), Madison, WI. Detailed information about the tree, harvesting, and transportation have been described previously.<sup>15,16</sup> Wood chips were produced at FPL and the chips were screened to retain chips between 6 and 38 mm. The screen chips were frozen at approximately –16 °C until use.

Commercial cellulase enzymes Cellic® CTec2 (abbreviated CTec2) were complimentary provided by Novozymes North America (Franklinton, North Carolina, USA). The cellulase activity of 147 FPU mL<sup>-1</sup> was calibrated using a literature method.<sup>17</sup> All chemicals including sodium acetate buffer, sulfuric acid, and sodium bisulfite were ACS reagent grade and purchased from Sigma-Aldrich (St. Louis, MO). High purity sodium LS (D748) from sulfite pulping of softwood was donated by LignoTech USA (Rothschild, WI).

Titanium dioxide (TiO<sub>2</sub>) powder was purchased from Kermel Chemistry Co. Ltd. (Tianjin, China) with purity of 99%. The manufacture specified average particle diameter of TiO<sub>2</sub> was 5.43 μm. An EyeTech Laser particle size analyzer (Ankersmid Co. Ltd., Holland) was used to analysis TiO<sub>2</sub> particle size in aqueous suspensions.

*Saccharomyces cerevisiae* YRH400 was engineered from a fungal strain for xylose fermentation.<sup>18</sup> YPD agar plates containing 10 g L<sup>-1</sup> yeast extract, 20 g L<sup>-1</sup> peptone, 20 g L<sup>-1</sup> glucose, and 20 g L<sup>-1</sup> agar, was used to grow the strain at 30 °C. A liquid YPD medium was used to culture the strain in a flask on a shaking bed at 90 rpm (Thermo Fisher Scientific, Model 4450, Waltham, MA) overnight at 30 °C. The cultured medium was monitored using optical density at 600 nm (OD<sub>600</sub>) by a UV-Vis spectrometer (Model 8453, UV-visible spectroscopy system, Agilent Technologies, Palo Alto, CA) and used to inoculate the fermentation culture.

### 2.2 SPORL pretreatment

SPORL pretreatments of lodgepole pine BD4 wood chips were conducted in a 23 L laboratory wood pulping digester using dilute sodium bisulfite solution of pH approximately 2.0 at two temperatures of 180 °C and 165 °C for 25 min and 75 min,

respectively. The required reaction time of 75 min at the lower pretreatment temperature of 165 °C was determined using eqn (1) when the same chemical loadings of C<sub>A</sub> and C<sub>B</sub> were maintained based on constant pretreatment severity measured by the combined hydrolysis factor (CHF; eqn (2)).<sup>10</sup>

$$t^{T^{165}} = \exp \left[ -\frac{E}{R} \left( \frac{1}{T^{165}} - \frac{1}{T^{180}} \right) \right] t^{T^{180}} \quad (1)$$

$$\text{CHF} = e^{\left( \alpha - \frac{E}{RT} + \beta C_A + \gamma C_B \right)} (C_A + C_B)t \quad (2)$$

Where  $E = 100\,000 \text{ J mole}^{-1}$  is apparent activation energy,  $R = 8.314$  is universal gas constant, and  $t^{T^{180}} = 25 \text{ min}$ . The digester was heated by a steam jacket and rotated at 2 rpm for mixing. Both pretreatments used 2000 g wood chips in oven dry (od) weight with fixed liquor to wood ratio (L/W) of 3 : 1. The sulfuric acid and sodium bisulfite charge on wood as mass fraction was 2.2% and 8.0%, respectively. These pretreatment conditions resulted in the same pretreatment severity of CHF = 22.5 to produce the same level of hemicellulose dissolution.<sup>10,19</sup> Therefore, comparisons can be made between these two pretreatments for ethanol production. At the end of each pretreatment, the digester was cooled down by flushing the heating steam jacket with tap water to terminate the reaction. The pretreated solids and spent liquor were directly transferred to a disk mill for size reduction without adding water. The resultant whole slurry was pressed in a screen box to separate solids from liquor for component mass balance determination. The yield of solids (unwashed) was determined from the oven dry weight of the material. Both the volume and weight of the collected pretreatment spent liquor were recorded. Duplicate pretreatments were conducted at each pretreatment temperature and the solid and liquor samples from replicate runs were respectively combined for downstream saccharification and fermentation evaluation.

### 2.3 Enzymatic hydrolysis

Washed pretreated solid substrate at 20 g L<sup>-1</sup> loading was used in enzymatic hydrolysis in a 50 mL of 50 mM acetate buffer setting on a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 50 °C and 200 rpm. Elevated pH 5.5, higher than commonly used pH 4.8–5.0, is to significantly reduce nonproductive cellulase binding to lignin to enhance lignocellulose saccharification.<sup>20–22</sup> NaOH of 5 wt% or acetic acid was used to adjust the pH of the substrate suspension to pH 5.5. The CTec2 loading was 10 FPU or 0.067 mL g<sup>-1</sup> glucan that is lower than most studies using softwoods. Aliquots of 1 mL enzymatic hydrolysate were taken periodically (3, 6, 9, 24, 48, and 72 h) for glucose analysis after centrifuging 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 value and standard deviation used as error bars in plots.

### 2.4 Quasi-simultaneous enzymatic saccharification and combined fermentation (SSCombF)

Quasi-simultaneous enzymatic saccharification and combined fermentation (SSCombF) of the enzymatic hydrolysate of the pretreated lodgepole pine solids and spent liquor were carried

out in 250 mL Erlenmeyer flasks using a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at three levels of unwashed solids mass fractions, 12, 15, and 18%. The matched amount of pretreatment spent liquor, determined based on the collected amounts of unwashed solids and liquor, *i. e.*, their respective yields from pretreatment, was added to the flasks. The mixture (or the pretreated lodgepole pine whole slurry) was adjusted to pH 6.2 with solid calcium hydroxide. Acetic acid/sodium acetate buffer (0.05 mole L<sup>-1</sup>) of pH 5.5 was added into the pH adjusted mixture to liquefy the water insoluble solids using CTec2 at 20 FPU g<sup>-1</sup> glucan (doubled the amount used in saccharification study discussed above). Elevated pH 5.5 is again used to reduce nonproductive cellulase binding to lignin and enhance saccharification.<sup>20–22</sup> Liquefaction of solid substrate was observed in about 18–22 h at 50 °C and 200 rpm. The mixture was then cooled down to 35 °C and the shaker speed was reduced to 90 rpm and inoculated with 2 mL of yeast seed. The initial optical density of the yeast for all fermentation experiments was controlled at OD<sub>600</sub> = 5. No nutrients were applied during fermentation. Aliquot samples of fermentation broth were taken periodically for mono-saccharides, inhibitor, and ethanol analyses. Reported results are the average of duplicate analyses. Replicate fermentation runs were conducted to ensure experimental repeatability. The standard deviations were used as error bars in plotting.

## 2.5 Analytical methods

The chemical compositions of the untreated and pretreated lignocelluloses were analyzed as described previously.<sup>15</sup> All lignocellulosic samples were Wiley milled (Model No. 2, Arthur Thomas Co, Philadelphia, PA, USA) to 20 mesh (~1 mm) and hydrolyzed in two stages using sulfuric acid volumetric concentration of 720 mL L<sup>-1</sup> at 30 °C for 1 h and 36 mL L<sup>-1</sup> at 120 °C for 1 h. Carbohydrates of the hydrolysates were analyzed by high performance anion exchange chromatography with pulsed amperometric detection (ICS-5000, Dionex). Klason lignin (acid insoluble) was quantified gravimetrically.<sup>23</sup> For fast analysis, glucose in the enzymatic hydrolysates were measured using a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH, USA).

The pretreatment spent liquor and fermentation broth samples were analyzed using a Dionex HPLC system (Ultimate 3000) for glucose, mannose, xylose, arabinose, and galactose using an RI (RI-101) detector and BioRad Aminex HPX-87P column (300 × 7.8 mm) operated at 80 °C. Double distilled water (d.d.w.) was used as eluent at a flow of 0.6 mL min<sup>-1</sup>. The same HPLC was used to analyze fermentation inhibitors (acetic acid, furfural and 5-hydroxymethylfurfural (HMF)) and ethanol using a UV detector (VWD-3400RS) and a BioRad Aminex HPX-87H column (300 × 7.8 mm) operated at 60 °C. Diluted sulfuric acid solution of 0.005 mole L<sup>-1</sup> was used as eluent at a flow rate of 0.6 mL min<sup>-1</sup>. Samples were centrifuged at 13 000g for 5 min. The supernatant was diluted by deionized water, and then filtered by a 0.22 μm filter prior to injection to the column. All sample injection volume was 20 μL.

## 2.6 LS purification and separation and LS dispersion properties

Lignin purification and separation was achieved through dialysis using Spectra/Pro® Biotech Cellulose Ester Dialysis Membrane bag (MWCO: 100–500 Da, Spectrum Laboratories, Inc., Rancho Dominguez, CA). The dispersion property of different LS was evaluated by examining the stability of TiO<sub>2</sub> particle in a dilute aqueous solution using a Turbiscan Lab Expert (Formulation, France). Detailed procedure for LS separation and dispersion measurements can be found in our previous study.<sup>14</sup>

## 2.7 Gel permeation chromatography (GPC)

Aqueous GPC was conducted using columns Ultrahydrogel™ 120 and Ultrahydrogel™ 250 and a UV detector 2487 at 280 nm (Waters Corp., Milford, MA). Polystyrene sulfonate with molecular weight ranged from 500 to 10 000 g mole<sup>-1</sup> was used for calibration. A 0.10 mole L<sup>-1</sup> NaNO<sub>3</sub> solution was used as eluent at 0.50 mL min<sup>-1</sup>. All LS samples after dialysis (*M<sub>w</sub>* < 500), filtrated by a 0.22 μm filter, were used for GPC analysis.

## 2.8 IR and <sup>1</sup>H-NMR analyses

Infrared (IR) spectra of LS were recorded between 4000 and 400 cm<sup>-1</sup> with 32 scans on a Nexus spectrometer (Thermo Nicolet, USA). Disks were prepared by mixing 2 mg of dried LS (after dialysis) with 200 mg KBr (for spectroscopy) in an agate mortar.

The <sup>1</sup>H-NMR spectra of LS were recorded with 30 mg LS (after dialysis) dissolved in 0.5 mL of dimethylsulfoxide (DMSO-*d*<sub>6</sub>) using a Bruker DRX-400 (maker and manufacture) spectrometer at 400 MHz.

# 3. Results and discussion

## 3.1 Comparison of wood component recovery

Wood chips were pretreated at 165 °C for 75 minutes or 180 °C for 25 minutes with the same chemical loadings; these two reaction conditions were chosen because they sum to the same combined severity factor. The pretreated wood chips were pressed and recovered as unwashed solids and spent liquor. Total solids recovery as initial untreated wood chip mass fraction was 96.6 and 85.7% for pretreatment conducted at 165 °C and 180 °C, respectively. Most of the total solids were retained with the unwashed wet solids because it contains all water insoluble components and approximately two thirds (67%) of the pretreatment spent liquor. High pretreatment temperature reduced the recovery of water insoluble solids, due to increased dissolution, and slightly increased recovery of dissolved solids including liginosulfonate (Table 1). Glucan recovery as wood glucan from the unwashed solids were 97 and 83% for pretreatment conducted at 165 and 180 °C, respectively. The total recoveries of individual saccharides following pretreatment at 165 °C were approximately 10% more than the respective value from pretreatment conducted at 180 °C.

The HMF and furfural production due to carbohydrate degradation as mass fractions of wood xylan and mannan were 5.2 and 7.6% at 165 °C, respectively, which is significantly lower

**Table 1** Chemical composition of the untreated lodgepole pine BD4 wood chips and wood component recoveries from SPORL pretreatments at two temperatures. Pretreatments were conducted at sulfuric acid and sodium bisulfite loading on wood of 2.2 and 8%, respectively, for 25 and 75 min at 180 °C and 165 °C, respectively

Temperature (°C)	Untreated wood <sup>d</sup>	Unwashed solids <sup>d</sup>		Collected Spent Liquor <sup>d</sup>		Total recovery (%)	
		165 <sup>d</sup>	180	165 <sup>d</sup>	180	165 <sup>d</sup>	180
Wet weight (kg)	2.281	6.227	5.620	1.726	2.250		
Solids content (%)	87.7	31.86	29.98	8.37	9.09		
Solids <sup>b</sup> (kg)	2.0	1.984; 90.0%	1.685; 76.4%	0.144; 6.6%	0.205; 9.3%	96.6%	85.7%
Klason lignin (%)	28.6	28.31; 99.0%	24.45; 85.5%	0.29; 1.0%	4.15; 14.5%	100.0%	100.0%
Arabinan (%)	1.7	0.58; 34.2%	0.39; 23.0%	0.06; 3.3%	0.07; 3.9%	37.6%	26.9%
Galactan (%)	2.9	1.43; 49.3%	1.04; 35.9%	0.40; 13.9%	0.55; 18.9%	63.1%	54.8%
Glucan (%)	41.9	40.64; 97.0%	34.68; 82.8%	0.60; 1.4%	1.03; 2.5%	98.4%	85.2%
Mannan (%)	11.7	5.98; 51.1%	4.21; 36.0%	1.70; 14.5%	2.39; 20.4%	65.6%	56.4%
Xylan (%)	5.5	2.86; 52.0%	1.87; 33.9%	0.53; 9.7%	0.70; 12.7%	61.6%	46.6%
HMF <sup>c</sup> (%)		0.43; 3.7%	0.72; 6.1%	0.18 (1.7); 1.5%	0.41 (3.0); 3.5%	5.2%	9.6%
Furfural <sup>c</sup> (%)		0.31; 5.7%	0.40; 7.3%	0.13 (1.1); 2.3%	0.23 (1.6); 4.2%	8.0%	11.5%
Acetic acid (%)		1.87	2.05	0.76	1.17		

<sup>a</sup> The numbers after “;” is wt% of theoretical based on untreated wood carbohydrate content. <sup>b</sup> In oven dry (od) weight. <sup>c</sup> Reported as of xylan and mannan for furfural and HMF, represent percent of xylan and mannan converted to furfural and HMF, respectively. The numbers in the parenthesis are concentrations measured in the collected spent liquor in g L<sup>-1</sup>. <sup>d</sup> Compositional data of untreated and SPORL pretreated at 165 °C biomass were reported previously.<sup>14</sup>

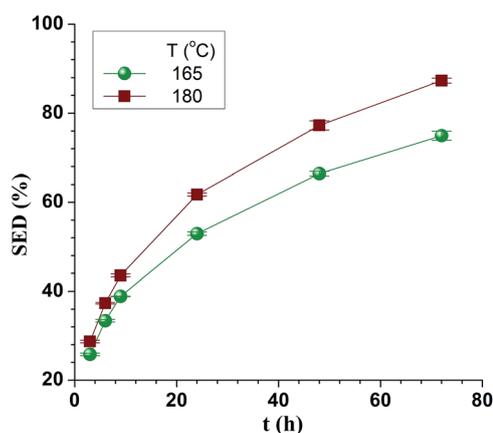
than 9.6 and 11.1% at 180 °C. The ratio of the measured furan (HMF + furfural) concentration between 180 °C and 165 °C is 1.64, close to the predicted value of 1.75 as calculated using the kinetic model developed previously.<sup>10</sup>

### 3.2 Comparison of enzymatic saccharification of washed solids

Substrate enzymatic digestibility (SED) for each pretreated sample was measured under standard conditions using washed solids at 20 g L<sup>-1</sup> solids loading, and defined as the percentage of substrate glucan enzymatically saccharified to glucose. While these conditions deviate from process conditions, they allow for comparison with literature values. SED at 72 h reduced from 87% to 75% when pretreatment temperature was reduced from 180 °C to 165 °C (Fig. 1). This suggests slightly reduced delignification and dissolution of hemicelluloses at 165 °C negatively impacted SED even though these two pretreatment were conducted at the same severities of CHF = 22.5.

### 3.3 Comparison of fermentation performance

The whole slurries produced from the wood chips were converted to ethanol using enzymatic liquefaction followed by simultaneous saccharification and combined fermentation (SSCombF). At 12% solids, pretreatment at the higher temperature (180 °C) facilitated greater cellulose digestion during the liquefaction step than 165 °C. The initial glucose concentrations following liquefaction were 52 g L<sup>-1</sup> and 44 g L<sup>-1</sup> for the higher and lower temperatures, respectively (Fig. 2a, yeast added at  $t = 0$ ). Within the first 18 h of fermentation, the initial glucose had been consumed and glucose concentration was constant for both runs; indicating that the rate of glucose release and consumption were equal thereafter. The higher initial glucose concentration resulted in improved ethanol production throughout the SSCombF. A final ethanol



**Fig. 1** Time-dependent substrate enzymatic digestibility (SED) of two SPORL-pretreated lodgepole pine at 180 °C and 165 °C after washing.

concentration of 32 g L<sup>-1</sup> was obtained from the slurry pretreated at 180 °C compared with 29 g L<sup>-1</sup> for 165 °C. The average glucose consumption and ethanol production at 24 h were -2.48 and 1.01 g L<sup>-1</sup> h<sup>-1</sup>, respectively, for the slurry produced at 180 °C compared with -2.27 and 0.89 g L<sup>-1</sup> h<sup>-1</sup> for the slurry produced at 165 °C (Table 2). However, the average mannose consumption in the 24 h for the slurry produced at 180 °C was -0.45 g L<sup>-1</sup> h<sup>-1</sup>, only slightly lower -0.54 g L<sup>-1</sup> h<sup>-1</sup> for the slurry produced at 165 °C (Table 2) as can be clearly seen from Fig. 3a. The HMF and furfural concentrations in the slurry from 180 °C were consistently higher than those in the slurry from 165 °C until all the furans were metabolized by YRH400 (Fig. 4a is shown in logarithmic scale for clarity).

At 15% solids loading, initial glucose production in SSCombF slurries from the 180 °C and 165 °C pretreatments were similar (Fig. 2b). Initial glucose concentrations following

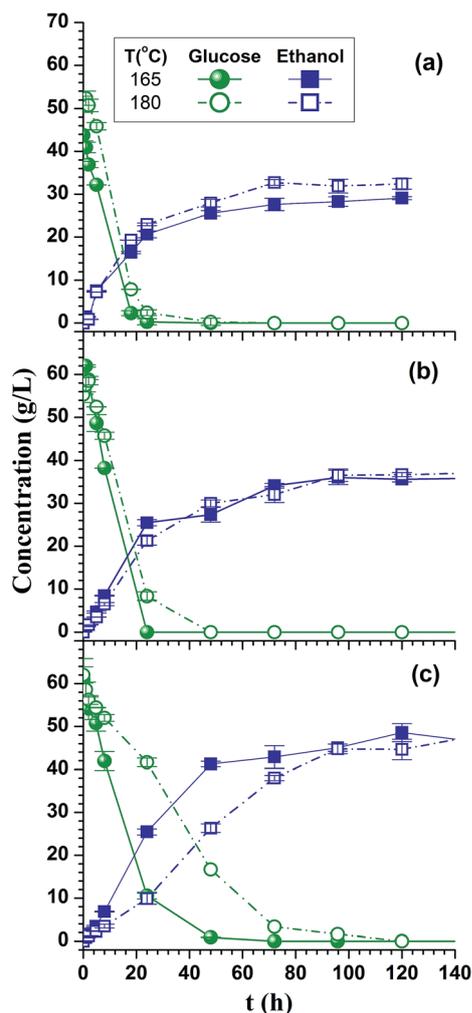


Fig. 2 Time-dependent glucose consumption and ethanol production during simultaneous enzymatic saccharification and combined fermentation (SSComBF) of SPORL-pretreated lodgepole pine whole slurry at three solids loadings. Comparison between two substrates produced at 180 °C and 165 °C. Unwashed solids loadings: (a) 12%; (b) 15%; (c) 18%.

liquefaction were approximately  $60 \text{ g L}^{-1}$  for both slurries. Glucose consumption and ethanol production for fermenting the slurry pretreated at 165 °C were  $-2.61 \text{ g L}^{-1} \text{ h}^{-1}$  and  $1.06 \text{ g L}^{-1} \text{ h}^{-1}$ , respectively, slightly higher than the corresponding values for the slurry from 180 °C,  $-2.27 \text{ g L}^{-1} \text{ h}^{-1}$  and  $0.87 \text{ g L}^{-1} \text{ h}^{-1}$  (Table 2). This reversed the trend observed from the fermentation runs at 12% solids loadings, suggesting the increased furan concentrations may have slowed ethanol production for the slurry from 180 °C pretreatment. Furthermore, both glucose consumption and ethanol production for the slurry pretreated at 165 °C in the first 24 h were higher than the corresponding value for fermentation of the slurry from 180 °C at 15% (Table 2). No obvious differences in mannose consumption between the two fermentations were observed (Fig. 3b). The same final ethanol concentration of approximately  $38 \text{ g L}^{-1}$  was produced from both slurries after 96 h fermentation.

When the solids loading was increased to 18% in SSComBF, the advantage of pretreating at 165 °C over 180 °C became readily apparent. Both glucose consumption and ethanol production rates for the slurry produced at 165 °C are significantly higher than the corresponding value for the slurry produced at 180 °C (Fig. 2c). For the slurry produced at 180 °C, the average glucose consumption within the first 24 h was reduced from  $-2.27$  to  $-0.76 \text{ g L}^{-1} \text{ h}^{-1}$  or by almost 70%. The average ethanol production within the first 24 h was reduced from  $0.87$  to  $0.40 \text{ g L}^{-1} \text{ h}^{-1}$  or more than 50%. However, for the slurry produced at 165 °C, both glucose consumption and ethanol productivity were essentially unchanged when fermentation solids loading was increased for 15 to 18%. Mannose consumption was significantly reduced comparing fermenting the slurry pretreated at 180 °C versus 165 °C (Fig. 3c); the average mannose consumption rates within the first 24 h were  $-0.14$  and  $-0.54 \text{ g L}^{-1} \text{ h}^{-1}$  for the slurries from 180 °C and 165 °C, respectively (Table 2). Both fermentation runs have a lag phase in mannose consumption of 8 and 6 h for slurry from 180 °C and 165 °C pretreatment, respectively. The same terminal ethanol concentration of approximately  $47 \text{ g L}^{-1}$ , however, was produced from both slurries.

No significant difference in xylose consumption was observed between fermenting the slurry from 180 and 165 °C at all solids levels as shown in Fig. 3a–c, perhaps due to the low xylose concentration of below  $7 \text{ g L}^{-1}$ . *Saccharomyces* yeasts do not express xylose-specific transporters and uptake of xylose into the cell is inefficient, especially at low xylose concentrations. Additionally, xylose fermentation is severely inhibited by acetate used in the buffer solution. It does not take much acetate to cause problems ( $<2 \text{ g L}^{-1} \text{ h}^{-1}$ ). The average rates of furan metabolization were approximately the same for all fermentation runs (Table 2 and Fig. 4a–c).

### 3.4 Comparison of ethanol yield

Ethanol yield in terms of g per g sugar in the pretreated whole slurry as well as liter per tonne wood base were calculated as shown in Table 2. The results indicate that improved substrate digestibility by using a higher pretreatment temperature of 180 °C increased the ethanol yield (g per g sugar) at a low SSComBF solids loading of 12%, i.e., 0.43 compared with 0.39 for the slurry pretreated at 165 °C. This advantage disappeared at the higher solids loading of 18% most likely due to the elevated level of fermentation inhibitors. Higher temperature pretreatment at 180 °C also reduced carbohydrate yield (Table 1) compared with that at 165 °C, which negatively impacted ethanol yield in terms of liter per tonne wood. The ethanol yield in L per tonne wood from the 180 °C pretreated slurry is consistently lower than that from the 165 °C at all three solids fermentation loadings. A maximum ethanol yield of 306 L per tonne wood and final titer of  $47.1 \text{ g L}^{-1}$  was achieved when SSComBF was conducted at 18% unwashed solids. Examining the time-dependent furan concentration profile, a further increase in solids loading for SSComBF is probably feasible as both HMF and furfural were completely metabolized within 48 h. Using the furan level for the slurry from 180 °C

**Table 2** Comparisons of the performance of SSCombF at three solids loadings between two lodgepole pine BD4 whole slurry samples pretreated at 180 and 165 °C

SSCombF solids (%)	12%		15%		18%	
Pretreatment <i>T</i> (°C)	165	180	165	180	165 <sup>c</sup>	180
<b>Average fermentation performance measure in the first 24 h unless indicated (g L<sup>-1</sup> h)</b>						
Ethanol productivity	0.89	1.01	1.06	0.87	1.03	0.40
Glucose consumption	-1.94	-2.48	-2.61	-2.27	-2.10	-0.76
Mannose consumption	-0.54	-0.45	-0.41	-0.40	-0.54	-0.14
HMF metabolization	-0.056 (18 h)	-0.063	-0.039	-0.061	-0.051	-0.046
Furfural metabolization	-0.059 (2 h)	-0.088 (5 h)	-0.059 (5 h)	-0.070 (8 h)	-0.058 (5 h)	-0.060 (8 h)
<b>Terminal maximal ethanol production</b>						
Ethanol concentration (g L <sup>-1</sup> )	29.1 ± 0.4	32.4 ± 1.2	36.0 ± 1.7	38.0 ± 0.3	47.1 ± 2.1	47.2 ± 1.8
Ethanol yield <sup>a</sup> (g per g sugar)	0.391 ± 0.005	0.431 ± 0.017	0.375 ± 0.045	0.393 ± 0.003	0.396 ± 0.018	0.394 ± 0.015
Ethanol yield (L per tonne wood)	302 ± 4	285 ± 11	290 ± 13	260 ± 2	306 ± 14	260 ± 10
Ethanol yield <sup>b</sup> (% theoretical)	70.8 ± 0.9	67.0 ± 2.6	68.0 ± 3.1	60.9 ± 0.5	71.8 ± 3.3	61.1 ± 2.3

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

<sup>b</sup> Theoretical yield (426 L per tonne wood) based on total glucan, mannan, xylan in the untreated wood. <sup>c</sup> Data appearing in this subcolumn were published previously as part of a different study.<sup>14</sup>

pretreatment as the limit for effective fermentation without detoxification, we can estimate the maximum limit for SSCombF solids loading of the slurry produced at 165 °C to be approximately 30%. A potential ethanol titer of 75 g L<sup>-1</sup> can be achieved without detoxification assuming a similar fermentation efficiency.

### 3.5 Comparison of LS molecular weight distribution

The dissolved lignin in spent liquor is in the form of LS, a directly marketable co-product. The sulfonic acid group content and molecular weight (MW) are two properties that can affect the performance of LS for a given application. The LS from SPORL-pretreated lodgepole pine at 165 °C, LS-SP165, and at 180 °C, LS-SP180, both have very high sulfonic acid group contents as measured by sulfur content (Table 3). The sulfur content of LS-SP180 is much higher than that of the high purity commercial softwood LS D-748. However, both LSs from SPORL pretreatment have much smaller molecular weights than the commercial product (Fig. 5). Pretreatment at 165 °C for 75 min produced a LS with a higher MW than that produced at 180 °C for 25 min. The peak MW of LS-SP165 is approximately 10% of that of D-748.

### 3.6 Spectral characteristics LS from SPORL

Infrared (IR) spectroscopy is a useful technique for studying lignin structure. The IR spectra of the LSs from SPORL spent liquor along with high purity commercial LS D-748 are shown in Fig. 6a. Based on literature assignments,<sup>24–26</sup> the broad band at 3440 cm<sup>-1</sup> is attributed to the O–H stretching in phenolic and aliphatic structures. The bands at 2937 and 1465 cm<sup>-1</sup> are C–H stretching in methyl and methylene of side chains. Weak shoulder peak at 1714 cm<sup>-1</sup> is associated with the unconjugated carboxyl stretching; peak at 1642 cm<sup>-1</sup> originates from conjugated carboxyl stretching. The peak at 1515 and 1420 cm<sup>-1</sup> are attributed to aromatic skeletal vibrations. However, there is no

obvious vibrational characterization for guaiacyl (G) and syringyl (S) ring, such as the G ring and C=O stretch around 1270 cm<sup>-1</sup>, the S ring around 1328 cm<sup>-1</sup>. A shoulder of the G ring and C=O stretch around 1270 cm<sup>-1</sup> can be seen from the D-748 spectrum. The peaks at 1140 and 1113 cm<sup>-1</sup> arise from the C–H in-plate deformation in G ring and S ring, respectively. Moreover, the carbohydrate arising vibrations are associated with other vibrations in the region of 1300–500 cm<sup>-1</sup>. The peak at 1042 cm<sup>-1</sup> is attributed to the S=O stretching, C–H deformation and C–OH bending. The peak at 621 cm<sup>-1</sup> is due to C–S stretching. All peak intensities for LS-SP165 from 165 °C SPORL pretreatment are lower than those for LS-SP180 from 180 °C pretreatment. The commercial LS D-748 that has much sharper peaks at 1515 and 1420 cm<sup>-1</sup> from aromatic skeletal vibrations, as well as at 1642 cm<sup>-1</sup> from conjugated carboxyl stretching than LSs from SPORL. The peaks at 1140 and 1113 cm<sup>-1</sup> of D-748 spectrum from the C–H in-plate deformation in G ring and S ring were shifted to high wavenumbers.

The <sup>1</sup>H-NMR spectra were obtained from the same amount of samples (30 mg LS in 0.5 mL DMSO-*d*<sub>6</sub>). The signal strength in Table 4 are normalized by the signal of DMSO-*d*<sub>6</sub> (2.56–2.44 ppm). As shown in Fig. 5 and Table 4, the resonance between 7.06 and 6.78 ppm and between 6.78 and 6.41 ppm is attributed to aromatic protons in guaiacyl (G) and syringyl (S) units, respectively. The signal strength represents the presence of relative contents of S and G units.<sup>27</sup> The low resonance of methoxyl protons between 3.58–3.16 ppm for the D-748 spectrum suggests the difference in demethylation reactions of aromatic methoxyl groups between sulfite pulping and SPORL pretreatment. The signal at 5.00–4.35 ppm, assigned to the H<sub>α</sub> and H<sub>β</sub> in β-O-4 units,<sup>28</sup> is decreased as pretreatment temperature increases, suggesting increased cleavage of β-O-4 linkages at high temperatures. Although the cleavage of α-aryl ether bonds is mainly responsible for fragmentation of lignin, acid-induced hydrolysis of arylglycerol-β-arylether substructures also exists during sulfite pulping.<sup>29,30</sup> This can be clearly seen from

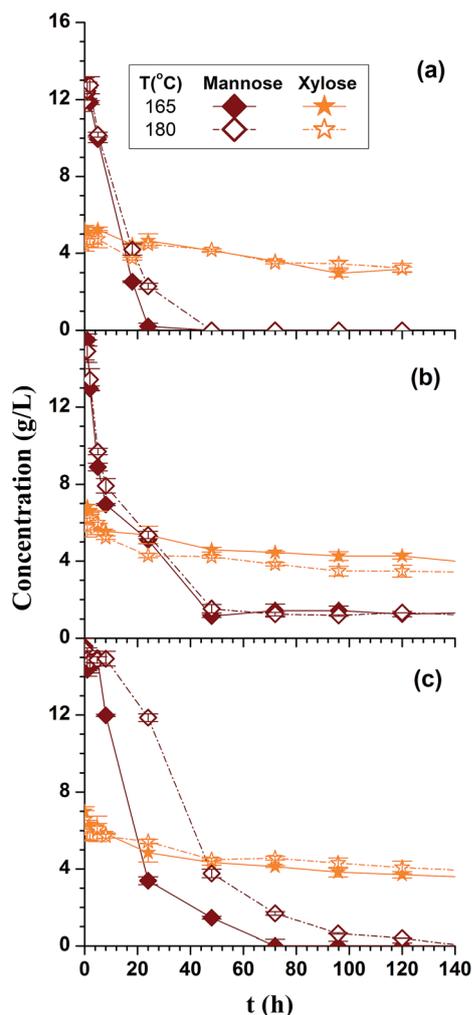


Fig. 3 Time-dependent mannose and xylose consumptions during simultaneous enzymatic saccharification and combined fermentation (SSCombF) of SPORL-pretreated lodgepole pine whole slurry at three solids loadings. Comparison between two substrates produced at 180 °C and 165 °C. Unwashed solids loadings: (a) 12%; (b) 15%; (c) 18%.

the very low signals between 5.00–4.35 for D-748 from sulfite pulping. The signal between 5.75–5.45 ppm and 3.20–2.90 ppm is attributed to the  $H_{\alpha}$  and  $H_{\beta}$  in  $\beta$ -5 and  $H_{\beta}$  in  $\beta$ - $\beta$  structure, respectively. Lignin condensation reactions can result in the formation of diphenylmethane substructures involving C-1 or C-6 and C-5 of aromatic nuclei, while some substructure in lignin can also undergo internal condensation to form a cyclohexene ring, such as  $\beta$ -O-4 and  $\beta$ - $\beta$  units.<sup>31</sup> Apparently, lignin condensation occurred in SPORL pretreatment but pretty much absent in sulfite pulping due to low temperature based on the signal strength between 5.75–5.45 ppm and 3.20–2.90 ppm (Table 4). It is also possible that the large MW of D748 may prevent differentiation of lignin structure using <sup>1</sup>H-NMR.

### 3.7 Dispersibility of LS from SPORL

LS is used as a dispersant in many commercial applications. We compared the dispersion properties of the two LSs from SPORL

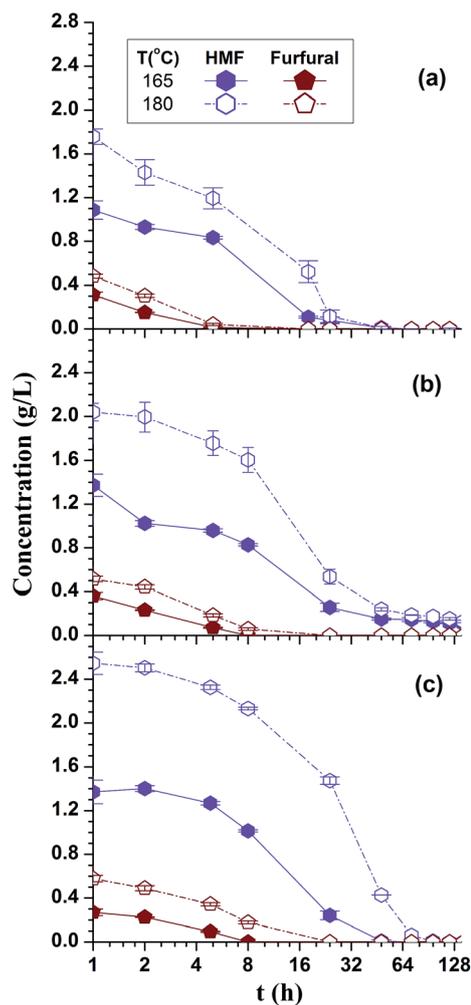


Fig. 4 Time-dependent HMF and furfural metabolization during simultaneous enzymatic saccharification and combined fermentation (SSCombF) of SPORL-pretreated lodgepole pine whole slurry at three solids loadings. Comparison between two substrates produced at 180 °C and 165 °C. Unwashed solids loadings: (a) 12%; (b) 15%; (c) 18%.

Table 3 Comparisons of elemental contents between a high purity commercial softwood LS and LSs from SPORL-pretreated lodgepole at 180 and 165 °C

LS	N (%)	C (%)	H (%)	Sulfur (wt%)
LS-SP180	<0.14	24.99 ± 0.050	3.44 ± 0.003	11.33 ± 0.32
LS-SP165	0.51 ± 0.121	41.93 ± 0.392	5.15 ± 0.090	6.04 ± 0.33
D-748	0.12 ± 0.006	44.07 ± 0.032	5.45 ± 0.051	6.01 ± 0.39

pretreatment with that of commercial LS D-748 by evaluating the mean particle size of TiO<sub>2</sub> in an aqueous solution. Without the application of LS (control), TiO<sub>2</sub> mean particle size increased linearly with time due to agglomeration as shown in Fig. 7. The increase was approximately 50%, from 17 to 25 μm in 60 min. The mean particle size was increased only by 5.5% and 8.6% with the application of LS-SP180 and LS-SP165, respectively, in the same 60 min period. These increments are lower

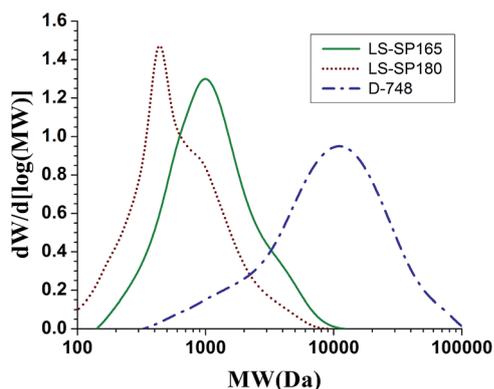


Fig. 5 Comparison of molecular weight distribution between a commercial high purity softwood lignosulfonate (LS) and LSs produced from SPORL pretreatments of lodgepole pine at 180 °C and 165 °C.

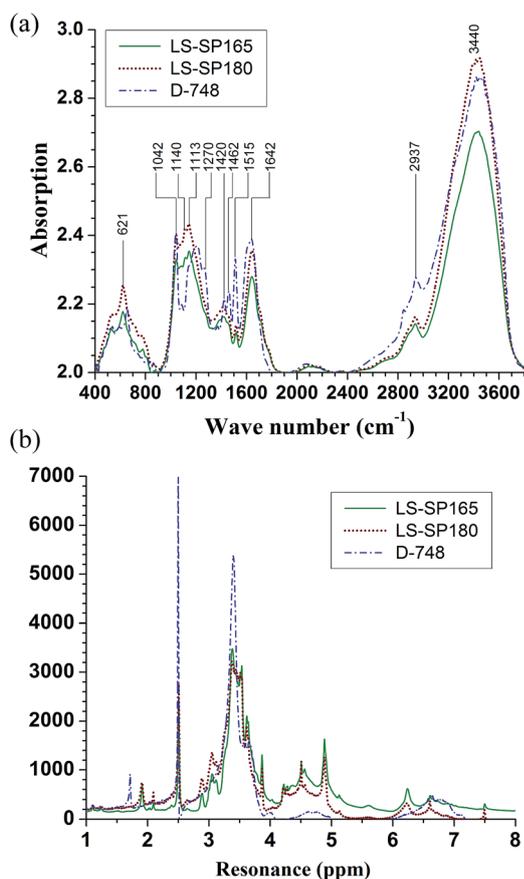


Fig. 6 Comparisons of NMR resonance and infrared (IR) absorption spectra between a commercial high purity softwood lignosulfonate (LS) and LSs produced from SPORL pretreatments of lodgepole pine at 180 °C and 165 °C. (a) IR spectra; (b)  $^1\text{H-NMR}$ .

than the 14.1% achieved applying a high purity commercial LS D-748. More importantly, the initial particle size were 2.6 and 2.8  $\mu\text{m}$  with the application of LS-SP180 and LS-SP165, respectively, smaller than the 4.5  $\mu\text{m}$  when applying D-748. These results indicate that LSs from the present SPORL pretreatments

Table 4 Signal assignment of  $^1\text{H-NMR}$  spectra of LSs

Signal (ppm)	Assignment	LS-SP180	LS-SP165	D-748
7.06–6.78	$\text{H}_2, \text{H}_5, \text{H}_6$ in guaiacyl (G) units	0.11	0.71	0.43
6.78–6.41	$\text{H}_2, \text{H}_6$ in syringyl (S) units	0.47	1.12	0.84
5.75–5.45	$\text{H}_\alpha, \text{H}_\beta$ in $\beta$ -5' substructures	0.07	0.61	—
5.00–4.75	$\text{H}_\alpha$ in $\beta$ -O-4' structures	1.95	2.65	0.13
4.75–4.35	$\text{H}_\beta$ in $\beta$ -O-4' structures	1.2	1.8	0.25
3.58–3.16	H in methoxyls	8.01	8.43	6.7
3.20–2.90	$\text{H}_\beta$ in $\beta$ - $\beta'$ structures	1.85	1.36	—
2.56–2.44	DMSO	1	1	1
2.15–2.00	H in aromatic acetates	0.47	0.33	0.32
1.97–1.71	H in aliphatic acetates	0.82	0.51	0.49
1.62–0.75	Aliphatic H	1.53	1.34	1.2

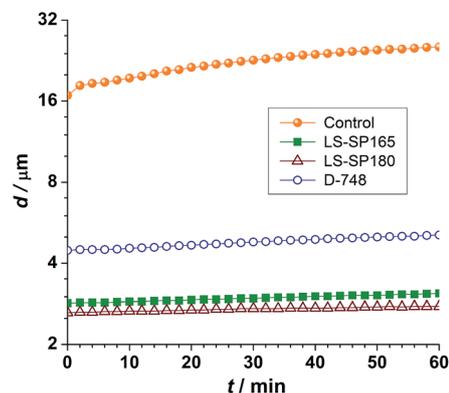


Fig. 7 Comparison of time-dependent  $\text{TiO}_2$  particle size with and without the application of lignosulfonate (LS) from SPORL pretreatments and a commercial source.

have better dispersion properties than high purity commercial softwood LS D-748 despite their smaller molecular weight.

## 4. Conclusions

At similar pretreatment severities, as determined by the combined hydrolysis factor (CHF) for hemicellulose dissolution, a lower temperature pretreatment at 165 °C with a longer duration of 75 min produced less sugar degradation products such as furan compared with pretreating at 180 °C for 25 min. The lower temperature SPORL pretreatment at 165 °C produced a solid substrate with slightly less enzymatic digestibility as measured by enzymatic cellulose saccharification using 2% washed solids compared with the substrate produced at 180 °C. As a result, glucose consumption and ethanol production was reduced when simultaneous enzymatic saccharification and combined fermentation (SSComBF) of the pretreated whole slurry was conducted at low unwashed solids loadings of 12%. However, the reduced amount of furan formation associated with low pretreatment temperature using SPORL facilitated SSComBF at high solids loadings of 18%. SPORL pretreatment at 165 °C also produced a higher carbohydrate yield than that at 180 °C and an overall higher ethanol yield per tonne wood at all

solids loadings. SSCombF at 18% solids loading produced an ethanol yield of 306 L per tonne wood at titer of  $47.1 \text{ g L}^{-1}$  without detoxification using a lodgepole pine tree killed by mountain pine beetles. Lignosulfonates (LSs) produced by SPORL at 180 and 165 °C are highly sulfonated though they have a lower molecular weight than a commercial high purity softwood LS. LSs from SPORL treated wood chips had better dispersity than the commercial LS from softwood.

## Acknowledgements

This work was supported by a USDA Small Business Innovative Research (SBIR) Phase II project (Contract Number: 2010-33610-21589) to Biopulping International, and the Chinese Scholarship Council (CSC), and the International Science and Technology Cooperation Program of China (ISTCP): 2013DFA41670. The funding from these programs made the visiting appointment of Zhou at the USDA Forest Products Laboratory (FPL) possible. We would like to acknowledge Jerry Gargulak of LignoTech for providing us the D748 Lignosulfonate; Fred Matt for conducting carbohydrate measurements; and Bruce Dien and Ron Hector of USDA Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL for providing us the YRH400 yeast and insightful discussion of fermentation performance. All authors have no conflict of interest in publishing this paper.

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