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Pilot-scale demonstration of SPORL for bioconversion of lodgepole pine to bioethanol and lignosulfonate

Abstract: The process sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL) has been the focus of this study. Pilot-scale (50 kg) pretreatment of wood chips of lodgepole pine (*Pinus contorta* Douglas ex Loudon) killed by mountain pine beetle (*Dendroctonus ponderosae* Hopkins) were conducted at 165°C with a dilute sulfite solution of pH 2 for bioconversion to ethanol and lignosulfonate (LS). The pretreatment duration was optimized in laboratory bench scale experiments with a certain severity based on a combined hydrolysis factor (CHF). The sodium bisulfite loading was 8% and the liquor to wood ratio 3. The pretreated solids were disk milled together with the spent liquid and the resultant slurry with a 25% solids content was directly (without detoxification) submitted to a simultaneous enzymatic saccharification and fermentation (SSF) with *Saccharomyces cerevisiae* YRH400 at cellulase loading of 35 ml kg⁻¹ of untreated wood. At solids loading of 20%, the alcohol yield was 288 l t⁻¹ wood (with a final concentration of 52.2 g l⁻¹), which corresponds to a 72.0% theoretical yield based on total glucan, mannan, and xylan. The LS from SPORL was highly sulfonated and its molecular weight was lower than that of a purified commercial softwood LS, and therefore it has a high potential as a directly marketable co-product.

Keywords: biofuel, enzymatic saccharification/hydrolysis, forest biorefinery, high solids fermentation, lignosulfonate, process scale-up

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Introduction

Woody biomass can be sustainably produced in large quantities in many regions of the world (Gan and Smith 2006; Perlack and Stokes 2011) and it has many advantages over herbaceous biomass in terms of logistics in harvesting, transportation, and storage (Zhu and Pan 2010). Producing sugars from woody biomass through enzymatic saccharification of structural carbohydrates can be an additional income source of the pulp and paper industry in the scope of the biorefinery concept as sugars are versatile and marketable raw materials (Bozell and Petersen 2010). Effective and commercially scalable pretreatment technologies are still a challenge in view of the difficult accessibility of wood to enzymes (Lynd et al. 2008; Zhu and Pan 2010). The research goals are high sugar yields, low toxicant formation, and low nonproductive cellulase binding by lignin to avoid solids washing and facilitate fermentation of pretreated whole slurry at high solids (Pan et al. 2005; Monavari et al. 2010; Zhu and Pan 2010; Yamamoto et al. 2011; Modenbach and Nokes 2012; Agarwal et al. 2013; Hoyer et al. 2013; Lan et al. 2013a; Tunc et al. 2014).

Sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL) was developed in our laboratory based on modified sulfite pulping (Zhu et al. 2009). By means of SPORL, woody biomass in forms of chips is pretreated in a dilute sulfite solution of approximately pH 2 in a temperature range higher than typical acidic sulfite pulping (Figure 1) to facilitate substantial hemicellulose removal even with a shorter reaction time than sulfite pulping (Wang et al. 2009, 2012; Zhu et al. 2009; Leu et al. 2013) critical to enzymatic saccharification (Leu and Zhu 2013). As a result, only partial delignification was achieved through lignin sulfonation. The sulfite solution can be prepared by bubbling SO₂ into a hydroxide solution or using acid to adjust the pH of a sulfite (with a base)

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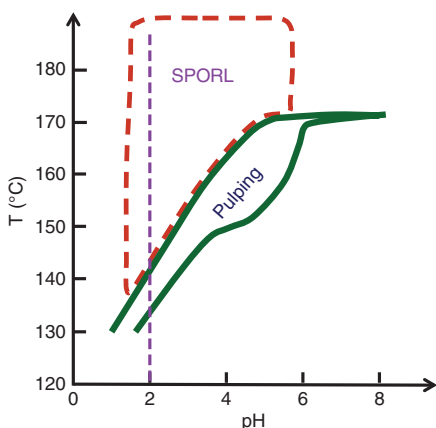


Figure 1: Comparison of operating conditions between SPORL and sulfite pulping in the temperature–pH diagram.

solution. Typical total SO_2 loading is much lower than in acidic sulfite pulping (Zhu et al. 2015). Robust ethanol production with high concentration was achieved by direct saccharification and fermentation of the pretreated whole slurry at high solid contents without washing and detoxification (Zhou et al. 2013b, 2014b). All reported studies, however, were conducted at laboratory bench scale experiments with <2 kg wood chips.

Mountain pine beetles (*Dendroctonus ponderosae* Hopkins) have affected more than half a million hectares of coniferous forests in Colorado alone and other regions of the Pacific North America. Tree mortality affects fire behavior, poses a safety hazard from falling trees, and to cope with beetle-killed lodgepole pine (BKLP, *Pinus contorta* Douglas ex Loudon) is a challenge for forest management (Jenkins et al. 2008; Klutsch et al. 2009) and its utilization as the wood is prone to checking (Woo et al. 2005). The utilization of BKLP for biofuel production, especially from wood dead for several years, is a good option to obtain value-added products. Previous studies demonstrated that such woods are more susceptible to chemical pretreatment for sugar and biofuel production through enzymatic saccharification and fermentation (Pan et al. 2008; Luo et al. 2010).

The objective of the present study is to demonstrate the ethanol production from BKLP by SPORL in a pilot-scale wood pulping digester. Additionally, the properties of the lignosulfonate (LS) dissolved by SPORL for direct marketing should be evaluated.

Materials and methods

Trees were harvested from the Canyon Lakes Ranger District of the Arapaho–Roosevelt National Forest in north central Colorado [GPS

location: 13 T 458469 4492172 (NAD27), elevation 2620 m, about 28 cm in diameter at breast height (DBH), killed by mountain pine beetle]. The trees were still fully foliated with brown needles confirming previous year attack. Emergence holes on the surface bark indicated that the beetles had left the tree and dispersed in the forest. The trees were cut into logs and the logs were manually debarked on site. Each log was individually wrapped in a plastic bag to avoid any potential beetle emergence during shipping to the USDA Forest Service, Forest Products Laboratory (FPL), Madison, WI. All wood logs were chipped at FPL in a laboratory chipper. The wood chips were then screened to remove all particles >38 mm and <6 mm in length. The thickness of the accepted chips ranged from 1 to 5 mm. The chips were kept frozen at -16°C until processing.

Commercial cellulase Cellic® CTec3 (abbreviated CTec3) was provided by Novozymes North America (Franklinton, NC, USA). The cellulase filter paper activity was 217 FPU ml^{-1} as calibrated according to Wood and Bhat (1988). All chemicals such as Folin Ciocalteu's phenol reagent (2 N), vanillin, sodium acetate, acetic acid, sulfuric acid, and sodium bisulfite were in ACS reagent grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). High-purity sodium LS (D-748) from acid sulfite pulping of softwood was donated by Ligno-Tech USA (Rothschild, WI, USA).

Saccharomyces cerevisiae YRH400 (Hector et al. 2011) was provided by the USDA Agricultural Research Service, National Center for Agriculture Utilization Research, Peoria, IL, USA. The strain was first grown on YPD agar plates as described previously (Zhou et al. 2013b) on a shaking bed incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA, USA). The concentration of the cultured biomass was monitored by measuring the optical density at 600 nm (OD_{600}) using a UV-Vis spectrometer (Model 8453, Agilent Technologies, Palo Alto, CA, USA). The cultured medium was inoculated for fermentation.

SPORL process scale-up design: The key in process scale-up design is to balance sugar yield against degradation to inhibitory compounds such as furans to achieve maximal bioethanol productivity through high solids processing. Usually, the H-factor (Vroom 1957), based on the measured thermal energy input, serves to scale up alkaline pulping. For SPORL process scale-up, a combined hydrolysis factor (CHF) (Zhu et al. 2012) is expedient:

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

where C_A and C_B are the concentrations of chemical A (sulfuric acid) and chemical B (sodium bisulfite) used in the present study, respectively; α , β , and γ are adjustable parameters; $E=100\,000 \text{ J mol}^{-1}$ is the apparent activation energy for lodgepole pine (Zhou et al. 2013b); R is the universal gas content of $8.314 \text{ J mole}^{-1} \text{ K}^{-1}$; t is time in min; and T is in degree Kelvin (K). Hemicellulose removal can be accurately predicted by CHF alone (Zhu et al. 2012; Zhou et al. 2013b).

$$X_R = (1-\theta)e^{-\text{CHF}} + \theta e^{-f\text{CHF}} \quad (2)$$

where X_R is fraction of hemicellulose remaining in pretreated solids, f is the ratio of the hydrolysis reaction rate constants between the slow and fast hemicelluloses reaction, and θ is the initial fraction of slow-reacting hemicelluloses.

The basic design principle was to maintain the same pretreatment severity measured by CHF, that is, the same level of hemicellulose removal according to Eq. (2). A previous laboratory study indicated that sugar yield can be maintained as long as CHF is maintained (Zhu et al. 2012; Zhou et al. 2013b). A low temperature

of $t_{up} = 165^\circ\text{C}$ was chosen for scale-up to 50 kg wood chips. The pretreatment duration for t_{up} can be calculated to be 75 min under the conditions of optimized laboratory pretreatment T_{op} (180°C) and t_{op} (25 min) with $\text{CHF} = 22.5$ (Zhou et al. 2013b).

$$t_{up} = \exp \left[\frac{E}{R} \left(\frac{1}{T_{up}} - \frac{1}{T_{op}} \right) \right] t_{op} \quad (3)$$

The rationales for choosing 165°C were (i) the limitation of the 50 kg reactor; (ii) the consideration of reducing sugar degradation to facilitate high solids fermentation without detoxification; and (iii) reducing reactor material corrosion and potentially capital cost for commercialization. Sugar degradation reactions have a higher activation energy than hemicellulose dissolution (Kamireddy et al. 2014; Zhang et al. 2014); as a result, a lower amount of sugar degradation products was formed at lower pretreatment temperature under the same pretreatment severity CHF (Zhang et al. 2014).

Pilot-scale pretreatment and substrate production: A total of 49.95 kg BKLP wood chips with a moisture content (MC) of 19.9%, equivalent to 40 kg in oven dry (OD) weight, were first loaded into the 390 l pilot-scale wood pulping digester. A vacuum of 20 mm mercury was created in the digester. Dilute sulfite liquor was prepared with 3.2 kg sodium bisulfite and 0.883 kg sulfuric acid mixed with 110 l city cold water in a plastic barrel. The liquor had pH approximately 2. The liquor (110 l) transferred into the digester gave a liquor to OD wood ratio (L/W, l kg^{-1}) 3:1 and a sodium bisulfite charge on wood 8%. The digester was then rotated at 2 rpm while heated by steam in a jacket. The final temperature (165°C) was reached in 38 min and maintained for 60 min. The calculated 75-min holding time had to be shortened because the temperature ramping time of 38 min was longer than the 10 min in the laboratory bench digester. At the end of pretreatment, the digester contents were discharged into a blow tank through a stainless steel pipe. Additional air blow was applied to complete discharging. Volatiles including SO_2 were vented to a wet scrubber (Figure 2). After sitting for 2 days to allow any remaining SO_2 to escape, both the solids and spent liquor were collected. The

amount of freely drainable pretreatment spent liquor collected from the blow tank was 39.95 kg. The pH of the liquor was 1.42 measured at 4°C . The amount of wet solids collected was 109.45 kg at a MC of 31.85%, or 34.95 kg OD weight.

The fraction of the pretreated wet solids of 10 kg was milled with 2.74 kg spent liquor (neutralized first; determined based on the ratio of collected spent liquor and wet solids) in an atmospheric disk refiner with a disk plate gap of 1.0 mm (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH, USA) without adding any water. The disk plates had a pattern of D2B-505. A sample of milled solids was washed for chemical composition analysis, yield determination, and enzymatic hydrolysis. The resultant BKLP whole slurry was neutralized to a pH of ca. 6.2 with solid lime for enzymatic saccharification and fermentation.

Enzymatic hydrolysis of the washed solids was conducted at 2% (w/v) in 100 ml of 50 mM acetate buffer of pH 5.5 on a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA, USA) at 50°C and 200 rpm. An elevated pH of 5.5, higher than the commonly used pH of 4.8–5.0 can reduce nonproductive cellulase binding to lignin and enhance lignocellulose saccharification (Lan et al. 2013b; Lou et al. 2013; Wang et al. 2013). The CTec3 enzyme loading was 10 FPU g^{-1} glucan. Samples of enzymatic hydrolysate were taken periodically and centrifuged at 13 000 g for 5 min for glucose analysis. Replicate hydrolysis runs were conducted. Each sample was analyzed twice. Mean values were presented with standard deviations (StD) as error bars in plots.

Quasi-simultaneous enzymatic saccharification and fermentation (Q-SSF): Q-SSF of the pretreated whole slurry at 20% unwashed solids loading (equivalent 15.1% water insoluble solids, WIS) was carried out in 125 ml Erlenmeyer flasks in a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA, USA). Acetic acid/sodium acetate buffer (50 mM) of pH 5.5 was added to the pH-adjusted whole slurry to conduct enzymatic hydrolysis with CTec3 at 35 ml (or 7600 FPU) kg^{-1} of untreated wood. The total mass input per Q-SSF run was 50 g. Liquefaction of solids was observed in about 24–26 h at 50°C and 200 rpm. The mixture was then cooled down to 35°C and inoculated with *S. cerevisiae* YRH400 yeast seed while the shaker speed was reduced to 90 rpm. Different amounts of cultured inoculum were added to achieve yeast seed loadings of 0.1, 0.4, and 0.6 mg dry cell g^{-1} substrate, equivalent to calculated optical density $\text{OD}_{600\text{ nm}} = 1, 3.5,$

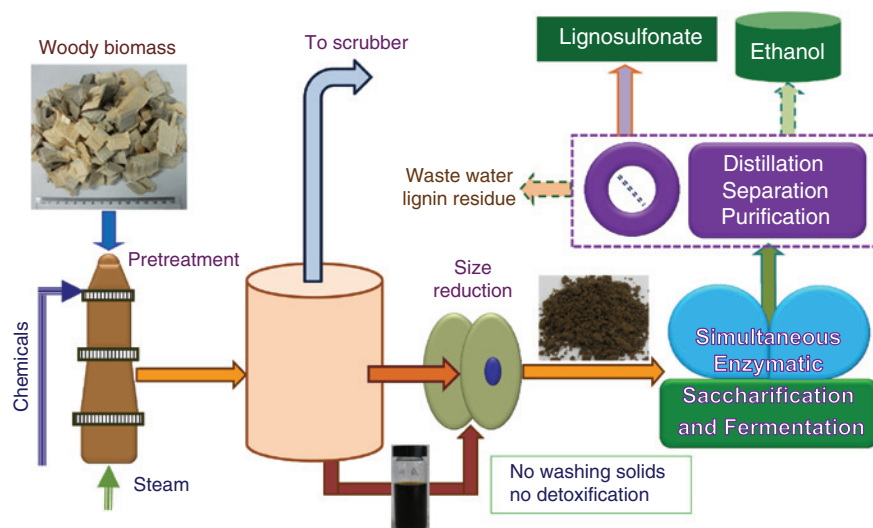


Figure 2: A schematic diagram shows the process flow of scale-up pretreatment of BKLP by SPORL and downstream saccharification and fermentation. Process boxed by dashed lines was partially conducted.

and 5, respectively, in different fermentation runs. Nutrients were not supplemented. Samples of the fermentation broth were taken periodically for analysis. Duplicate fermentation runs were conducted to ensure experimental repeatability. Each sample was analyzed twice. The reported data were averages of two fermentation runs. The error bars in plotting are StD.

Analytical methods: The chemical compositions of the untreated and pretreated lignocelluloses were analyzed according to Luo et al. (2010). Carbohydrates of the hydrolysates from two-step acid hydrolysis of solids were analyzed by high HP-AEC combined with pulsed amperometric detection (ICS-5000, Dionex, Sunnyvale, CA, USA). Klason lignin (acid insoluble) was quantified gravimetrically (Dence 1992). For fast analysis, glucose in the enzymatic hydrolysates was measured by a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH, USA).

Fermentation samples were analyzed for monomeric sugars, inhibitors, and ethanol by means of a HPLC system (Ultimate 3000, Thermo Scientific, Sunnyvale, CA, USA) equipped with an RI (RI-101) and UV (VWD-3400RS) detector and with a BioRad Aminex HPX-87P and a HPX-87H column operated as described previously (Zhou et al. 2013b).

LS molecular weight determination: The spent liquor was first filtered over Whatman filter paper followed by ultrafiltration (142-mm Millipore Hazardous Waste Filtration System, Millipore, Ireland) with an ultrafiltration membrane (Ultracel® 1 KDa Ultrafiltration Discs, Millipore Corporation, Billerica, MA). The membrane cut-off molecular weight was 1000 Da to remove impurities such as sugars, furans, and organic acids. The conductivity of the effluent was monitored by an YSI Conductance Meter (Model 35, YSI Inc., Yellow Springs, OH, USA). Glucose in the purified LS and the effluent was monitored by an YSI glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH, USA). The ultrafiltration was terminated when the conductance and the glucose concentration of the effluent was below 1.0 mS cm⁻¹ and 0.2 g l⁻¹, respectively. The purified LS was analyzed by a multidetector GPC system consisting of an Agilent 1100 HPLC equipped with a UV detector, an Optilab® T-REX™

RI detector (Wyatt Technology Corp., Santa Barbara, CA, USA), a DAWN® HELEOS™ II (Wyatt Technology Corp., Santa Barbara, CA, USA) multiangle light scattering (MALS) detector, and a GPC column (Ultrahydrogel™ 250, 7.8×300 mm, Waters, Milford, MA, USA). Sodium nitrate at 0.1 mol l⁻¹ was used as eluent at 0.5 ml min⁻¹. The samples were filtered through 0.2 µm PTFE (Alltech) before analysis. ASTRA 6 software (Wyatt Technology Corp., Santa Barbara, CA, USA) was used for data collection and analysis.

The phenolic group content of LS was determined according to Zhou et al. (2013a). The sulfur content of LS was analyzed by inductively coupled plasma (ICP) mass spectrometry. The solid substrate suspensions were shaken well before sampling. Aliquots of samples were digested at 145°C for 15 min in a microwave oven (MDS-2000, CEM Corp., Matthews, NC, USA) with about 5 ml of HNO₃ and 3 ml of 30% H₂O₂ before ICP optical emission spectrometry analysis. The reported data were averages of three analyses.

Results and discussion

Wood component recovery from pilot-scale pretreatment

The chemical composition of the BKLP wood chips are listed in Table 1. Each wood component was recovered from two streams after SPORL: (i) unwashed wet solids that consisted of all water-insoluble materials along with part of the pretreatment spent liquor retained and (ii) freely drainable spent liquor. Total solids recovery was 102.9% based on initial OD wood chips of 40 kg without taking account the chemicals applied. The amount of total soluble LS was

Table 1: Chemical composition of the untreated BKLP wood chips and wood component recoveries from the pilot-scale SPORL pretreatment in a 390 l wood pulping digester. Pretreatment was conducted at sulfuric acid and sodium bisulfite loadings on wood of 2.2% and 8%, respectively, for 75 min at 165°C.

	Untreated wood	Unwashed solids ^a	Collected spent liquor ^a	Total re-covary (%)	Washed solids ^a
Wet weight (kg)	49.95	109.45	39.95		
Solids content (%)	80.1	31.9	15.8		
Solids (kg) ^b	40.0	34.86; 87.2%	6.31; 15.8%	102.9	26.40; 66.0%
Klason lignin (%)	29.85±0.01	22.48±0.39; 75.3%	2.32; 7.8%	83.1	23.01±0.33; 77.1%
Arabinan (%)	1.76±0.02	0.55±0.01; 31.2%	0.00	31.2	0.00
Galactan (%)	3.56±0.04	1.54±0.02; 44.3%	0.74; 20.8%	64.1	0.12±0.11; 7.0%
Glucan (%)	39.00±0.03	36.95±1.53; 94.7%	1.13; 2.9%	97.6	37.46±0.94; 88.4%
Mannan (%)	9.46±0.11	5.95±0.14; 61.4%	2.49; 26.4%	87.8	1.42±0.16; 21.3%
Xylan (%)	7.23±0.01	3.64±0.11; 50.4%	1.16; 16.0%	66.4	1.57±0.12; 37.6%
HMF (%) ^c		0.30; 3.1%	0.11 (0.8); 1.1%	4.3	
Furfural (%) ^c		0.36; 5.0%	0.13 (1.0); 1.8%	6.8	
Acetic acid (%)		1.17	0.43 (4.3)		

^aThe first numbers of the chemical component data are based on 100 kg of untreated wood; the % data are theoretical yields based on total solids or component of untreated wood.

^bBased on oven dry (od) basis of untreated wood.

^cConversion rates of the compounds indicated. The numbers in the parenthesis are concentration measured in the collected spent liquor in g l⁻¹.

determined as Klason lignin based on the balance of the amount lignin remained in the washed solids. The partition of the LS in the two streams is based on the amounts of liquor recovered as freely drainable liquor and liquor remained in the wet solids. This method accounted for 83.1% of the Klason lignin. The wet solids contained 65% of the spent liquor and all water insolubles. Glucan loss was minimal with a recovery of 97.6%. Mannan recovery was around 88%, whereas recoveries of other hemicelluloses as monosaccharides were ca. 65%, with the exception of arabinan. The productions of HMF and furfural were only 1.1% and 1.8% expressed as the amount of mannan and xylan in the untreated wood, respectively.

Enzymatic digestibility of washed solids

Enzymatic hydrolysis efficiency of washed solids represents the effectiveness of pretreatment in removing the recalcitrance of lignocelluloses. The substrate enzymatic digestibility (SED), defined as the percentage of glucan in the washed solids enzymatically saccharified to glucose, achieved a value of 78% after 72 h with a low CTec3 loading of 10 FPU g⁻¹ glucan, or 17 ml kg⁻¹ untreated wood as shown in Figure 3, indicating that the pilot-scale pretreatment led to a good saccharification efficiency even at a low cellulase loading.

Fermentation of undetoxified pretreated whole slurry

The whole slurry of SPORL pretreated BKLP at the pilot-scale was directly used for Q-SSF at 20% unwashed solids

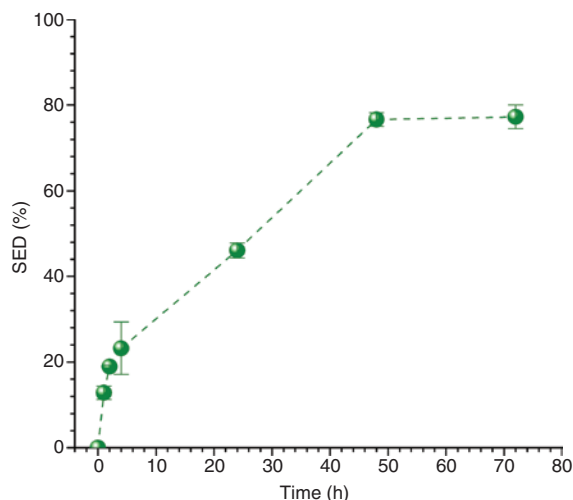


Figure 3: Time-dependent enzymatic saccharification efficiency of washed solids of BKLP pretreated by SPORL at pilot-scale at a low CTec3 loading of 10 FPU/g glucan.

loading without detoxification with *S. cerevisiae* YRH400. The initial glucose concentrations after enzymatic liquefaction was 60 g l⁻¹ (Figure 4a, yeast added at t=0). Glucose consumption was rapid by YRH400 for fermentation runs with yeast loadings of 0.4 and 0.6 mg dry cell g⁻¹ substrate, corresponding to OD_{600 nm}=3.5 and 5.0, respectively. It had a delay of 48 h, however, at yeast loading=0.1 mg g⁻¹ (OD_{600 nm}=1.0). Furthermore, glucose consumption was significantly reduced. The average glucose consumption in the first 48 h was only -0.14 g l⁻¹ h⁻¹ compared with -0.71 g l⁻¹ h⁻¹ at yeast loading of 0.4 mg g⁻¹ (Table 2), or a decrease by 80%. As a result, ethanol production also had a delay of 48 h. The final ethanol concentration was only

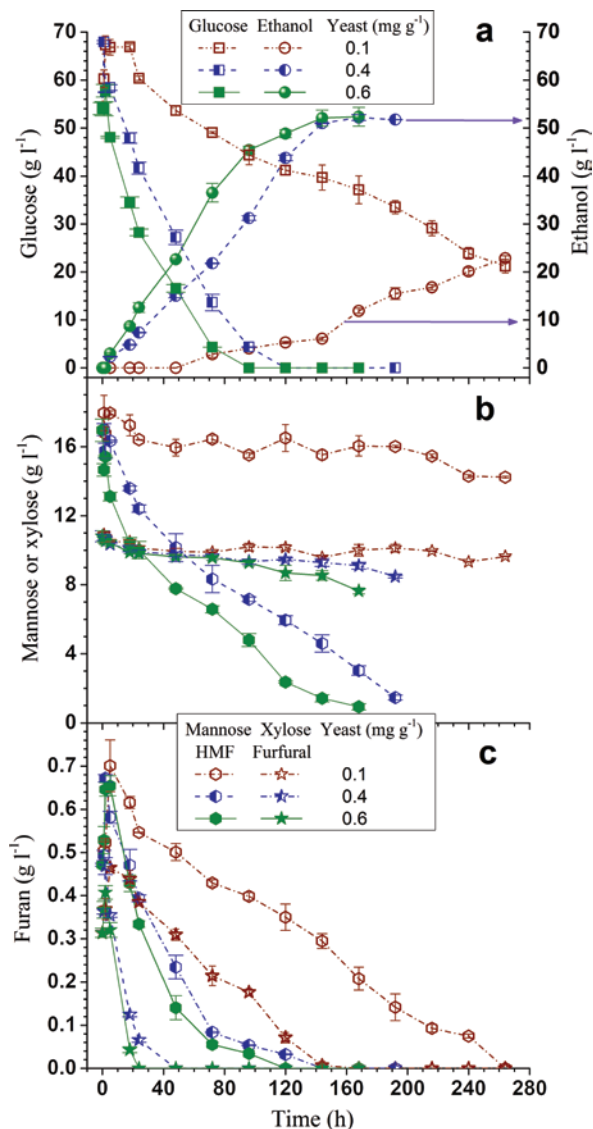


Figure 4: Time-dependent sugar, ethanol, and furan concentration profiles in the fermentation broth using the whole biomass slurry from the scale-up SPORL pretreatment at 20% total solids loading. (a) glucose and ethanol (b) mannose and xylose (c) furans.

Table 2: Performance of Q-SSF at three yeast loadings for BKLP SPORL pretreated at 390 l pilot-scale wood pulping digester.

	Yeast loading (mg dry cell g ⁻¹ substrate; OD _{600 nm})		
	0.1; 1.0	0.4; 3.5	0.6; 5.0
Average in the first 48 h or (g l ⁻¹ h ⁻¹)			
EtOH productivity	0.00	0.31	0.49
Glucose consumption	-0.14	-0.71	-0.86
Mannose consumption	-0.03	-0.15	-0.17
Xylose consumption	-0.019	-0.022	-0.024
HMF metabolization	-0.002	-0.009	-0.012
Furfural metabolization	-0.000 (24 h)	-0.018 (24 h)	-0.019 (24 h)
Final maximal EtOH production			
EtOH concentration (g l ⁻¹)	22.9±0.5 ^c	52.2±0.6 ^d	52.4±2.0 ^e
EtOH yield (g g ⁻¹ sugar) ^a	0.156±0.003	0.357±0.004	0.359±0.013
EtOH yield (l t ⁻¹ wood)	126.2±3.0	288.2±3.4	289.2±10.8
EtOH yield (% theoretical) ^b	31.4±0.7	71.7±0.8	71.9±2.7

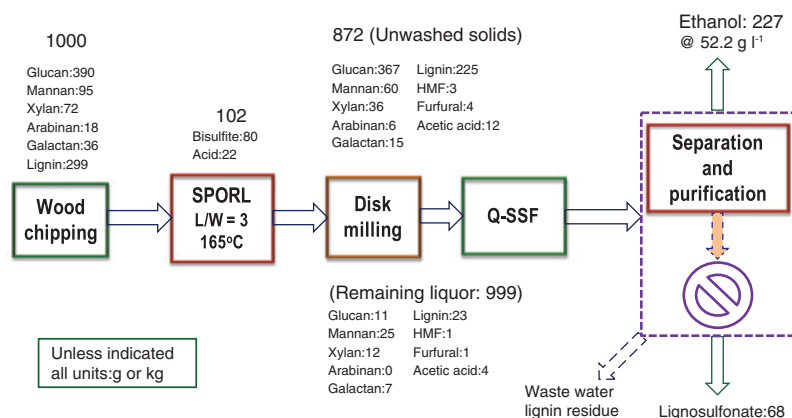
^aBased on total glucan, mannan, xylan.^bTheoretical yield (401.9 l t⁻¹ wood from BKLP) of total glucan, mannan, xylan in the untreated wood.^{c,d,e}Maximal EtOH production after 264, 1168 and 1168 h, respectively.

23 g l⁻¹ compared with 52 g l⁻¹ at yeast loading of 0.4 mg g⁻¹, or increased by 54%. Further increase yeast loading from 0.4 to 0.6 mg g⁻¹ did not substantially improve fermentation with only 20% increase in ethanol productivity in the first 48 h (Table 2). The terminal ethanol concentration, however, was the same (52 g l⁻¹), which was reached at a similar time of 144 h for both yeast loadings (Figure 4a).

YRH400 was found to consume mannose but substantially slower than glucose. Mannose concentration was close to zero after <200 h fermentation at yeast loading of 0.4 mg g⁻¹ (Figure 4b). The average mannose consumption rate was -0.15 g l⁻¹ h⁻¹ in the first 48 h compared with -0.71 g l⁻¹ h⁻¹ in consuming glucose (Table 2). However, xylose consumption was very limited even though YRH400 is

capable of fermenting xylose. This was most likely due to the low xylose concentration of 10 g l⁻¹ (Figure 4b) and the presence of furan (Table 1). This was especially true at low yeast cell loadings of 0.1 mg g⁻¹ when xylose consumption was almost zero (Figure 4b). This low xylose consumption was also observed in our previous studies (Zhou et al. 2014a,b) because *S. cerevisiae* relies on hexose transporters with a low affinity for xylose (Kotter and Ciriacy 1993).

YRH400 metabolized furan in the whole slurry of SPORL pretreated BKLP fairly rapidly at yeast loading of 0.4 mg g⁻¹ or higher (Figure 4c). Furthermore, furfural was metabolized faster than HMF even at the same concentration level. The lower furan concentrations also facilitated metabolization.

**Figure 5:** A block diagram shows process mass balance for the pilot-scale pretreatment of BKLP with subsequent saccharification and fermentation. Process boxed by dashed lines was partially conducted.

Ethanol yield and process mass balance

The overall process mass balance is presented in Figure 5 for the fermentation run at yeast loading of 0.4 mg g^{-1} (or $\text{OD}_{600 \text{ nm}}=3.5$). Ethanol yield was $288 \pm 3 \text{ l (227 kg) t}^{-1}$ wood, or equivalent to a theoretical yield of 72% based on the sum of wood's glucan, mannan, and xylan content. Terminal ethanol concentration was $52 \pm 1 \text{ g l}^{-1}$ (Table 2). These data are very similar to the study conducted at 2 kg (OD) wood chip (from similar pine beetle killed lodgepole pine BD4) scale in a 23 l reactor under similar conditions (Zhou et al. 2013b), that is, $306 \pm 14 \text{ l t}^{-1}$ wood or theoretical 72% with a concentration of 47 g l^{-1} . The quoted study was based on solids loading 18% (13.7% WIS) with 50 g wet mass and yeast loading at $\text{OD}_{600 \text{ nm}}=5.0$ in Q-SSF. BKLP had a slightly lower glucan and mannan content than BD4, which resulted in a slightly lower ethanol yield in liters per tonne wood. Increasing yeast loading to 0.6 mg g^{-1} in the present study did not improve ethanol yield, perhaps due to low concentrations of furan and acetic acid in the BKLP spent liquor (Table 1).

Very limited literature data on high titer ethanol production from softwoods without detoxification are available. The results from this study obtained by SPORL were compared with a recent study based on SO_2 steam explosion of spruce (Hoyer et al. 2013) as presented in Table 3. Despite the higher thermal energy input at 205°C in SO_2

steam explosion (Zhu and Zhuang 2012), along with supplementation of nutrients and 50 times higher yeast loading, the SO_2 steam explosion study produced a lower ethanol titer of 47.8 g l^{-1} and the theoretical yield was 72% (based on wood glucan and mannan), whereas in the present SPORL study the yield were 52.2 g l^{-1} (82.5% of the theory). Thus the advantages of SPORL are obvious.

LS properties

SPORL solubilized 23% or 68 kg wood lignin per tonne wood that can be recovered as LS as a co-product (Figure 5). The actual LS yield will be higher than 23% because sodium and sulfur on LS are not included in the mass balance presented in Figure 5. The yield of LS can be improved by slightly increasing sodium bisulfite loading to facilitate delignification as demonstrated previously (Zhang et al. 2014). Commercial LS is produced from sulfite pulping and has a variety of applications (Gargulak and Lebo 1999). The molecular weight (M_w), sulfonic acid group, and OH_{phen} contents as the key parameters for the performance of LS are listed in Table 4 for LSs from the pilot-scale SPORL pretreatment of BKLP, LS-SP-BKLP, and a commercial source D-748. The maximum M_w of LS-SP-BKLP is ca. four times lower than that of D-748 with a slightly more narrow distribution. The ratio between

Table 3: Comparison of ethanol production between the present study by SPORL from lodgepole pine and a literature work based on SO_2 steam explosion from spruce.

	SPORL lodgepole pine (present study)	SO_2 Steam explosion spruce ^a
Pretreatment		
Temperature ($^\circ\text{C}$)	165	205
Duration (min)	60	6–7
Liquor to wood ratio (l kg^{-1})	3.0	~3.0
Q-SSF		
Water insoluble solids (WIS) (%)	15.1	13.7
Total wet mass (g)	50	1300
Mixing mode	Shaking bed: poor	Mechanical mixing: good
Cellulase ($\text{FPU g}^{-1} \text{ WIS}$)	11.5 CTec3	10.0 CTec2+ β -glucosidase
Yeast (g dry cell/l)	0.1	5
Nutrients for fermentation		
$(\text{NH}_4)_2\text{HPO}_4$ (g l^{-1})	None	0.5
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (g l^{-1})	None	0.025
Yeast extract (g l^{-1})	None	1.0
Liquefaction time (h)	24–26	22
Final ethanol production		
Ethanol concentration (g l^{-1})	52.2	47.8
Ethanol yield (% of theoretical) ^b	82.5%	72.0%

^aHoyer et al. 2013.

^bTheoretical yield based on glucan and mannan (xylan excluded) in the untreated wood.

Table 4: Comparisons of molecular weights and sulfur and OH_{phen} contents between the LS from SPORL pretreated BKLP at pilot-scale and a commercial LS.

LS	M_w (Da)	M_n (Da)	M_w/M_n	Sulfur (%)	OH _{phen} (mmol g ⁻¹)
LS-SP-BKLP	10 334	5040	2.056	6.17±0.18	1.71±0.03
D-748	43 110	19 002	2.269	6.13±0.13	1.63±0.01

the M_w and M_n (dispersity) is 2.1 compared with 2.3 for D-748. Most sulfur in LS is associated with the sulfonic acid group. LSs with higher sulfur contents are better dispersants for coal water and cement slurries (Yang et al. 2007; Ouyang et al. 2009). This is also true for dispersant in gypsum paste (Matsushita and Yasuda 2005). Sulfur content of LS-SP-BKLP was nearly the same as that of D-748 (6.1%). The OH_{phen} contents of LS influence both physical and chemical properties (Adler 1977) and are the reactive groups for chemical and biological modifications. LS-SP-BKLP has a slightly higher OH_{phen} content (1.7 mmol g⁻¹) than that of D-748 (1.6 mmol g⁻¹).

Conclusions

The SPORL pilot-scale process is efficient for bioconversion of BKLP, which gives rise to ethanol with a high titer and yield and lignosulfonate (LS) as co-product. The pilot-scale pretreatment at a relatively low temperature of 165°C reduced sugar degradation to fermentation inhibitors. This facilitated simultaneous enzymatic saccharification and fermentation of undetoxified whole slurry of SPORL pretreated BKLP at a solid loading of 20%. A terminal ethanol yield of 288 l t⁻¹ wood, at a titer of 52.2 g l⁻¹, was achieved with *S. cerevisiae* YRH400 at 0.4 mg dry yeast cell g⁻¹ solids, whereas the cellulase CTec3 dosage was 35 ml kg⁻¹ untreated wood. The LS produced by SPORL has a low molecular weight, but is equally sulfonated as that of a commercial LS and therefore can be directly marketed. Because SPORL was developed based on sulfite pulping, it can be applied within the same infrastructure of existing pulp mills with low risk for commercialization. The directly marketable LS can improve process economics comparing with competing technologies. The ongoing studies are focusing on further adaptation of SPORL to production conditions of commercial sulfite mills.

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Conflict of interest statement: Zhu and Gleisner are co-inventors of a US patent application on SPORL.

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