

SPORL PRETREATMENT OF DOUGLAS-FIR FOREST RESIDUE FOR PRECOMMERCIAL SCALE PRODUCTIONS OF SUGAR/BIOFUELS

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LIST OF ACRONYMS

CHF	combined hydrolysis factor (Eq. (1))
HMF	hydroxymethylfurfural
SPORL	Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses
WIS	water insoluble solids determined through thorough easing
WWS	whole slurry solids

EXECUTIVE SUMMARY

This part of the report demonstrated the robust performance of SPORL for bioconversion of forest harvest residue to fermentable sugars. The performance measure included not only sugar yield but also process scalability and flexibility. Process data from process optimization at 150 g scale were used to develop a kinetic based reaction severity factor, the combined hydrolysis factor (CHF), for process scale-up design. Scale-up studies were conducted at 50 kg scale on a 390 liter digester at the USDA Forest Products Laboratory. An industry scale pilot plant trail run was conducted before the precommercial scale run. High temperature of approximately 185 °C, minimal magnesium bisulfite loading of 12% on wood (in oven dry), and a short residence time of approximately 40 min were used to meet the capability of ZeaChem's precommercial scale facility.

INTRODUCTION

Woody biomass is an important feedstock for sustainable production of biobased fuels and chemicals. It is available in large quantities and from many regions of US (US Department of Energy, 2011). It also has advantages over herbaceous biomass with relatively high density for easing transportation and flexible harvesting schedule to eliminate long term storage (Zhu and Pan, 2010). Biochemical conversion of woody biomass can be a viable pathway by making full use of well-developed technologies and feedstock supply chains in the pulp and paper industry while avoiding feedstock competition with the existing forest products industry by using underutilized woody biomass such as forest harvest residues. Due to the natural strong recalcitrance of woody biomass to microbial deconstruction, a pretreatment step is required for subsequent release of sugars from structural carbohydrates. Few

pretreatment process have demonstrated good performance in removing the recalcitrance of woody biomass especially those from softwoods (Zhu and Pan, 2010). The Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL) demonstrated robust performance for efficient bioconversion of woody biomass in laboratory studies (Zhu et al., 2009). SPORL was selected by NARA for further development for the production of 1000 gallons of bio-jet fuel from Douglas-fir forest residue through the sponsorship of USDA-NIFA. Here we report progress made in the last 5 years in two fronts of developing the SPORL process: (1) fundamental understanding in removing the strong recalcitrance; (2) SPORL process integration and scale-up.

TASK 1: OPTIMIZATION SPORL AND/OR OTHER PRETREATMENTS FOR DOUGLAS-FIR/DOUGLAS-FIR RESIDUES AT LAB BENCH SCALE (150 G /2KG)

The optimization study, which was partially supported by a USDA SBIR research project, developed a combined hydrolysis factor (CHF, eq. (1)) that can optimize pretreatment duration for a given temperature at given chemical loadings (see equation (2) below) (Zhu et al., 2012). Further study indicated that a low pretreatment temperature was favored to reduce sugar degradation to inhibitors (Eq. (3) and Figures SP-1.1a and SP-1.1b) (Zhang et al., 2014; Zhou et al., 2014b).

Where C_A and C_B are the concentrations of chemical A (SO_2) and chemical B (hydroxide) used in pretreatment, respectively; α , β and γ are adjustable parameters, $E = 100,000$ J/mole is the apparent activation energy of xylan dissolution for softwoods. E_d is the apparent activation energy of sugar degradation, R is the universal gas constant of 8.314 J/mole/K, and T is absolute temperature (K). D is defined as the sum of the concentration of HMF and furfural.

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

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

$$\frac{D_{T_1}}{D_{T_2}} = \frac{k_d^{T_1}}{k_d^{T_2}} \frac{t^{T_1}}{t^{T_2}} = \exp \left[\frac{E - E_d}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \right] \quad (3)$$

Table SP-1.1 lists the optimal pretreatment duration at a given temperature and the corresponding relative inhibitor formation calculated based on eq. (2) and (3). The advantage of low T pretreatment is obvious in terms of reducing sugar degradation to inhibitors; however, a very long reaction is needed.

This data provided great flexibility in site selection for the pretreatment of 60 tonnes of Douglas-fir forest residue for producing 1000 gallons of biojet.

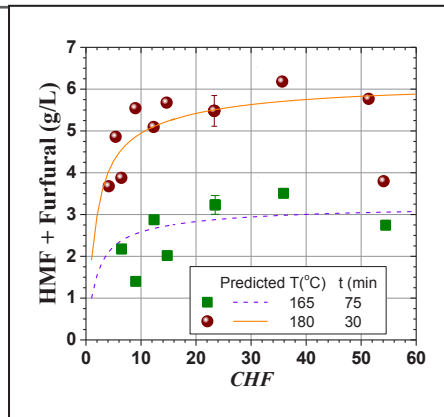


Figure SP-1.1a. Correlation between the HMF and furfural concentration in the pretreatment hydrolysate with the combined hydrolysis factor (CHF) in comparisons with model predictions at 165°C and 180°C.

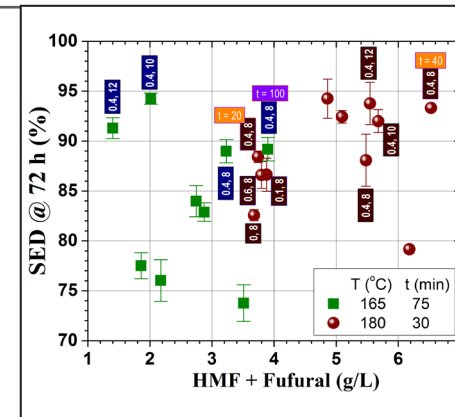


Figure SP-1.1b. Comparisons of substrate cellulose enzymatic digestibility (SED) and furan (HMF+Furfural) production from SPORL pretreatments at 165°C and 180°C.

Table SP-1.1. Calculated optimal pretreatment duration and relative inhibitor formation at SO_2 and hydroxide loadings of approximately 6.6 and 6.5 wt% on wood, respectively.

T (°C)	Time (min)	Relative Inhibitor
180	26	1.000
173	39	0.776
170	47	0.694
165	75	0.575
155	123	0.389
145	240	0.258

To further reduce inhibitor formation, we also developed a pH profiling process for SPORL pretreatment (Cheng et al., 2015; Zhu and Gleisner, 2016). The purpose of a pH profiling run is to reduce sugar degradation to furans by delaying acid application in pretreatment. The basic concept is described in the below diagram (Figure SP-1.2). Compared with a control SPORL run, all pretreatment conditions (temperature, chemical loading, reaction time, etc.) were identical to a control

run, except the same amount acid as those used in the control run were at a time t_i rather than at time 0 as in the control run. We achieved equivalent enzymatic saccharification efficiency and glucose yield but at significantly reduced furan formation as shown in Figure SP-1.3. This suggests that the pH profiling technique can be applied to SPORL pretreatment to reduce furan formation to further facilitate fermentation along with low temperature pretreatment as reported previously. Pilot scale pH-profiling runs were conducted. This is very desirable for high T pretreatment to reduce pretreatment duration as well as inhibitor formation.

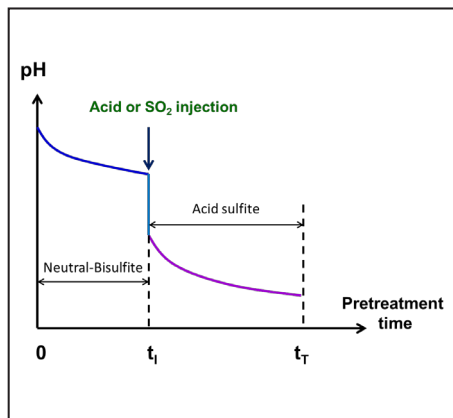


Figure SP-1.2. Schematic of the SPORL-pH Profiling process

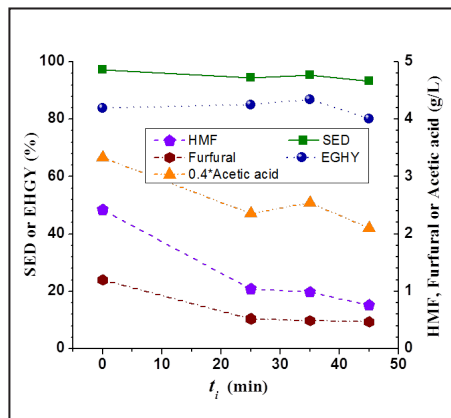


Figure SP-1.3. Effect of delayed acid application in SPORL pretreatment on substrate enzymatic digestibility (SED), enzymatic hydrolysis glucose yield (EHGY), and furan and acids acid formation

To meet sulfite mill operating requirements for a potential large scale trial run at low temperature but substantially high SO_2 loading, for example, SO_2 on wood > 25 wt% on wood pilot-scale runs (50 kg) were conducted using $\text{Mg}(\text{OH})_2$ with SO_2 at 140°C . It was found that a 60 min pretreatment time was sufficient to produce substrate with excellent enzymatic digestibility and ultralow sugar degradation to inhibitor. This indicates that increased SO_2 loading can substantially reduce pretreatment time at low temperatures with little sugar degradation to inhibitor as will be discussed below (Gu et al., 2016).

TASK 2: OPTIMIZATION SPORL AND/OR OTHER PRETREATMENTS AT FPL PILOT PLANT FACILITY (50 KG/RUN)

Pilot scale run of 50 kg FS-10 Douglas-fir forest residue was conducted using calcium bisulfite at 145°C using the FPL pilot scale digester (Zhu et al., 2015). The technical issues addressed in this study are: (1) demonstrating SPORL process using commercial pulp mill chemistry i.e., bubbling SO_2 into a hydroxide solution to produce the sulfite solution rather than using H_2SO_4 and sodium bisulfite as reported previously for ease of pretreatment experiments in the laboratory; (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. The pretreatment time was determined by maintaining the same pretreatment severity measured using the combined hydrolysis factor (CHF) reported previously, according to Eq. (2) described in the previous task, and using the optimal pretreatment time of 25-30 min at 180°C for softwood determined at the 0.15 kg lab scale reported previously (Zhou et al., 2013). Eq. (2) indicates the required reaction time, t^{T145} , for the pilot-scale pretreatment at 145°C to be 230 – 275 min.

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 SO_2 , was produced in a stirred barrel by bubbling SO_2 , regulated at a gauge pressure of 34.5 kPa, into a hydroxide solution of 139 L containing 1.25 kg (95% purity) of $\text{Ca}(\text{OH})_2$. After 37 minutes 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.

As schematically show in Figure SP-2.1, 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). At the current test conditions, this required a total liquor volume of 150 L, total SO_2 mass concentration in the sulfite solution of 2.3 wt%, and free SO_2 and $\text{Ca}(\text{HSO}_3)_2$ charge on wood 2.48 and 6.46 wt%, respectively. This translates to a total 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 minutes 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 SO_2 was 0.43 wt% compared with calculated values of 1.93 wt% and 0.74 wt%, respectively, based on the amounts of $\text{Ca}(\text{OH})_2$, SO_2 , and steam applied. The lower measured 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 through a stainless steel pipe. An additional air blow was applied to ensure all contents were discharged. Volatiles including SO_2 were vented to a wet scrubber (Figure SP-2.1). 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 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 (Figure SP-2.1). 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 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



Figure SP-2.1. A schematic process flow diagram shows Douglas-fir forest residue harvesting, transportation, pretreatment and downstream saccharification and fermentation.

neutralization using lime.

Enzymatic saccharification of the washed solids was conducted to evaluate the effectiveness of SPORL pretreatment in removing the recalcitrance of Douglas-fir forest residue 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% (Figure SP-2.2). 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 and can act as a surfactant to enhance enzymatic saccharification. 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.

The pretreated liquor had very low levels of fermentation inhibitors and could be directly fermented at 16.7 wt% total solids loading without detoxification using *Saccharomyces cerevisiae* YRH-400 (a strain developed by USDA-ARS). The ethanol yields from 284 L/tonne at 41.9 g/L were obtained. The sample was found to be fairly well fermentable by Gevo's yeast strain without detoxification.

We have developed a pilot-scale membrane system for purify lignosulfonate. The spent liquor was first centrifuged to remove particulates. Any remaining

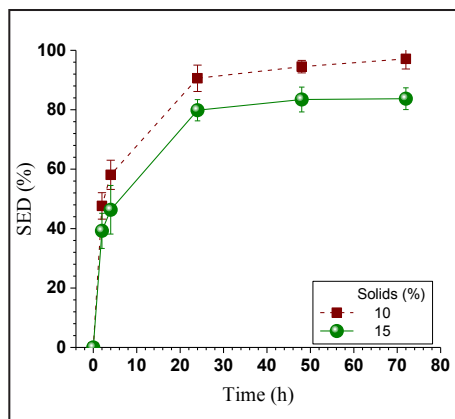


Figure SP-2.2. Time-dependent enzymatic saccharification efficiency of washed SPORL pretreated FS-10 at pilot-scale at two solids loadings with CTec3 dosage of 15 FPU/g glucan.

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 ultrafiltration (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 SP-2.1. 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 SP-2.1 indicated that Ca-LS could be extracted with high recovery and purity by UF. The purified Ca-LS had a similar molecular weight and polydispersity to the commercial lignosulfonate D748 (Borregaard LignoTech, Rothschild, WI, USA) based on GPC MALS measurements. However, GPC UV measurements indicate that the purified Ca-LS had a smaller molecular weight with lower polydispersity than D-748 (Table SP-2.1).

The Ca-LS is highly sulfonated with sulfur content of 69.2 ± 0.9 mg/g, which is higher than the commercial LS D-748 of 60.1 ± 3.9 mg/g. 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.

To reduce pretreatment time using SPORL at low temperatures and to evaluate the potential of using existing sulfite mills for large scale production without modifying the chemistry practiced for sulfite pulping, we evaluated high SO_2 loading for pretreating a Douglas-fir forest residue to achieve near zero furan formation using the same sulfite chemistry at an existing sulfite pulp mill in the U.S. Pacific Northwest. The pretreatment temperature of 140°C and total SO_2 loading of 32 wt% on wood (most of which is recovered in the mill) were the operating conditions of

Table SP-2.1. Characterization of purified calcium lignosulfonate solution

Ultrafiltration experiment				
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 ^a	69.6	7.6	43.2	86.8
< 4 kDa	24.9	84.3	41.5	19.3
GPC (MALS) analysis				
Sample	Mw ^b	Mn ^c	Mw/Mn ^d	
4-200 kDa	23430	12910	1.8	
D748	24660	14190	1.7	
GPC (UV) analysis				
4-200 kDa	2704	1092	2.5	
D-748	13113	3293	4.0	
^a The fraction of 4-200 kDa is the purified calcium lignosulfonate sample				
^b Number-average molecular weight				
^c Weight-average molecular weight				
^d Polydispersity				

the sulfite pulp mill. Magnesium is used as the metal base and is fully recovered in the sulfite mill, an advantage compared with other metals. The pretreatments using the same 50 kg Douglas-fir forest residue and using calcium bisulfite at low SO_2 loading were carried out similar to that described above. The results were compared with those from calcium bisulfite pretreatment (Gu et al., 2016).

Table SP-2.2 lists the pretreatment conditions using magnesium in comparison with the calcium bisulfite pretreatment with a low SO_2 loading. The letter “R” in the run label stands for mixing achieved through rotating the digester, while “C” stands for through liquor circulation. Component recovery data are reported in Table SP-2.3. Overall, a prolonged reaction under the same temperature and chemical loadings resulted in low whole slurry solids (WSS) and water insoluble solids (WIS) yields. Major carbohydrate recoveries from WSS were not much affected under the conditions experimented. However, prolonged pretreatment reduced carbohydrate yield from WIS due to dissolution. This is corroborated by the sugar and inhibitor concentrations in the spent liquor (Table SP-2.2). Prolonged pretreatment also increased sugar degradation to inhibitors (Table SP-2.2). The low lignin and high carbohydrate yields of the C-t60 WIS were probably due to experimental errors in sampling for carbohydrate analyses. The results were compared with a low SO_2 SPORL using the same FS-10 at the same pilot scale reactor with Ca as metal base C-t240- SO_2 6 (Tables SP-2.2 and SP-2.3). It appears that C-t240- SO_2 6 most resembles C-t120 in terms of component recovery in WIS and the sugar and inhibitor concentrations in the spent liquors.

Table SP-2.2. List of SPORL conditions along with major carbohydrate dissolution and inhibitor formation represented by concentrations in the collected spent liquor in pretreating Douglas-fir forest residue FS-10

Run Labels	R-t50	C-t60	C-t120	R-t240-SO ₂ 6 (Zhu et al. 2015)
Pretreatment conditions				
T (°C)	140	140	140	145
Heat-up time to T (min)	40	32	36	37
Time at T (min)	50	60	120	240
Metal base	Mg	Mg	Mg	Ca
Total SO ₂ on wood (wt%)	32	32	32	6.6
	27.4 (measured)	28.6 (measured)	26.3 (measured)	6.6 (measured)
Combined SO ₂ on wood (wt%) ^a	4.4	4.4	4.4	4.1
	3.2 (measured)	4.8 (measured)	3.6 (measured)	4.1 (measured)
Liquor to wood ratio (L/kg)	4.0	4.0	4.0	3.55
Mixing mechanism	Digester rotation	Liquor circulation	Liquor circulation	Digester rotation
Carbohydrate dissolution and inhibitor formation -Concentration in spent liquor (g/L)				
Glucose	7.29	6.72	9.93	9.40
Mannose	20.70	17.66	18.39	20.64
Xylose	10.36	8.29	9.91	8.54
Acetic acid	4.73	4.15	5.43	3.18
Levulinic acid	0.12	0.09	0.24	ND
HMF	0.04	0.08	0.32	0.26
Furfural	0.16	0.33	1.00	0.69

^a SO₂ in Mg(HSO₃)₂ or Ca(HSO₃)₂

Table SP-2.3. Component recovery of FS-10 Douglas-fir forest residue from SPORL at pilot-scale. Data for Run R-t240-SO₂6 from (Zhu et al. 2015)

Run Label	Untreated FS-10				Recovery from Pretreated WSS (%)				Recovery from WIS (%)			
	R-t50	C-t60	C-t120	R-t240-SO ₂ 6	R-t50	C-t60	C-t120	R-t240-SO ₂ 6	R-t50	C-t60	C-t120	R-t240-SO ₂ 6
Wet weight (kg)	56.2	66.41	56.65	61.75								
Solids content (%)	89.0	75.29	88.22	81.4								
Solids (kg) ^a	50.02	50.00	49.98	50.26	101.1	100.1	91.0	98.4	67.3	53.8	55.3	57.5
Klason lignin (%)			29.30		85.2	59.1	63.0	79.6	72.4	32.9	55.9	55.0
Arabinan (%)			1.04		58.3	63.5	56.9	47.3	0.0	0.0	0.0	0.0
Galactan (%)			2.00		91.0	112.6	100.1	91.0	0.0	0.0	0.0	6.6
Glucan (%)			40.97		88.3	84.3	85.3	92.3	92.1	83.0	78.8	80.5
Mannan (%)			9.67		68.0	73.8	66.8	69.2	9.7	16.7	7.4	7.5
Xylan (%)			5.70		55.0	61.8	55.9	49.2	14.2	17.0	5.8	8.1

^a Calculation of solids recovery from WSS and WIS did not include SO₂.

Table SP-2.4. Fermentation of un-detoxified SPORL pilot scale pretreated whole slurry of FS-10: average ethanol productivity and rates of sugar consumption, along with maximal ethanol production.

	R-t50	C-t60	C-t120	R-t240-SO ₂ 6
Solids Loading (wt%)	20	20	20	16.7
Fermentation performance (g/L/h)				
Ethanol productivity	1.204	1.923	1.266	1.523
Glucose consumption	-3.089	-2.258	-3.237	-3.634
Mannose consumption	-0.512	-0.521	-0.454	-0.572
Xylose consumption	-0.160 (48h)	-0.119 (48h)	-0.030 (48h)	-0.048 (48h)
Terminal maximal ethanol production				
Time to reach maxima (h)	48	72	48	72
Ethanol concentration (g/L)	54.6±0.6	56.3±0.5	54.5±2.0	41.9±0.3
Ethanol yield (g/g sugar)	0.487±0.005	0.504±0.004	0.460±0.017	0.412±0.003
Ethanol yield (L/tonne wood)	315.5±3.5	321.6±2.9	289.0±10.6	284±2.0
Ethanol yield (% theoretical)	77.6±0.9	79.1±0.7	71.1±2.6	70.0±0.5

The effectiveness of the high SO₂ loading pretreatments were evaluated through quasi-simultaneous enzymatic saccharification and fermentation (Q-SSF) at 20 wt% total solid content using *S. cerevisiae* YRH-400. The low temperature pretreatment of 140 °C allowed Q-SSF without detoxification due to very low inhibitor levels (Table SP-2.4). Glucose and mannose consumptions were rapid for all three runs (Figure SP-2.3a and Table SP-2.4). Fermentations were completed in 48-72 h. Comparing R-t50 and C-t60, little differences were observed except C-t60 had a slightly higher terminal ethanol concentration (Figure SP-2.3a) and yield (Table SP-2.4). This is most likely due to the better substrate digestibility of C-t60 as the two pretreatments produced similar levels of inhibitors (Table SP-2.2). Ethanol yield from C-t120 was lower than R-t50 and C-t60 (Table SP-2.4). Xylose consumption in C-t120 was also minimal and lower than those for R-t50 and C-t60 (Figure SP-2.3b). This is most likely due to higher inhibitor concentrations in the spent liquor of C-t120 than R-t50 and C-t60 (Table SP-2.2), as xylose consumption is known to be sensitive to inhibitors (Almeida et al. 2011; Helle et al. 2003; Zhou et al. 2014a).

Compared with the low SO₂ loading run R-t240-SO₂6 (Zhu et al., 2015), the three high SO₂ loading runs, R-t50, C-t60, and C-t120 all produced a higher terminal

ethanol concentration after factoring in the lower fermentation solids loading in R-t240-SO₂6 (Table SP-2.4). The unused glucose at the end of fermentation observed in R-t240-SO₂6 may explain this difference. Low fermentation inhibitors in R-t50 and C-t60 allowed ethanol yields of 316 and 322 L/tonne FS-10, equivalent to 77.6% and 79.1% theoretical based on glucan, mannan and xylan in FS-10, higher than the 284 L/tonne FS-10 or 70% theoretical from R-t240-SO₂6. C-t120 had similar fermentation performance as R-t240-SO₂6, in terms of sugar consumption and ethanol yield, perhaps due to the similar levels of fermentation inhibitors in the spent liquors (Table SP-2.2).

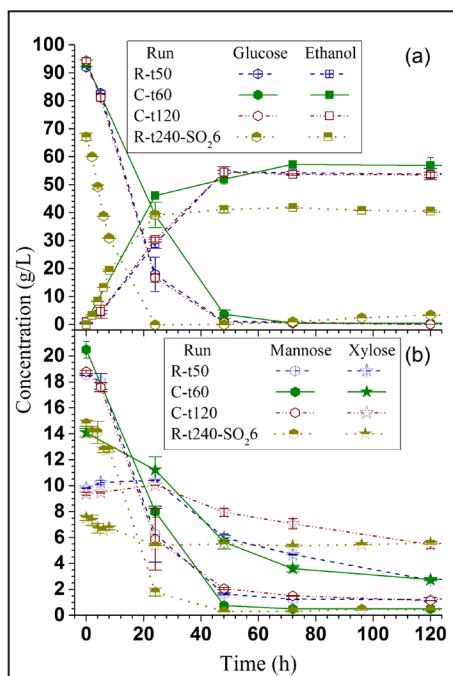


Figure SP-2.3. Time-dependent sugar and ethanol concentrations in the fermentation broth of the SPORL pretreated FS-10 whole slurry at 20.0 % solids loading. (a) Glucose and ethanol; (b) mannose and xylose.

TASK 3: IDENTIFICATION AND EXPERIMENTAL VERIFICATION OF OPERATING CONDITIONS THAT ARE SUITABLE FOR INDUSTRIAL TRIAL RUNS

Several industrial facilities were identified for potential 60 tonne FS-10 pretreatment. The first facility is a sulfite pulp mill using Mg(HSO₃)₂ with very high SO₂ loading of approximately 60-80 g/L (or >25 wt% on wood) in the pulping liquor at 140°C. The mill stated that the chemical formulation cannot be modified for pretreating 60 tonne FS-10. The second facility has limited SO₂ handling capabilities, so a low SO₂ loading is desired, however, 240 min is too long for the facility to handle. A high temperature of 170°C is required, based on the scaling factor CHF reported in the previous quarter, to meet the 45 min longest residence time of the facility. The third facility has too low of a capacity of 1 ton/day, though it has excellent capability for SO₂ handling and sugar concentration, therefore not serious consideration.

After selecting the ZeaChem facility for the 60 tonne FS-10 pretreatment, a trial run at Andritz industrial pilot scale facility (Springfield OH) was conducted. The ZeaChem facility was designed by Andritz. The Andritz Springfield facility is just a small version of the ZeaChem facility. We first conducted a laboratory study at FPL using 170°C for 45 min and low Mg bisulfite loading. Base Mg was chosen because it performed better in enzymatic saccharification and fermentation as determined by Gevo. Mg(HSO₃)₂ and H₂SO₄ were used instead of MgO+SO₂ because both Andritz and ZeaChem have limited capability to handle SO₂. Mg(HSO₃)₂ charge on wood (oven dry) of 12% was used based on a previous study (Leu et al. 2013). After an initial adjustment, the final sample from Andritz trial run was successfully fermented by Gevo. A final T of 180° C was used.

Conclusions

With USDA NIFA support through NARA, we demonstrated the great performance of SPORL for biochemical converting softwood forest residue to fermentable sugars. The scalability of the SPORL process was demonstrated from 150 g to 2 kg, 50 kg, 1 tonne, and 60 tonnes forest residue. The flexibility of the SPORL process provided NARA flexibility in site selection for precommercial scale production. The study solved the problem of softwood recalcitrance for sugar production.

NARA OUTPUTS

Patent:

Zhu, J.Y., Gleisner, R., “Methods of Pretreating Lignocellulosic Biomass with Reduced Formation of Fermentation Inhibitors”, *U.S. Utility Patent No. 9,243,364 B2* (2016)

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2. Zhu, J.Y., (2016), “Understanding the role of lignin sulfonation on enzymatic saccharification of lignocelluloses”, Borregaard, Sarpsborg, Norway, April 20
3. Zhu, J.Y. (2016), “Recent progress in woody biomass conversion to biofuel and cellulose nanomaterials” Dept. of Chemistry, University of Jyväskylä, Jyväskylä, Finland, March 16.
4. Zhu, J.Y. (2016), “Recent progress in woody biomass conversion to biofuel and cellulose nanomaterials” Johan Gadolin Process Chemistry Center, Abo Academy University, Turku, Finland, March 14.

5. Zhu, J.Y. (2016), “Pretreatment of woody biomass: Our understanding”, VTT, Espoo, Finland March 7.
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34. Zhu, J.Y. (2012), “Fundamentals and practices for efficient production of biofuel from lignocelluloses,” presented at the 243rd ACS National Spring Meeting, San Diego, CA, Mar 25-29

Webinar

Zhu, J.Y., (2016), “Pretreatment of Woody Biomass for Biofuel Production”, Organized by NARA, Jan 19.

NARA OUTCOMES

1. Forest biomass has long been recognized as an efficient feedstock for bio refinery applications from harvesting, logistics, and supply chain, and cost point of view. But it was considered as an inferior feedstock for biochemical conversion due to its strong recalcitrance to enzymatic saccharification and was considered more suitable for thermal-chemical conversion. Our research work has changed this thinking by demonstrating robust performance in sugar/biofuel production in terms of yield and scalability from harvest forest residue. We developed substantial amount of knowledge encompassing upgrading forest residue (Zhang et al. 2012), pretreatment (Gu et al. 2016; Leu et al. 2013), reducing nonproduction cellulase binding to lignin (Lou et al. 2013), reaction kinetics based process scale-up design to reduce fermentation inhibitor formation and maximize sugar yield (Zhang et al. 2014). We developed a technological pathway for utilization of harvest forest residue through biochemical conversion, which provided flexibility for stakeholders such as landowners in marketing their product.
2. By utilizing underutilized woody biomass through this project, it avoided competing for feedstock with existing Forest Products Industry. This is critically important in changing the views and altitude of the stakeholders of the existing Forest Products Industry toward developing biofuels and biochemical. It made it much easier to get support from existing industry for technology development for biofuels and biochemical using lignocellulosic biomass.
3. The efficient utilization of underutilized woody biomass through our research made research and development in underutilized biomass feedstock relevant to biorefinery operations.
4. The knowledge we developed in fundamental understanding of lignin and cellulase interaction, cellulase accessibility to cellulose, process scale-up design using process kinetics based scaling factor, large scale production know-how will substantially contributed to future biorefinery development.

FUTURE DEVELOPMENT

Future development should be focused on

1. Valorization of enzymatic hydrolysis residue lignin, which is part of the lignin co-product team efforts through production of activated carbon. The focus here is to look into developing different products.
2. Focus on enzymatic hydrolysis and fermentation of the whole slurry, which has been done in ethanol production in the present project. It needs to extend to use the isobutanol yeast for producing isobutanol as a valuable chemical.

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