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Robust enzymatic saccharification of a Douglas-fir forest harvest residue by SPORL☆

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ABSTRACT

Forest harvest residues can be a cost-effective feedstock for a biorefinery, but the high lignin content of forest residues is a major barrier for enzymatic sugar production. Sulfite pretreatment to overcome strong recalcitrance of lignocelluloses (SPORL) was applied to a Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco var. *menziesii*) forest residue in a range of sulfite and acid loadings at 165 °C for 75 min with liquid to wood ratio of 3:1. Sodium bisulfite and sulfuric acid charge as mass fraction of oven dry biomass of 12% and 2.21%, respectively, was optimal in terms of enzymatic cellulose saccharification, sugar yield and formation of hydroxymethylfurfural (HMF) and furfural. Enzymatic glucose yield was 345 g kg^{-1}, or equivalent to 82.3% of theoretical at a cellulase (CTec2) dosage of 15 filter paper unit (FPU) per gram of glucan. HMF and furfural formation were low at approximately 2.5 g L^{-1} each in the pretreatment hydrolyzate. Delignification was important to achieve good cellulose saccharification efficiency, however, approximately 80–90% hemicellulose removal is also required. Substrate enzymatic digestibility (SED) was found to correlate to a combined parameter Z(CHF) of delignification and hemicellulose dissolution well, suggesting that the combined hydrolysis factor (CHF) – a pretreatment severity measure – can be used to predict saccharification of forest residue for scale-up studies to reduce numbers of experiments.

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1. Introduction

Forest harvest residues can be sustainably produced in large quantities in North America and various regions of the globe [1–3]. About 50 Mt of forest residues are available in the United States alone, of which it is estimated that 70% can be sustainably recovered annually [1–3]. A recent study by the U.S. National Academy of Sciences indicated that forest residues are one of the two most cost effective feedstock for biofuel production [4]. Forest residues have relatively high bulk densities and can be harvested year round which reduces on-site storage requirements, both of which are significant advantages over agriculture residues and herbaceous biomass in terms of improving supply chain logistics and reducing

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transportation costs [5,6]. However, forest residues are very recalcitrant to biochemical conversion through the biorefinery concept because bark and juvenile wood in the residues have high lignin content. Very limited studies are reported on bioconversion of forest residues [7]. Few process technologies reported satisfactory enzymatic saccharification yield from woody biomass including forest residues. Successfully addressing efficient bioconversion of forest residues has significant practical importance because feedstock sustainability and cost are the two critical factors that dictate the commercial viability of the biorefinery concept.

Some degrees of lignin removal as well as substantial removal of hemicelluloses through a pretreatment step are required for efficient enzymatic saccharification of lignocellulosic biomass with high lignin content [8]. Various pretreatment technologies, such as Organosolv, alkaline, and SO_2 catalyzed steam explosion, have been applied to softwood species with high lignin contents [6] and achieved some level of success [9–13]. Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL), though a relatively new process [14], demonstrated robust performances for sugar and biofuel production from very recalcitrant softwoods with excellent sugar and ethanol yields [15,16] and at high titer [17]. Recently, we demonstrated that lignosulfonate produced in the soluble stream (spent liquor) by SPORL pretreatment acts as non-ionic surfactant to enhance cellulose saccharification [18]. This facilitates simultaneous enzymatic saccharification and combined fermentation of the solids and soluble streams from pretreatment without either solid and liquid separation or washing of solids [17]. Furthermore, we found that elevated pH of 5.2–6.0 significantly alters the surface charge of insoluble sulfonated lignin from SPORL pretreatment, resulting in near zero nonproductive cellulase binding to lignin in the solid fraction [18–20]. These positive effects of lignin sulfonation by SPORL makes it uniquely suited for pretreating feedstock of very high lignin content such as forest residues.

The objective of this study is to evaluate the SPORL process for fermentable sugar production from a Douglas-fir forest residue. Douglas-fir forest residues represent one of the most recalcitrant lignocellulosic feedstock because of its softwood lignin structure and very high lignin content arising from the additional rich bark and juvenile wood content. Pretreatments were conducted in a range of severities using varied sulfite and acid dosages in a lab scale reactor. Both total sugar recovery and the production of fermentation inhibitors, such as 5-Hydroxymethyl furfural (HMF) were evaluated. This study can provide useful information to further improve the SPORL process for efficient bioconversion of forest residues in large scale studies for commercial applications in the future.

2. Materials and methods

2.1. Feedstock and chemicals

Douglas-fir forest residues used in this study were collected from roadside piles (Fig. 1) resulting from a regeneration harvest in a Douglas-fir stand located in western Oregon (44.24′ N and 123.42′ W) owned by Roseboro Resources

Fig. 1 – A typical forest residue pile (Lane County, Oregon) from which FS-03 was taken.

(Roseburg, OR, USA). The stand was harvested in Spring of 2011. The residues were still fairly green when ground on February 16, 2012 using a Peterson Pacific 4710B horizontal grinder with a combination of 76 mm and 102 mm grates, and a combination of 18 standard carbide and 18 chipper bits. The harvested residues were shipped to Weyerhaeuser Company at Federal Way, WA. The moisture content was 38.1% measured at arrival. The residues were composed of approximately 87% Douglas-fir and 6% hardwood based upon wood fiber identification. The collected residues were screened using a 3.2 mm woven wire screen to remove fines. The mass fraction of screen reject fines was approximately 15%. The ash content of the fines was very high at 15.3% in agreement with a previous study of chipped Douglas-fir residue sample [21]. The ash content of the screen accepts after fines removal was 1.2%. The screen accepts were then air-dried to 10.4% moisture. The dried accept chips (labeled for the project as FS-03) were shipped to the USDA Forest Products Lab. The received FS-03 was fractionated using a Williams horizontal sieve shaker (USPN 7905, Williams Standard, Williams Apparatus Company, Watertown, NY) with a set of sieves of sizes: 3.2, 4.8, 6.4, 9.5, 12.7, 15.9, 19.1, 22.2, 25.4, 28.6, and 31.8 mm to determine particle size distribution.

All chemicals, i.e. sulfuric acid and sodium bisulfite, acetic acid and sodium acetate, were ACS reagent grade and purchased from Sigma–Aldrich (St. Louis, MO). A commercial cellulase cocktail CTec2 was kindly provided by the Novozymes North America (Franklinton, NC). The CTec2 activity was 150 FPU cm^{-3}.

2.2. Substrate production

FS-03 Douglas-fir forest residue was pretreated using SPORL in lab bomb reactors. Three 1 L stainless bomb reactors were housed in an autoclave configuration in a 23 L laboratory rotating pulping digester as described previously [16,22]. The pulping digester was heated internally by steam and rotated at 0.21 rad s^{-1} for mixing. Our previous study indicated that SPORL pretreatment conducted at a low temperature of 165 °C is advantageous in reducing sugar degradation during SPORL pretreatment without affecting the enzymatic digestibility of the pretreated solid substrate [23]. Therefore all SPORL pretreatments were conducted at $T = 165$ °C with varied

pretreatment duration $t = 50–125$ min, chemical loadings of sodium bisulfite as mass fraction of oven dry (od) wood $B = 4–14\%$, and sulfuric acid concentration as volume fraction $= 0–0.8\%$ or as mass fraction $A = 0–4.42\%$ on oven dried solids as listed in Table 1. Replicate pretreatments were conducted for several pretreatment conditions. Each pretreatment was conducted in a bomb reactor using 150 g of oven dried solids mixed with dilute sodium bisulfite solution at a fixed liquid to solids ratio of 3:1. The pretreatment temperature was monitored using a thermocouple probe inside of the 23 L pulping digester by a wireless transmitter (Omega Engineering, Inc., CT) and a laptop computer. The temperature was controlled at 165 ± 3 °C by manually adjusting the steam flow through the digester. After pretreatment, the spent liquor was separated from the pretreated solids using a stainless steel mesh for determining mass balances of the solid and liquid fractions.

The solids were then disk milled in a 0.31 m disk refiner (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH) at atmospheric pressure using a pair of disk plates of pattern D2-B505 with plate gap of 1 mm and rotating at 269 rad s^{-1}. The collected pretreatment spent liquor was re-mixed with the pretreated solids at the inlet to milling, without adding any additional dilution water. The milled samples were placed into a canvas bag to separate the pretreatment liquor containing dissolved materials from the solids, by hydraulic pressure. A 100 g sample of the resultant wet solids was washed twice by mixing with 1 L of tap water to wash out the soluble components. The washed solids were filtered using a Whatman paper filter. The washed solid sample was collected for yield determination and chemical composition analysis.

2.3. *Pretreatment severity*

The combined severity factor (CSF) has been used to describe the severity of dilute acid pretreatment [24]. Unfortunately, CSF failed to provide good predictions of hemicellulose dissolution during pretreatments [25]. Furthermore, it cannot be applied to pretreatments with additional catalysts. We previously developed a combined hydrolysis factor (CHF) that can accurately predict hemicellulose dissolution for both SPORL and dilute acid pretreatment of aspen [26] and SPORL pretreatment of Douglas-fir [23] under a wide range of conditions:

Table 1 – SPORL pretreatment conditions for Douglas-fir forest harvest residue (FS-03).

Run no.	Run label[a]	Pretreatment condition[b]			Initial pH	CHF[c]
		Time (min)	Acid (volume fraction %)	Bisulfite (mass fraction %)		
1	t50-A4-B10R1	50	0.4	10	1.84	5.10
2	t50-A4-B10R2	50	0.4	10	1.80	5.10
3	t50-A4-B12	50	0.4	12	1.85	2.71
4	t75-A0-B10	75	0	10	4.14	1.22
5	t75-A2-B10	75	0.2	10	2.25	3.01
6	t75-A4-B4	75	0.4	4	1.61	39.38
7	t75-A4-B6	75	0.4	6	1.77	23.70
8	t75-A4-B8	75	0.4	8	1.91	13.44
9	t75-A4-B10R1	75	0.4	10	1.92	7.34
10	t75-A4-B10R2	75	0.4	10	1.91	7.34
11	t75-A4-B10R3	75	0.4	10	1.75	7.34
12	t75-A4-B10R4	75	0.4	10	1.73	7.34
13	t75-A4-B12R1	75	0.4	12	1.96	3.90
14	t75-A4-B12R2	75	0.4	12	1.80	3.90
15	t75-A6-B10	75	0.6	10	1.66	17.74
16	t100-A2-B10R1	100	0.2	10	2.08	3.92
17	t100-A2-B10R2	100	0.2	10	2.37	3.92
18	t100-A4-B6	100	0.4	6	1.57	30.92
19	t100-A4-B8	100	0.4	8	1.72	17.54
20	t100-A4-B10R1	100	0.4	10	1.70	9.57
21	t100-A4-B10R2	100	0.4	10	1.67	9.57
22	t100-A4-B12	100	0.4	12	1.81	5.09
23	t100-A4-B14	100	0.4	14	1.79	2.65
24	t100-A6-B10R1	100	0.6	10	1.45	23.15
25	t100-A6-B10R2	100	0.6	10	1.81	23.15
26	t100-A8-B10R1	100	0.8	10	1.27	55.56
27	t100-A8-B10R2	100	0.8	10	1.64	55.56
28	t125-A4-B10R1	125	0.4	10	1.71	11.81
29	t125-A4-B10R2	125	0.4	10	1.65	11.81
30	t125-A4-B12	125	0.4	12	1.75	6.28

a txx is pretreatment duration in min; Axx is sulfuric acid loading in cm^3 in 1000 cm^3 solution; Bxx is sodium bisulfite charge on wood (oven dry weight) in mass fraction %; Rxx is replicate number for the specified set of condition.
b All pretreatments were conducted at 165 °C with liquor to solids mass ratio = 3:1.
c CHF = Combined hydrolysis factor (Eq. (1)).

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

where C_A and C_B are the molar concentrations of chemical A (sulfuric acid) and chemical B (sodium bisulfite) used in pretreatment, respectively; α, β and γ are adjustable parameters, E is the apparent activation energy (J mol^{-1}), R is universal gas content of 8.314 J mol^{-1} K^{-1}, t in min, and T is absolute temperature (K). The values of $\alpha = 28.5$, $\beta = 17$; $\gamma = -10$, and $E = 100{,}000$ (J mole^{-1}) were used in our previous study for the bark-free Douglas-fir wood chips [23]. Slow and fast reaction hemicelluloses were incorporated into the kinetic analysis for predicting hemicellulose dissolution using CHF by the following equation:

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

where X_R is the fraction of hemicellulose remaining in the pretreated solids, θ is the fraction of slow hemicelluloses, f is the ratio of the rate constants between the slow and fast hemicellulose hydrolysis reactions. The slow hemicelluloses represent a small fraction of hemicelluloses intimately associated with cellulose that is hard to be hydrolyzed.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out at a solids mass fraction loading of 2%, and an enzyme dosage of 15 FPU (or 100 mm^3) per gram glucan, or approximately 0.5–0.6 mL CTec2 per gram biomass. The wet substrate was mixed into sodium acetate buffer to make a 50 mL mixture in a 125 mL flask. The pH of the mixture was first adjusted using lime and then controlled at 5.5 using acetate buffer rather than pH 5.0 commonly used in many laboratories throughout the world. Elevated pH of approximately 5.5 can significantly reduce nonproductive cellulase binding to bound lignin on solid substrates and enhance enzymatic saccharification [19,20]. The flasks were placed into a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 50 °C and agitated at 20.9 rad s^{-1} (i.e. 200 rpm). Hydrolyzate samples were collected at 3, 6, 9, 24, 48, 72 h for each experiments. Replicates of enzymatic hydrolysis were conducted for selected samples.

2.5. Analytical methods

The chemical compositions of the forest residues, and the SPORL pretreated substrates were analyzed as described previously [22]. Briefly, the biomass carbohydrates were hydrolyzed using sulfuric acid in two steps: concentration as volume fraction of 72% at 30 °C for 1 h followed by dilution to concentration as volume concentration of 3.6% at 120 °C for 1 h. The hydrolyzed sugars were analyzed using a Dionex HPLC system (ICS-3000, Dionex) equipped with integrated amperometric detector. Klason lignin was determined gravimetrically. Sugars, furan, and acetic acid concentrations in the pretreatment hydrolyzates were analyzed by another HPLC (Ultimate 3000, Thermo Scientific) equipped with a refractive index detector for carbohydrate and furan analyses, using a Biorad Aminex HPX-87P column with an ionic deashing guard column, as well as a UV–vis detector for acetic acid analysis using a Biorad Aminex HPX-87H column along with a cation H guard column. A commercial glucose analyzer (YSI 2700 S, YSI Inc., Yellow Springs, OH) was used for fast analysis of glucose concentrations in the enzymatic hydrolyzates.

3. Results and discussion

3.1. Analysis of the forest residue (FS-03)

FS-03 has a bark mass fraction of 3.5% measured by manually separating bark and wood of an aliquot sample, which is very close to 3.1% calculated from the Klason lignin and glucan contents of pure wood, pure bark, and the FS-03 forest residue according to a procedure developed previously [21]. Images of the FS-03 fractions are shown in Fig. 2(a) through Fig. 2(d). Despite initial fractionation of as-received moisture content material (rejecting particles less than 3.2 mm), drying of the forest residue resulted in an additional fraction of small particles, presumably from (1) the separation of small particles that adhered to large particles when wet, and (2) the breakup of the brittle bark particles due to drying. This fraction of small particles can be clearly seen from Fig. 2(a) and accounts for approximately 2% of the total mass. Because FS-03 was harvested by grinding, some relatively large particles were observed (Fig. 2(b)) which can pose problems for pretreatment using the small scale laboratory reactor. Therefore FS-03 was hammer milled before pretreatment. The large particles were cut manually to shorter length as shown in Fig. 2(c), to facilitate hammer milling. Hammer milling significantly reduced particle size (Fig. 2(d)) and the particle size distribution becomes relatively uniform compared with the initial FS-03 (Fig. 3). However hammer milling also produced a significant amount of small particles. The mass fraction with size less than 3.2 mm increased from approximately 2%–33% (Fig. 3).

The chemical compositions of FS-03, and the wood and the bark from FS-03 were analyzed (Table 2). FS-03 has higher lignin and lower glucan content than the commercial wood from which FS-03 was obtained due to: (1) the high lignin (38.4%) and low glucan (31.7%) content in the bark; and (2) the wood in forest residue is primarily from tree tops and branches which are juvenile wood with relatively high lignin and low glucan contents.

3.2. Effect of pretreatment on cell wall composition, inhibitor formation, and substrate enzymatic digestibility

Cell wall component losses, formation of fermentation inhibitors such as furan and acetic acid, and substrate enzymatic cellulose saccharification efficiency are important factors in determining the optimal pretreatment for a given feedstock. The effect of pretreatment time t was evaluated under constant mass charges of sodium bisulfite on wood $B = 10\%$ and sulfuric acid $A = 2.21\%$. t had a minimal effect on delignification and glucan loss (Fig. 4(a)). Increasing t increased the removals of hemicelluloses, xylan and mannan, which improved cellulose accessibility. This can be seen from the 20% increase in substrate enzymatic digestibility (SED, Fig. 4(a)), defined as the percentage of substrate glucan enzymatically saccharified to glucose, when pretreatment time t was doubled from 50 to 100 min. However, t also had

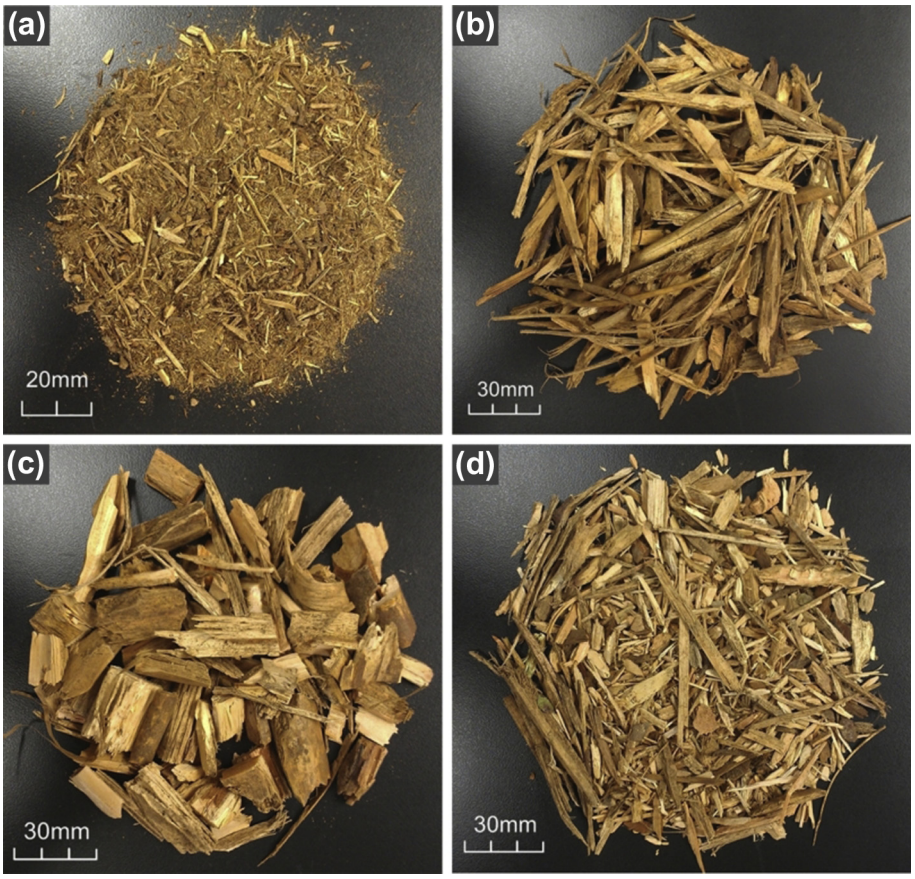

Fig. 2 – Images of different fractions and hammer-milled FS-03. (a) I < 3.2 mm; (b) 6.4 ≤ IV < 9.5; (c) 19.1 ≤ VIII < 22.2; (d) hammer-milled FS-03.

significant impact on furan formation (Fig. 4(a)). Both HMF and furfural increased almost linearly with t to approximately 4 g L^{-1} and then plateaued at 100 min. The formation of acetic acid was almost constant for the range of t studied.

Increasing B slightly reduced hemicellulose removal but significantly improved delignification and increased glucan loss under constant $A = 2.21\%$ and $t = 75$ min (Fig. 4(b)) as sulfite is known capable of degrading cellulose to produce weak sulfite pulp based on sulfite pulping. Lignin removal achieved 40% at $B = 12\%$. Partial delignification is important for improving enzymatic saccharification of lignocelluloses with high lignin content materials such as FS-03 [8]. SED was increased from 50 to 91% (Fig. 4(b)) when B was increased from 4 to 12% due primarily to the increased lignin removal from 0 to 40%. Increasing B increased pH of the pretreatment liquor at constant A, and as a result, furan formation and acetic acid decreased linearly as B increased (Fig. 4(b)). Both HMF and furfural concentrations were approximately 2.5 g L^{-1} at $B = 12\%$.

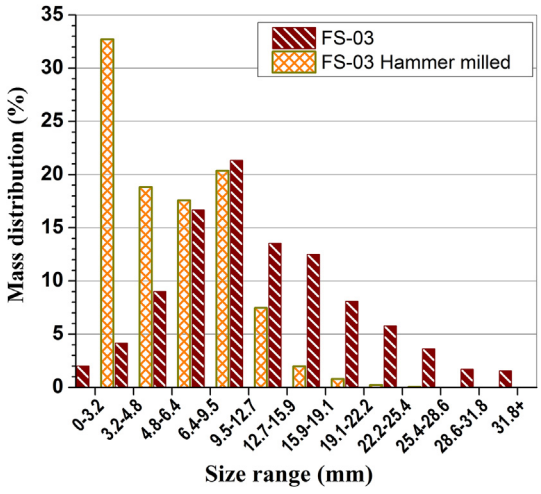

Fig. 3 – Particle size distributions of FS-03 and hammer-milled FS-03.

Low pH facilitates hemicellulose dissolution but causes lignin condensation. Increasing A under constant B and t resulted in improved xylan and mannan removal and decreased delignification (Fig. 4(c)). Xylan and mannan removal were increased from approximately 60% to over 90% when A was increased from 0 to 3.3%. Lignin removal, however, was reduced from approximately 50%–20%. Glucan loss was not affected by A because actual pH variation is small in the acid range investigated. The opposing directions of hemicellulose removal and delignification resulted in negligible effect on SED (Fig. 4(c)). Increasing A resulted in significant increase in furan production due to reduced pH, opposite to that observed from increasing sodium bisulfite loading.

Table 2 – Chemical compositions as mass fraction of the forest harvest residue (%).

Sample	Ash	Klason lignin	Arabinan	Galactan	Glucan	Xylan	Mannan	Total carbohydrates
FS-03	0.8	32.3	1.3	3.7	37.7	6.3	8.2	57.3
Wood of FS-03	0.2	30.9	0.9	3.1	39.9	6.6	9.6	60.1
Bark of FS-03	0.7	38.4	5.7	3.2	31.7	4.9	5.3	50.9

Both HMF and furfural were increased approximately from 0.7 g L^{-1} to 4 g L^{-1}.

3.3. *Predictions of hemicellulose removal and delignification using CHF*

CHF was developed using aspen with the consideration of both slow and fast xylan and shown to provide accurate prediction of xylan removal even at near complete xylan removal [26]. When CHF was applied to the current study of Douglas-fir forest residue (FS-03), fairly good prediction of hemicellulose removal was also obtained (Fig. 5(a)). The difference in the removal of xylan and mannan was apparent. This is probably due to the differences between these two hemicellulose types and how they are embedded in the cell matrix. Another possibility is due to the fact that bark has higher content of xylan and lower content of mannan compared with wood (Table 2). The high lignin content in bark may resulted in less hemicellulose dissolution than the hemicellulose in wood. Separating fittings of xylan and mannan produced better predictions of approximately ±3% (Fig. 5(a)).

Delignification was found to be inversely proportional to CHF and can be predicted to within ±6% (Fig. 5(b)) despite the fact that CHF was developed for predicting hemicellulose dissolution. This is probably due to the fact that all pretreatments were conducted in a narrow range of conditions, e.g., temperature was fixed at 165 °C. Furthermore, delignification was facilitated by sulfite but negatively impacted by acid through lignin condensation reactions, which are accurately captured by CHF, i.e., β is positive and γ is negative in Eq. (1). Fine tuning optimization experiments, especially in scale-up studies are often conducted in a narrow range, and therefore, can use CHF to predict delignification. These results indicate that CHF can be used for designing pretreatment processes, especially for scale-up studies where conducting numerous experiments are economically prohibitive.

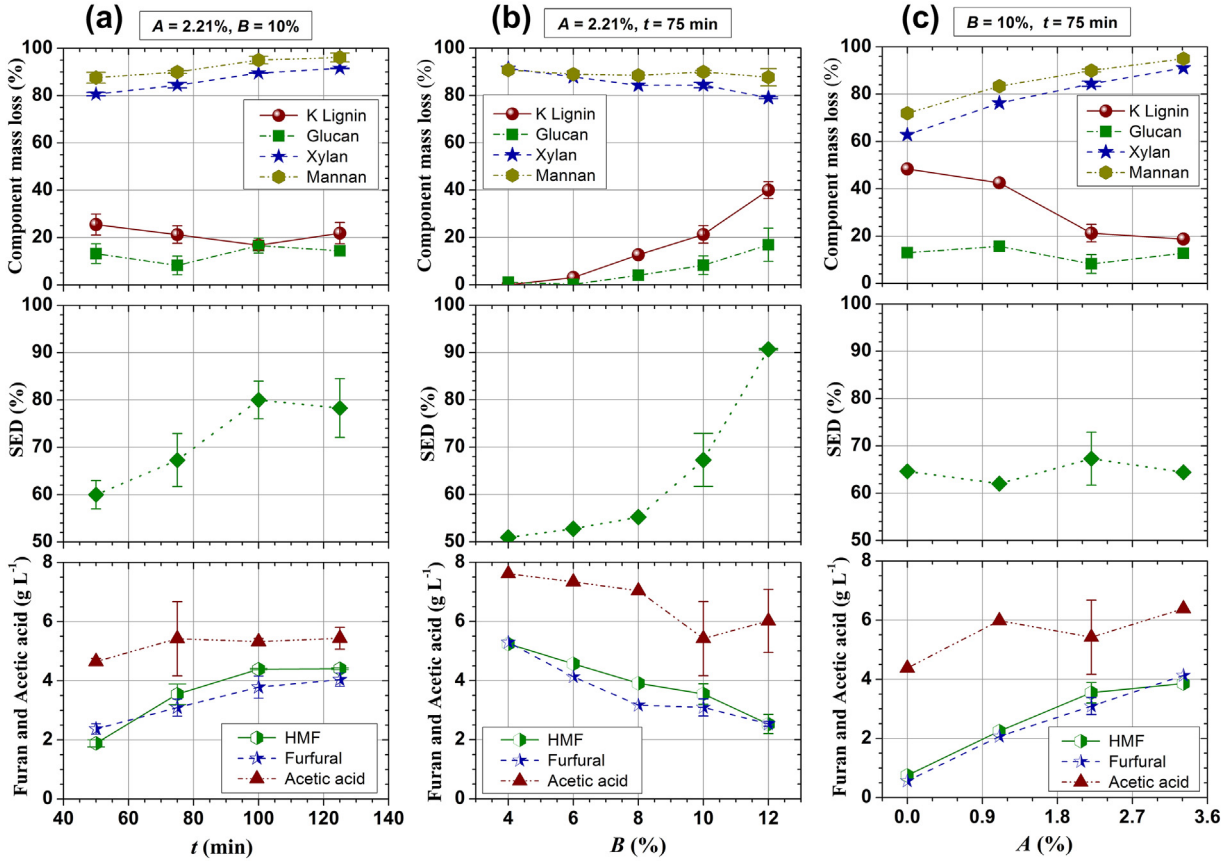

Fig. 4 – Effects of pretreatment conditions on cell wall component removal, substrate enzymatic digestibility (SED), and inhibitor formation. (a) Variation of pretreatment time t; (b) variation of sodium bisulfite loading B; (c) variation of sulfuric acid loading A.

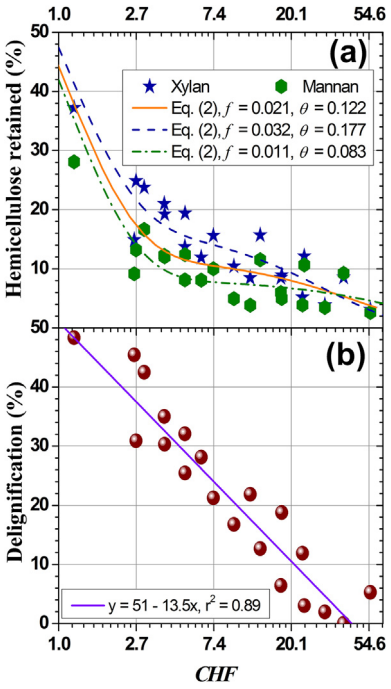

Fig. 5 – Correlations of hemicellulose removal and delignification with combined hydrolysis factor (CHF). (a) hemicellulose removal; (b) delignification.

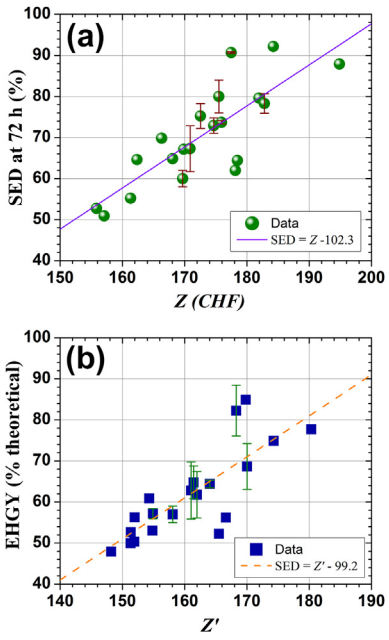

Fig. 6 – Correlations of substrate enzymatic digestibility (SED) and enzymatic hydrolysis glucose yield (EHGY) with combined factors of delignification and carbohydrate loss Z(CHF) and Z′, respectively. (a) SED with Z(CHF); (b) EHGY with Z′.

3.4. *Enzymatic cellulose saccharification and hemicellulose removal and delignification*

Cellulose accessibility is a key factor dictating enzymatic saccharification of lignocelluloses [8]. Delignification and removal of hemicelluloses can improve cellulose accessibility [27]. For lignocelluloses with low lignin content, hemicellulose removal is the dominant factor for improving enzymatic saccharification [8]. SED can be predicted using xylan removal or CHF [26]. Delignification becomes important for lignocelluloses with high lignin content such as softwoods [8]. FS-03 has a lignin content of 32.3% (Table 2) much higher than that of common softwoods around 28% [6]. The effects of delignification on SED can be clearly seen from Fig. 4(b). Based on the aforementioned effects of component removal from high lignin content substrates, a combined parameter Z can be developed consisting of delignification, hemicellulose removal and glucan loss. When the SEDs of the substrates are plotted against Z (Fig. 6(a)), a good correlation is found despite some data scattering. This combined factor is defined as:

$$Z = L \times \text{Delignification} + H \times \text{Hemicellulose removal} + G \times \text{Glucan loss} \quad (3a)$$

where hemicellulose removal is the mass weighted-average percent loss of mannan (M) and xylan (X). Least square fitting resulted in $L = 0.908$, $H = 1.671$, and $G = 0.089$. When comparing the magnitude of the terms in Eq. (3a). It is apparent that delignification is important to increase SED while hemicellulose removal is still critical and more important than delignification. Furthermore, glucan loss also contributes to improving SED due to improve cellulose accessibility as noticed in an early study using catalyzed steam explosion [28]. However, G is an order magnitude smaller than L and H and glucan loss was lower than delignification and hemicellulose dissolution. We can assume $G = 0$ and refit the data to result $L = 0.934$ and $H = 1.725$. Because both delignification and hemicellulose removal are functions of CHF as shown in Fig. 5(a) and (b), we have

$$Z(\text{CHF}) = 0.934 \times \text{Delignification} + 1.725 \times \text{Hemicellulose removal} \quad (3b)$$

This makes CHF much more meaningful and useful for prediction purpose.

Enzymatic hydrolysis glucose yield (EHGY) can also be correlated to delignification, hemicellulose removal, and glucan using Eq. (3a) as shown in Fig. 6(b). Glucan loss can increase SED, however, it reduced glucan recovery to result in a negative effect on EHGY. Least square fitting resulted in a different set of coefficients, i.e., $L = 0.944$, $H = 1.675$, and $G = -0.577$. We use Z's to represent this combined parameter,

$$Z' = 0.943 \times \text{Delignification} + 1.675 \times \text{Hemicellulose removal} - 0.577 \times \text{Glucan loss} \quad (3c)$$

3.5. *Overall mass balance and maximal sugar yield*

Based upon SED, EHGY, inhibitor formation, as well as total sugar yield, we determined that pretreatment condition $A = 2.21\%$, $B = 12\%$, and $t = 75$ min as the optimal pretreatment condition. An overall mass balance under this pretreatment

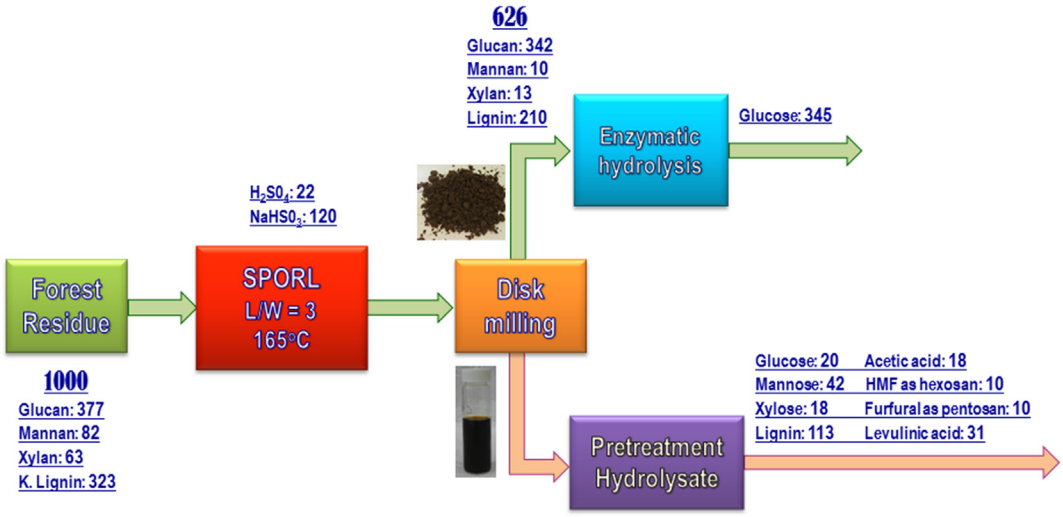

Fig. 7 – Overall mass balance of the optimal run at $T = 165$ °C, $t = 75$ min, $B = 12\%$, and $A = 2.2\%$ with liquid to solid ratio of 3:1. All numbers are expressed in gram.

(averaged of duplicate pretreatments) is shown in Fig. 7. A total of 365 g glucose was recovered from 1000 g FS-03, equivalent to 87.1% theoretical, which include EHGY of 345 g kg^{-1} wood equivalent to 82.3% theoretical. Mannose and xylose recovery from the pretreatment hydrolyzate was relatively low at approximately 50 and 30%, respectively. High sodium bisulfite loading of 12% to facilitate delignification reduced xylan removal to approximately 80%. Recoveries of mannose and xylose from enzymatic hydrolysis were not measured but are expected to increase the overall mannose and xylose recovery. The HMF and furfural concentrations in the pretreatment hydrolyzate were relatively low with each at approximately 2.5 g L^{-1}.

4. Conclusions

SPORL can effectively remove the strong recalcitrance of a Douglas-fir forest harvest residue to produce a good sugar yield. The optimal SPORL pretreatment condition was $T = 165$ °C for 75 min at liquor to solid ratio of 3:1 and sodium bisulfite and sulfuric acid loading of 12% and 2.21% on dry biomass, respectively. An enzymatic hydrolysis glucose yield of 87% theoretical was achieved at this condition with HMF and furfural concentration each at only 2.5 g L^{-1}. The combined hydrolysis factor (CHF) developed using aspen was capable of predicting dissolution of hemicelluloses for the Douglas-fir forest harvest residue. Delignification becomes important in order to achieve high enzymatic cellulose saccharification efficiency for the forest residue due to its high lignin content. Delignification can be correlated to CHF for the narrow range of pretreatment conditions investigated. Enzymatic cellulose saccharification can be predicted by CHF, which makes CHF a good factor for scale-up studies where numerous experiments are economically prohibitive.

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