

Robust Enzymatic Saccharification of a Douglas-fir Forest Harvest Residue by SPORL

Shao-Yuan Leu^{1,2}, J.Y.Zhu^{1*}, Roland Gleisner¹, John Sessions³, Gevan Marrs⁴

¹ USDA Forest Service, Forest Products Laboratory, Madison, WI

² Dept. Civil Environ. Eng., Hong Kong Polytechnic University, Kowloon, Hong Kong

³ College of Forestry, Oregon State University, Corvallis, OR

⁴ Weyerhaeuser NR Company, Federal Way, WA

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 minutes 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 of a dry biomass 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 hydrolysate. 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.

30

31 **Keywords:** forest harvest residue, pretreatment, enzymatic hydrolysis/saccharification,
32 biofuel, pretreatment severity

33

34 * Corresponding author: jzhu@fs.fed.us, (608) 231 – 9520 (Phone), (608) 231 – 9538 (fax)

35 This work was conducted on official government time of Zhu and Gleisner while Leu was a
36 postdoctoral fellow at the USDA Forest Service, Forest Products Lab.

37 **Introduction**

38 Forest harvest residues can be sustainably produced in large quantities in North America
39 and various regions of the globe [1-3]. About 50 Mt of forest residues are available in the United
40 States alone, of which it is estimated that 70 % can be sustainably recovered annually [1-3]. A
41 recent study by the U.S. National Academy of Sciences indicated that forest residues are one of
42 the two most cost effective feedstock for biofuel production [4]. Forest residues have relatively
43 high bulk densities and can be harvested year round which reduces on-site storage requirements,
44 both of which are significant advantages over agriculture residues and herbaceous biomass in
45 terms of improving supply chain logistics and reducing transportation costs [5, 6]. However,
46 forest residues are very recalcitrant to biochemical conversion through the biorefinery concept
47 because bark and juvenile wood in the residues have high lignin content. Very limited studies
48 are reported on bioconversion of forest residues [7]. Few process technologies reported
49 satisfactory enzymatic saccharification yield from woody biomass including forest residues.
50 Successfully addressing efficient bioconversion of forest residues has significant practical
51 importance because feedstock sustainability and cost are the two critical factors that dictate the
52 commercial viability of the biorefinery concept.

53 Some degrees of lignin removal as well as substantial removal of hemicelluloses through a
54 pretreatment step are required for efficient enzymatic saccharification of lignocellulosic biomass
55 with high lignin content [8]. Various pretreatment technologies, such as Organosolv, alkaline,
56 and SO₂ catalyzed steam explosion, have been applied to softwood species with high lignin
57 contents [6] and achieved some level of success [9-13]. Sulfite Pretreatment to Overcome the
58 Recalcitrant of Lignocelluloses (SPORL), though a relatively new process [14], demonstrated
59 robust performances for sugar and biofuel production from very recalcitrant softwoods with

60 excellent sugar and ethanol yields [15, 16] and at high titer [17]. Recently, we demonstrated that
61 lignosulfonate produced in the soluble stream (spent liquor) by SPORL pretreatment acts as non-
62 ionic surfactant to enhance cellulose saccharification [18]. This facilitates simultaneous
63 enzymatic saccharification and combined fermentation of the solids and soluble streams from
64 pretreatment without either solid and liquid separation or washing of solids [17]. Furthermore,
65 we found that elevated pH of 5.2 to 6.0 significantly alters the surface charge of insoluble
66 sulfonated lignin from SPORL pretreatment, resulting in near zero nonproductive cellulase
67 binding to lignin in the solid fraction [18-20]. These positive effects of lignin sulfonation by
68 SPORL makes it uniquely suited for pretreating feedstock of very high lignin content such as
69 forest residues.

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

79

80

81

82

83 2. Materials and methods

84 2.1 Feedstock and chemicals

85 Douglas-fir forest residues used in this study were collected from roadside piles (Fig. 1)
86 resulting from a regeneration harvest in a Douglas-fir stand located in western Oregon (44.24' N
87 and 123.42' W) owned by Roseboro Resources (Roseburg, OR, USA). The stand was harvested
88 in Spring of 2011. The residues were still fairly green when ground on February 16, 2012 using
89 a Peterson Pacific 4710B horizontal grinder with a combination of 76 mm and 102 mm grates,
90 and a combination of 18 standard carbide and 18 chipper bits. The harvested residues were
91 shipped to Weyerhaeuser Company at Federal Way, WA. The moisture content was 38.1 %
92 measured at arrival. The residues were composed of approximately 87 % Douglas-fir and 6 %
93 hardwood based upon wood fiber identification. The collected residues were screened using a 3.2
94 mm woven wire screen to remove fines. The mass fraction of screen reject fines was
95 approximately 15 %. The ash content of the fines was very high at 15.3 % in agreement with a
96 previous study of chipped Douglas-fir residue sample [21]. The ash content of the screen
97 accepts after fines removal was 1.2 %. The screen accepts were then air-dried to 10.4 %
98 moisture. The dried accept chips (labeled for the project as FS-03) were shipped to the USDA
99 Forest Products Lab. The received FS-03 was fractionated using a Williams horizontal sieve
100 shaker (USPN 7905, Williams Standard, Williams Apparatus Company, Watertown, NY) with a
101 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
102 determine particle size distribution.

103 All chemicals, i.e. sulfuric acid and sodium bisulfite, acetic acid and sodium acetate, were
104 ACS reagent grade and purchased from Sigma-Aldrich (St. Louis, MO). A commercial cellulase

105 cocktail CTec2 was kindly provided by the Novozymes North America (Franklinton, NC). The
106 CTec2 activity was 150 FPU cm^{-3} .

107 **2.2 Substrate production**

108 FS-03 Douglas-fir forest residue was pretreated using SPORL in lab bomb reactors. Three
109 1 L stainless bomb reactors were housed in an autoclave configuration in a 23 L laboratory
110 rotating pulping digester as described previously [16, 22]. The pulping digester was heated
111 internally by steam and rotated at 0.21 rad s^{-1} for mixing. Our previous study indicated that
112 SPORL pretreatment conducted at a low temperature of $165 \text{ }^\circ\text{C}$ is advantageous in reducing
113 sugar degradation during SPORL pretreatment without affecting the enzymatic digestibility of
114 the pretreated solid substrate [23]. Therefore all SPORL pretreatments were conducted at $T =$
115 165°C with varied pretreatment duration $t = 50 - 125$ minutes, chemical loadings of sodium
116 bisulfite as mass fraction of oven dry (od) wood $B = 4 - 14 \%$, and sulfuric acid concentration as
117 volume fraction $= 0 - 0.8\%$ or as mass fraction $A = 0 - 4.42 \%$ on oven dried solids as listed in
118 Table 1. Replicate pretreatments were conducted for several pretreatment conditions. Each
119 pretreatment was conducted in a bomb reactor using 150 g of oven dried solids mixed with dilute
120 sodium bisulfite solution at a fixed liquid to solids ratio of 3:1. The pretreatment temperature
121 was monitored using a thermocouple probed inside of the 23 L pulping digester by a wireless
122 transmitter (Omega Engineering, Inc., CT) and a laptop computer. The temperature was
123 controlled at $165 \pm 3^\circ\text{C}$ by manually adjusting the steam flow through the digester. After
124 pretreatment, the spent liquor was separated from the pretreated solids using a stainless steel
125 mesh for determining mass balances of the solid and liquid fractions.

126 The solids were then disk milled in a 0.31 m disk refiner (Andritz Sprout-Bauer
127 Atmospheric Refiner, Springfield, OH) at atmospheric pressure using a pair of disk plates of

128 pattern D2-B505 with plate gap of 1 mm and rotating at 269 rad s⁻¹. The collected pretreatment
129 spent liquor was re-mixed with the pretreated solids at the inlet to milling, without adding any
130 additional dilution water. The milled samples were placed into a canvas bag to separate the
131 pretreatment liquor containing dissolved materials from the solids, by hydraulic pressure. A 100
132 g sample of the resultant wet solids was washed twice by mixing with 1 L of tap water to wash
133 out the soluble components. The washed solids were filtered using a Whatman paper filter. The
134 washed solid sample was collected for yield determination and chemical composition analysis.

135 **2.3 Pretreatment severity**

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

$$142 \quad CHF = e^{\left(\alpha - \frac{E}{RT} + \beta C_A + \gamma C_B\right)} (C_A + C_B)t \quad (1)$$

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

$$150 \quad X_R = (1 - \theta)e^{-CHF} + \theta e^{-f CHF} \quad (2)$$

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

155 **2.4 Enzymatic hydrolysis**

156 Enzymatic hydrolysis was carried out at a solids mass fraction loading of 2 %, and an
157 enzyme dosage of 15 FPU (or 100 mm^3) per gram glucan, or approximately 0.5-0.6 mL CTec2
158 per gram biomass. The wet substrate was mixed into sodium acetate buffer to make a 50 mL
159 mixture in a 125 mL flask. The pH of the mixture was first adjusted using lime and then
160 controlled at 5.5 using acetate buffer rather than pH 5.0 commonly used in many laboratories
161 throughout the world. Elevated pH of approximately 5.5 can significantly reduce nonproductive
162 cellulase binding to bound lignin on solid substrates and enhance enzymatic saccharification [19,
163 20]. The flasks were placed into a shaking incubator (Thermo Fisher Scientific, Model 4450,
164 Waltham, MA) at 50 °C and agitated at 20.9 rad s^{-1} (i.e. 200 rpm). Hydrolysate samples were
165 collected at 3, 6, 9, 24, 48, 72 hours for each experiments. Replicates of enzymatic hydrolysis
166 were conducted for selected samples.

167 **2.5 Analytical methods**

168 The chemical compositions of the forest residues, and the SPORL pretreated substrates were
169 analyzed as described previously [22]. Briefly, the biomass carbohydrates were hydrolyzed
170 using sulfuric acid in two steps: concentration as volume fraction of 72% at 30 °C for one hour
171 followed by dilution to concentration as volume concentration of 3.6% at 120 °C for one hour.
172 The hydrolyzed sugars were analyzed using a Dionex HPLC system (ICS-3000, Dionex)
173 equipped with integrated amperometric detector. Klason lignin was determined gravimetrically.

174 Sugars, furan, and acetic acid concentrations in the pretreatment hydrolysates were analyzed by
175 another HPLC (Ultimate 3000, Thermo Scientific) equipped with a refractive index detector for
176 carbohydrate and furan analyses, using a Biorad Aminex HPX-87P column with an ionic
177 deashing guard column, as well as a UV-Vis detector for acetic acid analysis using a Biorad
178 Aminex HPX-87H column along with a cation H guard column. A commercial glucose analyzer
179 (YSI 2700 S, YSI Inc., Yellow Springs, OH) was used for fast analysis of glucose concentrations
180 in the enzymatic hydrolysates.

181

182 **Results and discussion**

183 **3.1 Analysis of the forest residue (FS-03)**

184 FS-03 has a bark [mass fraction](#) of 3.5 % measured by manually separating bark and wood
185 of an aliquot sample, which is very close to 3.1 % calculated from the Klason lignin and glucan
186 contents of pure wood, pure bark, and the FS-03 forest residue according to a procedure
187 developed previously [21]. Images of the FS-03 fractions are shown in Fig. 2a through Fig. 2d.
188 Despite initial fractionation of as-received moisture content material (rejecting particles less than
189 3.2 mm), drying of the forest residue resulted in an additional fraction of small particles,
190 presumably from (1) the separation of small particles that adhered to large particles when wet,
191 and (2) the breakup of the brittle bark particles due to drying. This fraction of small particles can
192 be clearly seen from Fig. 2a and accounts for approximately 2 % of the total mass. Because FS-
193 03 was harvested by grinding, some relatively large particles were observed (Fig. 2b) which can
194 pose problems for pretreatment using the small scale laboratory reactor. Therefore FS-03 was
195 hammer milled before pretreatment. The large particles were cut manually to shorter length as
196 shown in Fig. 2c, to facilitate hammer milling. Hammer milling significantly reduced particle

197 size (Fig. 2d) and the particle size distribution becomes relatively uniform compared with the
198 initial FS-03 (Fig. 3). However hammer milling also produced a significant amount of small
199 particles. The mass fraction with size less than 3.2 mm increased from approximately 2 % to 33
200 % (Fig. 3).

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

206 **3.2 Effect of pretreatment on cell wall composition, inhibitor formation, and substrate** 207 **enzymatic digestibility**

208 Cell wall component losses, formation of fermentation inhibitors such as furan and acetic
209 acid, and substrate enzymatic cellulose saccharification efficiency are important factors in
210 determining the optimal pretreatment for a given feedstock. The effect of pretreatment time t
211 was evaluated under constant mass charges of sodium bisulfite on wood $B = 10\%$ and sulfuric
212 acid $A = 2.21\%$. t had a minimal effect on delignification and glucan loss (Fig. 4a). Increasing t
213 increased the removals of hemicelluloses, xylan and mannan, which improved cellulose
214 accessibility. This can be seen from the 20 % increase in substrate enzymatic digestibility (SED,
215 Fig. 4a), defined as the percentage of substrate glucan enzymatically saccharified to glucose,
216 when pretreatment time t was doubled from 50 to 100 minutes. However, t also had significant
217 impact on furan formation (Fig. 4a). Both HMF and furfural increased almost linearly with t to
218 approximately 4 g L^{-1} and then plateaued at 100 minutes. The formation of acetic acid was
219 almost constant for the range of t studied.

220 Increasing B slightly reduced hemicellulose removal but significantly improved
221 delignification and increased glucan loss under constant $A = 2.21\%$ and $t = 75$ minutes (Fig. 4b)
222 as sulfite is known capable of degrading cellulose to produce weak sulfite pulp based on sulfite
223 pulping. Lignin removal achieved 40 % at $B = 12\%$. Partial delignification is important for
224 improving enzymatic saccharification of lignocelluloses with high lignin content materials such
225 as FS-03 [8]. SED was increased from 50 to 91 % (Fig. 4b) when B was increased from 4 to 12
226 % due primarily to the increased lignin removal from 0 to 40 %. Increasing B increased pH of
227 the pretreatment liquor at constant A , and as a result, furan formation and acetic acid decreased
228 linearly as B increased (Fig. 4b). Both HMF and furfural concentrations were approximately 2.5
229 g L^{-1} at $B = 12\%$.

230 Low pH facilitates hemicellulose dissolution but causes lignin condensation. Increasing
231 A under constant B and t resulted in improved xylan and mannan removal and decreased
232 delignification (Fig. 4c). Xylan and mannan removal were increased from approximately 60 %
233 to over 90 % when A was increased from 0 to 3.3 %. Lignin removal, however, was reduced
234 from approximately 50 % to 20 %. Glucan loss was not affected by A because actual pH
235 variation is small in the acid range investigated. The opposing directions of hemicellulose
236 removal and delignification resulted in negligible effect on SED (Fig. 4c). Increasing A resulted
237 in significant increase in furan production due to reduced pH, opposite to that observed from
238 increasing sodium bisulfite loading. Both HMF and furfural were increased approximately from
239 0.7 g L^{-1} to 4 g L^{-1} .

240 **3.3 Predictions of hemicellulose removal and delignification using *CHF***

241 *CHF* was developed using aspen with the consideration of both slow and fast xylan and
242 shown to provide accurate prediction of xylan removal even at near complete xylan removal

243 [26]. When *CHF* was applied to the current study of Douglas-fir forest residue (FS-03), fairly
244 good prediction of hemicellulose removal was also obtained (Fig. 5a). The difference in the
245 removal of xylan and mannan was apparent. This is probably due to the differences between
246 these two hemicellulose types and how they are embedded in the cell matrix. Another possibility
247 is due to the fact that bark has higher content of xylan and lower content of mannan compared
248 with wood (Table 2). The high lignin content in bark may resulted in less hemicellulose
249 dissolution than the hemicellulose in wood. Separating fittings of xylan and mannan produced
250 better predictions of approximately $\pm 3\%$ (Fig. 5a).

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

261 **3.4 Enzymatic cellulose saccharification and hemicellulose removal and delignification**

262 Cellulose accessibility is a key factor dictating enzymatic saccharification of
263 lignocelluloses [8]. Delignification and removal of hemicelluloses can improve cellulose
264 accessibility [27]. For lignocelluloses with low lignin content, hemicellulose removal is the
265 dominant factor for improving enzymatic saccharification [8]. *SED* can be predicted using xylan

266 removal or *CHF* [26]. Delignification becomes important for lignocelluloses with high lignin
267 content such as softwoods [8]. FS-03 has a lignin content of 32.3 % (Table 2) much higher than
268 that of common softwoods around 28 % [6]. The effects of delignification on *SED* can be clearly
269 seen from Fig. 4b. Based on the aforementioned effects of component removal from high lignin
270 content substrates, a combined parameter *Z* can be developed consisting of delignification,
271 hemicellulose removal and glucan loss. When the *SEDs* of the substrates are plotted against *Z*
272 (Fig. 6a), a good correlation is found despite some data scattering. This combined factor is
273 defined as:

$$274 \quad Z = L \times \text{Delignification} + H \times \text{Hemicellulose removal} + G \times \text{Glucan loss} \quad (3a)$$

275 Where hemicellulose removal is the mass weighted-average percent loss of mannan (*M*) and
276 xylan (*X*). Least square fitting resulted in $L = 0.908$, $H = 1.671$, and $G = 0.089$. When
277 comparing the magnitude of the terms in Eq. (3a). It is apparent that delignification is important
278 to increase *SED* while hemicellulose removal is still critical and more important than
279 delignification. Furthermore, glucan loss also contributes to improving *SED* due to improve
280 cellulose accessibility as noticed in an early study using catalyzed steam explosion [28].
281 However, *G* is an order magnitude smaller than *L* and *H* and glucan loss was lower than
282 delignification and hemicellulose dissolution. We can assume $G = 0$ and refit the data to result L
283 $= 0.934$ and $H = 1.725$. Because both delignification and hemicellulose removal are functions of
284 *CHF* as shown in Figs. 5a and 5b, we have

$$285 \quad Z(\text{CHF}) = 0.934 \times \text{Delignification} + 1.725 \times \text{Hemicellulose removal} \quad (3b)$$

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

287 Enzymatic hydrolysis glucose yield (*EHGY*) can also be correlated to delignification,
288 hemicellulose removal, and glucan using Eq. (3a) as shown in Fig. 6b. Glucan loss can increase

289 *SED*, however, it reduced glucan recovery to result in a negative effect on *EHGY*. Least square
290 fitting resulted in a different set of coefficients, i.e., $L = 0.944$, $H = 1.675$, and $G = -0.577$. We
291 use Z 's to represent this combined parameter,

$$292 \quad Z' = 0.943 \times \text{Delignification} + 1.675 \times \text{Hemicellulose removal} - 0.577 \times \text{Glucan loss}$$

293 $(3c)$

294 **3.5 Overall mass balance and maximal sugar yield**

295 Based upon *SED*, *EHGY*, inhibitor formation, as well as total sugar yield, we determined
296 that pretreatment condition $A = 2.21$ %, $B = 12$ %, and $t = 75$ minutes as the optimal pretreatment
297 condition. An overall mass balance under this pretreatment (averaged of duplicate
298 pretreatments) is shown in Fig. 7. A total of 365 g glucose was recovered from 1000 g FS-03,
299 equivalent to 87.1 % theoretical, which include *EHGY* of 345 g kg⁻¹ wood equivalent to 82.3 %
300 theoretical. Mannose and xylose recovery from the pretreatment hydrolysate was relatively low
301 at approximately 50 and 30 %, respectively. High sodium bisulfite loading of 12 % to facilitate
302 delignification reduced xylan removal to approximately 80%. Recoveries of mannose and
303 xylose from enzymatic hydrolysis were not measured but are expected to increase the overall
304 mannose and xylose recovery. The HMF and furfural concentrations in the pretreatment
305 hydrolysate were relatively low with each at approximately 2.5 g L⁻¹.

306

307 **Conclusions**

308 SPORL can effectively remove the strong recalcitrance of a Douglas-fir forest harvest
309 residue to produce a good sugar yield. The optimal SPORL pretreatment condition was $T =$
310 165°C for 75 min at liquor to solid ratio of 3:1 and sodium bisulfite and sulfuric acid loading of
311 12 % and 2.21 % on dry biomass, respectively. An enzymatic hydrolysis glucose yield of 87%

312 theoretical was achieved at this condition with HMF and furfural concentration each at only 2.5 g
313 L⁻¹. The combined hydrolysis factor (*CHF*) developed using aspen was capable of predicting
314 dissolution of hemicelluloses for the Douglas-fir forest harvest residue. Delignification becomes
315 important in order to achieve high enzymatic cellulose saccharification efficiency for the forest
316 residue due to its high lignin content. Delignification can be correlated to *CHF* for the narrow
317 range of pretreatment conditions investigated. Enzymatic cellulose saccharification can be
318 predicted by *CHF*, which makes *CHF* a good factor for scale-up studies where numerous
319 experiments are economically prohibitive.

320

321 **Acknowledgement**

322 The authors thank the financial support of the Agriculture and Food Research Initiative
323 Competitive grant (No. 2011-68005-30416), USDA National Institute of Food and Agriculture
324 (NIFA) through the Northwest Advanced Renewables Alliance (NARA). The authors also thank
325 Novozymes North America for providing the cellulase enzyme; Fred Matt of USFS-FPL for
326 conducting the chemical composition analysis. Drs. Xuejun Pan and Troy Runge of University of
327 Wisconsin-Madison for allowing us access to their lab analytical equipment. We also would like
328 to acknowledge the photo credit of the forest residue pile (Fig. 1) by Rene Zamora, Graduate
329 Research Assistant, College of Forestry, Oregon State University.

330

References

- 331
- 332 [1] Perlack RD, Stokes BJ, [Leader Authors](#). DOE. 2011. U.S. Billion-Ton Update: Biomass
333 Supply for a Bioenergy and Bioproducts Industry. Oak Ridge (TN): Oakridge National
334 Laboratory, 2011 [August](#). 227 p. [Prepared of the US DOE under](#) Contract No.: DE-AC05-
335 00R22725.
- 336 [2] Kirschbaum MUF. To sink or burn? A discussion of the potential contributions of forests to
337 greenhouse gas balances through storing carbon or providing biofuels. *Biomass Bioenergy*
338 2003;24(4-5):297-310.
- 339 [3] Gan J, Smith CT. Availability of logging residues and potential for electricity production
340 and carbon displacement in the USA. *Biomass Bioenergy* 2006;30(12):1011-20.
- 341 [4] [Committee on Economic and Environmental Impacts of Increasing Biofuels Production](#).
342 Renewable Fuel Standard: Potential Economic and Environmental Effects of US Biofuel
343 Policy. [Washington DC](#): National Academies Press: 2011
344 http://www.nap.edu/catalog.php?record_id=13105
- 345 [5] Stephen JD, Mabee WE, Saddler JN. Biomass logistics as a determinant of second-
346 generation biofuel facility scale, location, and technology selection. *Biofuels Bioprod*
347 *Biorefin* 2010;4:503-18.
- 348 [6] Zhu JY, Zhuang XS. Conceptual net energy output for biofuel production from
349 lignocellulosic biomass through biorefining. *Prog Energy Combust Sci* 2012;38(4):583-98.
- 350 [7] Kim KH, Tucker M, Nguyen Q. Conversion of bark-rich biomass mixture into fermentable
351 sugar by two-stage dilute acid-catalyzed hydrolysis. *Bioresour Technol* 2005;96(11):1249-
352 55.

- 353 [8] Leu SY, Zhu JY. Substrate-related factors affecting enzymatic saccharification of
354 lignocelluloses: Our recent understanding. *Bioenergy Res* 2013;6(2):405-15.
- 355 [9] Iakovlev M, van Heiningen A. Efficient fractionation of spruce by SO₂-ethanol-water
356 treatment: Closed mass balances for carbohydrates and sulfur. *ChemSusChem*
357 2012;5(8):1625-37.
- 358 [10] Kumar L, Chandra R, Saddler JN. Influence of steam pretreatment severity on post-
359 treatments used to enhance the enzymatic hydrolysis of pretreated softwoods at low enzyme
360 loadings. *Biotechnol Bioeng* 2011;108(10):2300-11.
- 361 [11] Monavari S, Bennato A, Galbe M, Zacchi G. Improved one-step steam pretreatment of SO₂-
362 impregnated softwood with time-dependent temperature profile for ethanol production.
363 *Biotechnol Progr* 2010;26(4):1054-60.
- 364 [12] Pan XJ, Xie D, Yu R, Saddler JN. The bioconversion of mountain pine beetle killed
365 lodgepole pine to fuel ethanol using the organosolv process. *Biotechnol Bioeng*
366 2008;101(1):39-48.
- 367 [13] Zhao YL, Wang Y, Zhu JY, Ragauskas A, Deng YL. Enhanced enzymatic hydrolysis of
368 spruce by alkaline pretreatment at low temperature. *Biotechnol Bioeng* 2008;99(6):1320-28.
- 369 [14] Zhu JY, Pan XJ, Wang GS, Gleisner R. Sulfite pretreatment (SPORL) for robust enzymatic
370 saccharification of spruce and red pine. *Bioresour Technol* 2009;100(8):2411-18.
- 371 [15] Tian S, Luo XL, Yang XS, Zhu JY. Robust cellulosic ethanol production from SPORL-
372 pretreated lodgepole pine using an adapted strain *S. cerevisiae* without detoxification.
373 *Bioresour Technol* 2010;101:8678-85.

- 374 [16] Zhu JY, Zhu W, OBryan P, Dien BS, Tian S, Gleisner R, et al. Ethanol Production from
375 SPORL-Pretreated Lodgepole Pine: Preliminary Evaluation of Mass Balance and Process
376 Energy Efficiency. *Appl Microbiol Biotechnol* 2010;86(5):1355-65.
- 377 [17] Lan TQ, Gleisner R, Zhu JY, Dien BS, Hector RE. High titer ethanol production from
378 SPORL-pretreated lodgepole pine by simultaneous enzymatic saccharification and
379 combined fermentation. *Bioresour Technol* 2013;127:291-97.
- 380 [18] Wang ZJ, Lan TQ, Zhu JY. Lignosulfonate and elevated pH can enhance enzymatic
381 accharification of lignocelluloses. *Biotechnol Biofuels* 2013;6:9.
- 382 [19] Lan TQ, Lou H, Zhu JY. Enzymatic saccharification of lignocelluloses should be conducted
383 at elevated pH 5.2 - 6.2. *BioEnergy Res* 2013;6(2):476-85.
- 384 [20] Lou H, Zhu JY, Lan TQ, Lai H, Qiu X. pH-induced lignin surface modification to reduce
385 nonspecific cellulase binding and enhance enzymatic saccharification of lignocelluloses.
386 *ChemSusChem* 2013; 6(5):919-27.
- 387 [21] Zhang C, Zhu JY, Gleisner R, Sessions J. Fractionation of Forest Residues of Douglas-fir
388 for Fermentable Sugar Production by SPORL Pretreatment. *Bioenergy Res* 2012;5(4):978-
389 88.
- 390 [22] Luo X, Gleisner R, Tian S, Negron J, Horn E, Pan XJ, et al. Evaluation of mountain beetle
391 infested lodgepole pine for cellulosic ethanol production by SPORL pretreatment. *Ind Eng*
392 *Chem Res* 2010;49(17):8258-66.
- 393 [23] Zhang C, Houtman CJ, Zhu JY. Using Low Temperature to Balance Enzymatic
394 Saccharification and Furan Formation during SPORL Pretreatment of Douglas-fir. *AIChE J*
395 2013 (submitted).

- 396 [24] Chum HL, Johnson DK, Black SK, Overend RP. Pretreatment-catalyst effects of the
397 combined severity parameter. *Appl Biochem Biotechnol* 1990;24/25:1-14.
- 398 [25] Abatzoglou N, Chornet E, Belkacemi K, Overend RP. Phenomenological kinetics of
399 complex systems: the development of a generalized severity parameter and its application to
400 lignocellulosics fractionation. *Chem Eng Sci* 1992;47(5):1109-22.
- 401 [26] Zhu W, Houtman CJ, Zhu JY, Gleisner R, Chen KF. Quantitative predictions of
402 bioconversion of aspen by dilute acid and SPORL pretreatments using a unified combined
403 hydrolysis factor (CHF). *Process Biochem* 2012;47:785-91.
- 404 [27] Wang QQ, He Z, Zhu Z, Zhang Y-HP, Ni Y, Luo XL, et al. Evaluations of cellulose
405 accessibilities of lignocelluloses by solute exclusion and protein adsorption techniques.
406 *Biotechnol Bioeng* 2012;109(2):381-89.
- 407 [28] Clark TA, Mackie KL, Dare PH, McDonald AG. Steam explosion of the softwood *Pinus*
408 *radiata* with sulphur dioxide addition. II. Process characterization. *J Wood Chem Technol*
409 1989;9(2):135-66.

410

411

412

413

414 **List of Figures**

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

416 Fig. 2 Images of different fractions and hammer-milled FS-03. (a) $I < 3.2$ mm; (b) $6.4 \leq IV <$
417 9.5 ; (c) $19.1 \leq VIII < 22.2$; (d) hammer-milled FS-03

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

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

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

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

427 Fig. 7 Overall mass balance of the optimal run at $T = 165^{\circ}\text{C}$, $t = 75$ minutes, $B = 12$ %, and $A =$
428 2.2 % with liquid to solid ratio of 3:1. All numbers are expressed in gram.

429

430

431 **List of Tables:**

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

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

434

435

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

Run No.	Run Label ¹	Pretreatment Condition ²			Initial pH	CHF ³
		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

¹ *t*_{xx} is pretreatment duration in min; *A*_{xx} is sulfuric acid loading in cm³ in 1000 cm³ solution; *B*_{xx} is sodium bisulfite charge on wood (oven dry weight) in mass fraction %; *R*_{xx} is replicate number for the specified set of condition.

² All pretreatments were conducted at 165°C with water to solids mass ratio = 3:1

³ CHF = Combined hydrolysis factor (Eq. (1))

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

Figure 1
[Click here to download high resolution image](#)



Figure 2
[Click here to download high resolution image](#)

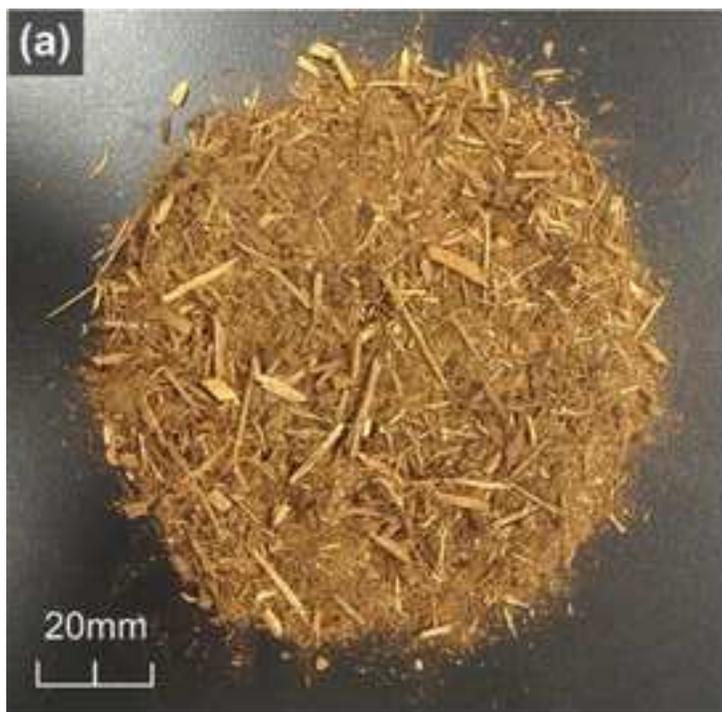


Figure 3
[Click here to download high resolution image](#)

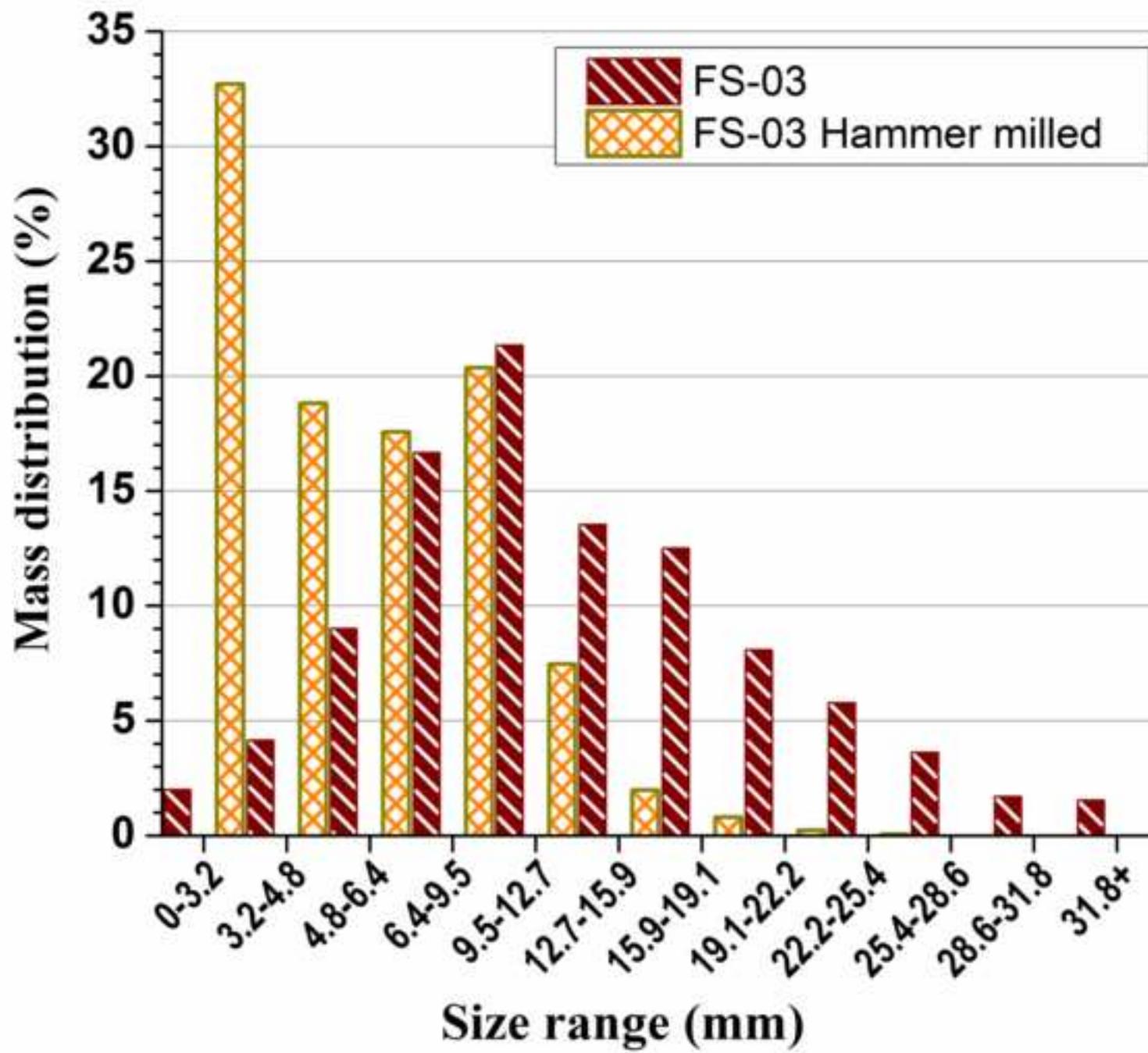


Figure 4a

[Click here to download high resolution image](#)

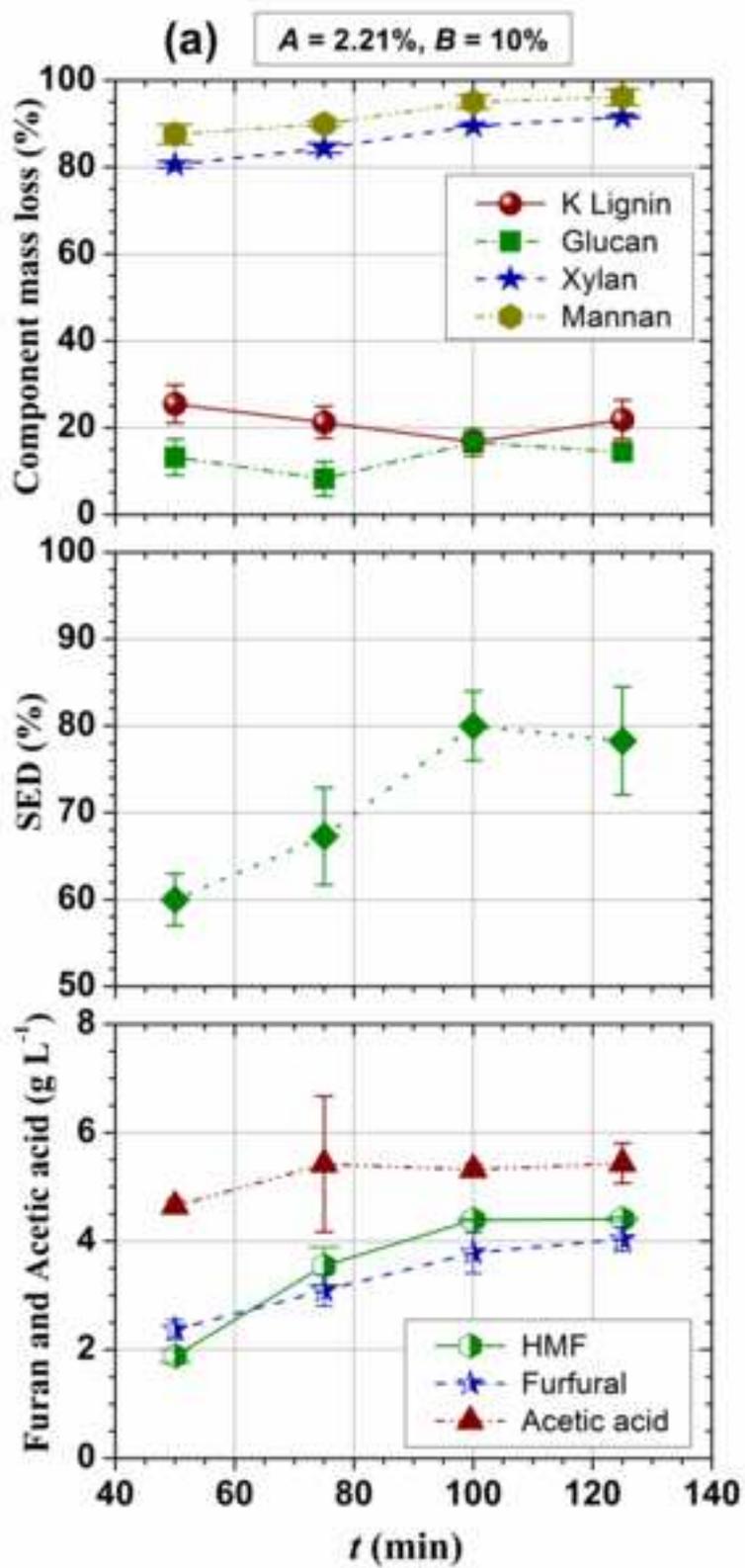


Figure 4b

[Click here to download high resolution image](#)

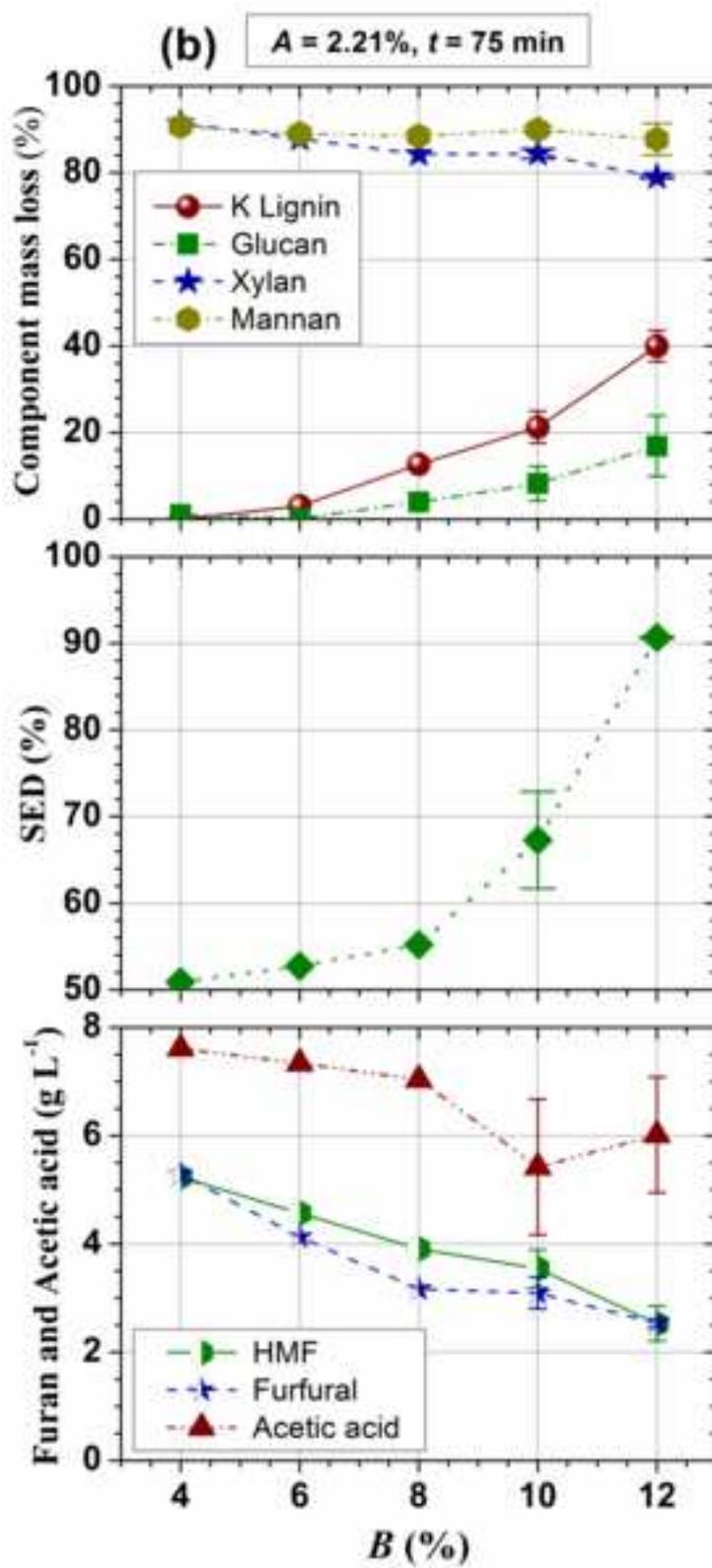


Figure 4c

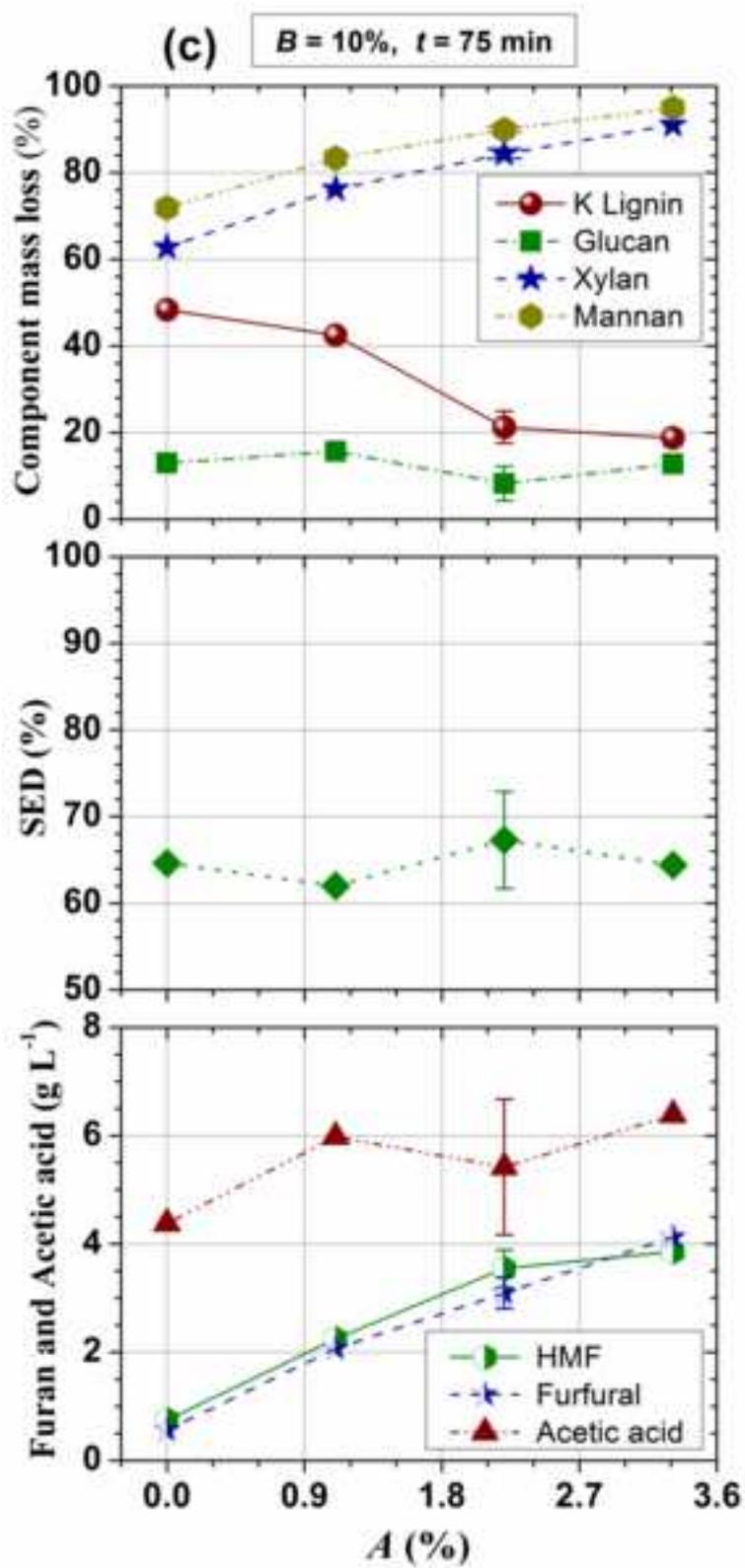
[Click here to download high resolution image](#)

Figure 5

[Click here to download high resolution image](#)

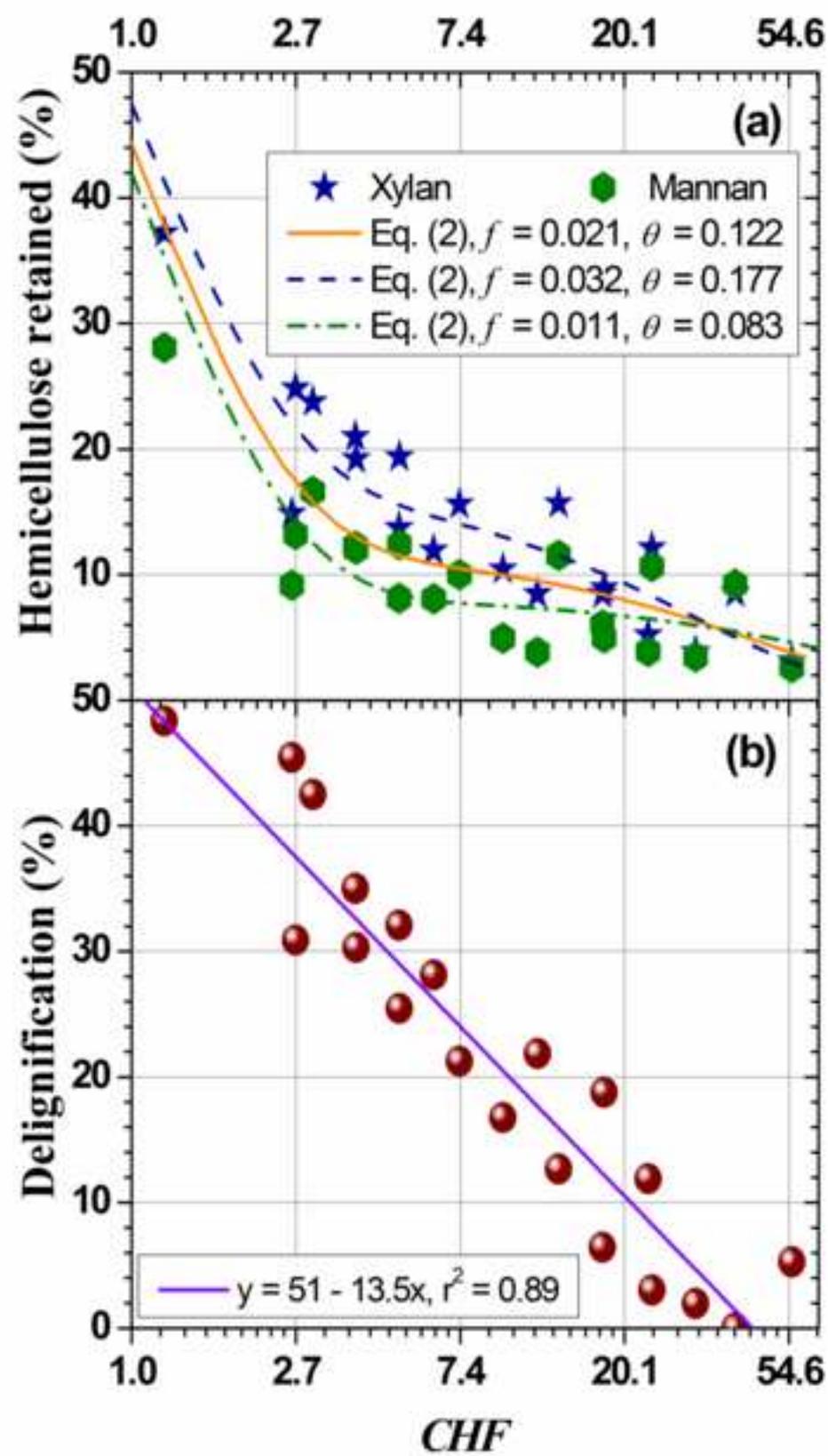


Figure 6

[Click here to download high resolution image](#)

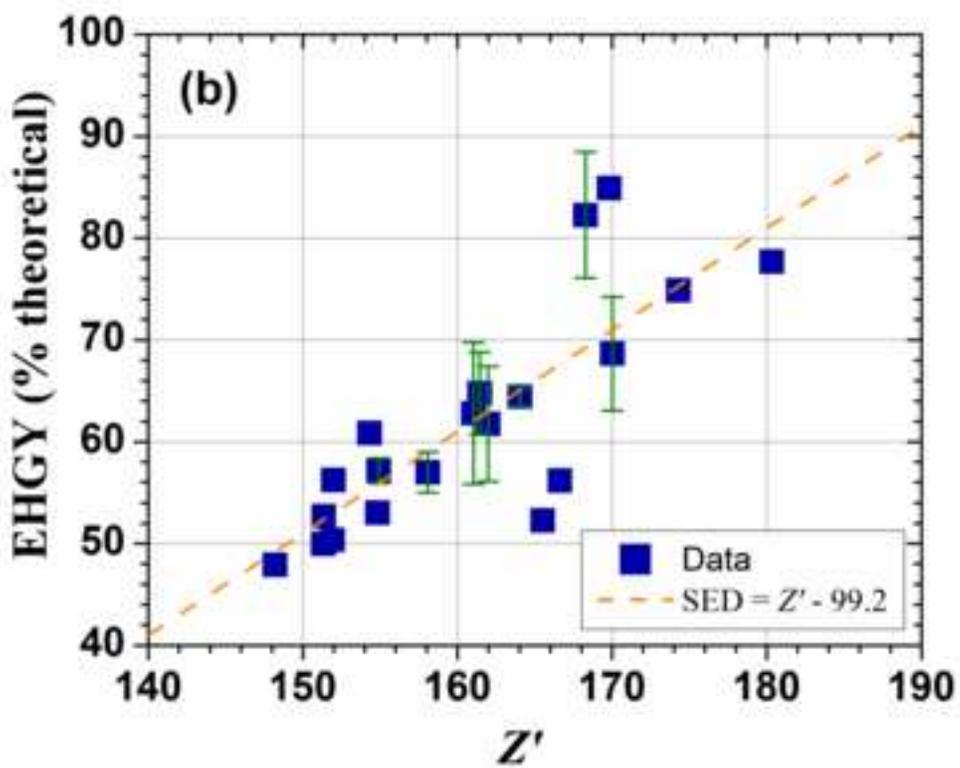
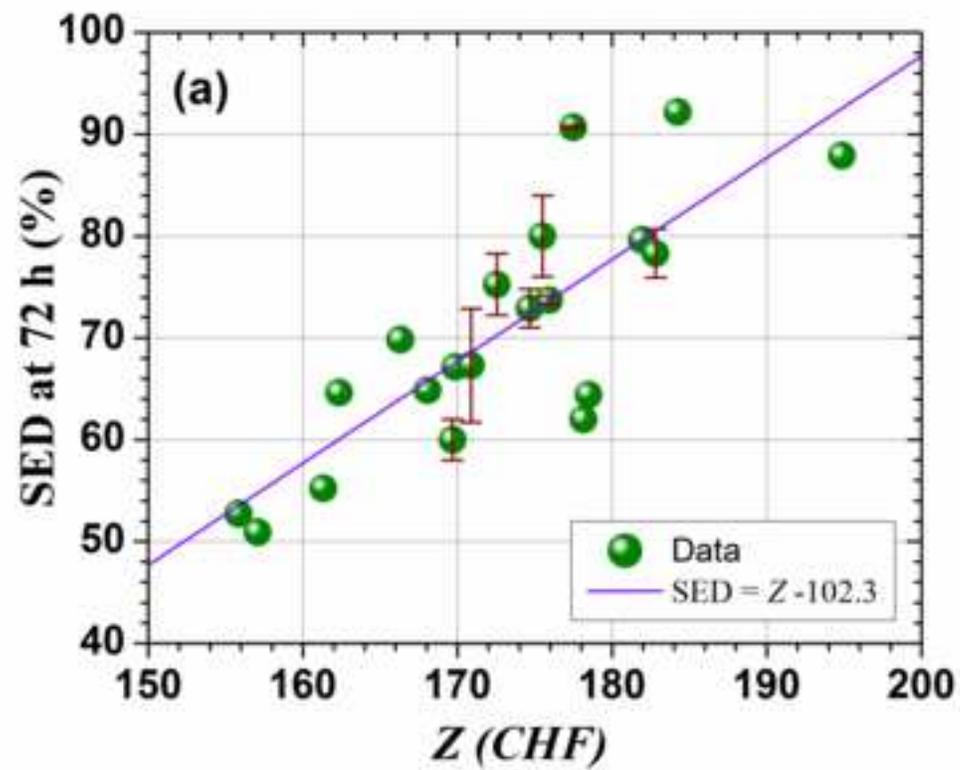


Figure 7
[Click here to download high resolution image](#)

