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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 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.

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Keywords: forest harvest residue, pretreatment, enzymatic hydrolysis/saccharification,

biofuel, pretreatment severity

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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 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 Recalcitrant of Lignocelluloses (SPORL), though a relatively new process [14], demonstrated 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.

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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 (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

105   cocktail CTec2 was kindly provided by the Novozymes North America (Franklinton, NC). The

106   CTec2 activity was 150 FPU cm^{-3}.

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 ºC 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   165ºC 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±3°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    $$CHF = e^{\left(\alpha - \frac{E}{RT} + \beta\, C_A + \gamma\, C_B\right)}(C_A + C_B)t \qquad (1)$$

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

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

Results and discussion

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 ±3% (Fig. 5a).

251   Delignification was found to be inversely proportional to *CHF* and can be predicted to

252 within ±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 165ºC. 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

12

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          $Z = L \times$ Delignification $+ H \times$ Hemicellulose removal $+ G \times$ Glucan loss   (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   *Z(CHF) = 0.934 ×* Delignification *+ 1.725 ×* Hemicellulose removal   (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     $Z' = 0.943\times$ Delignification $+ 1.675 \times$ Hemicellulose removal $- 0.577 \times$ 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 hydroysate 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.

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Acknowledgement

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Table 1

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; 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.

[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

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

Figure 2
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Figure 3
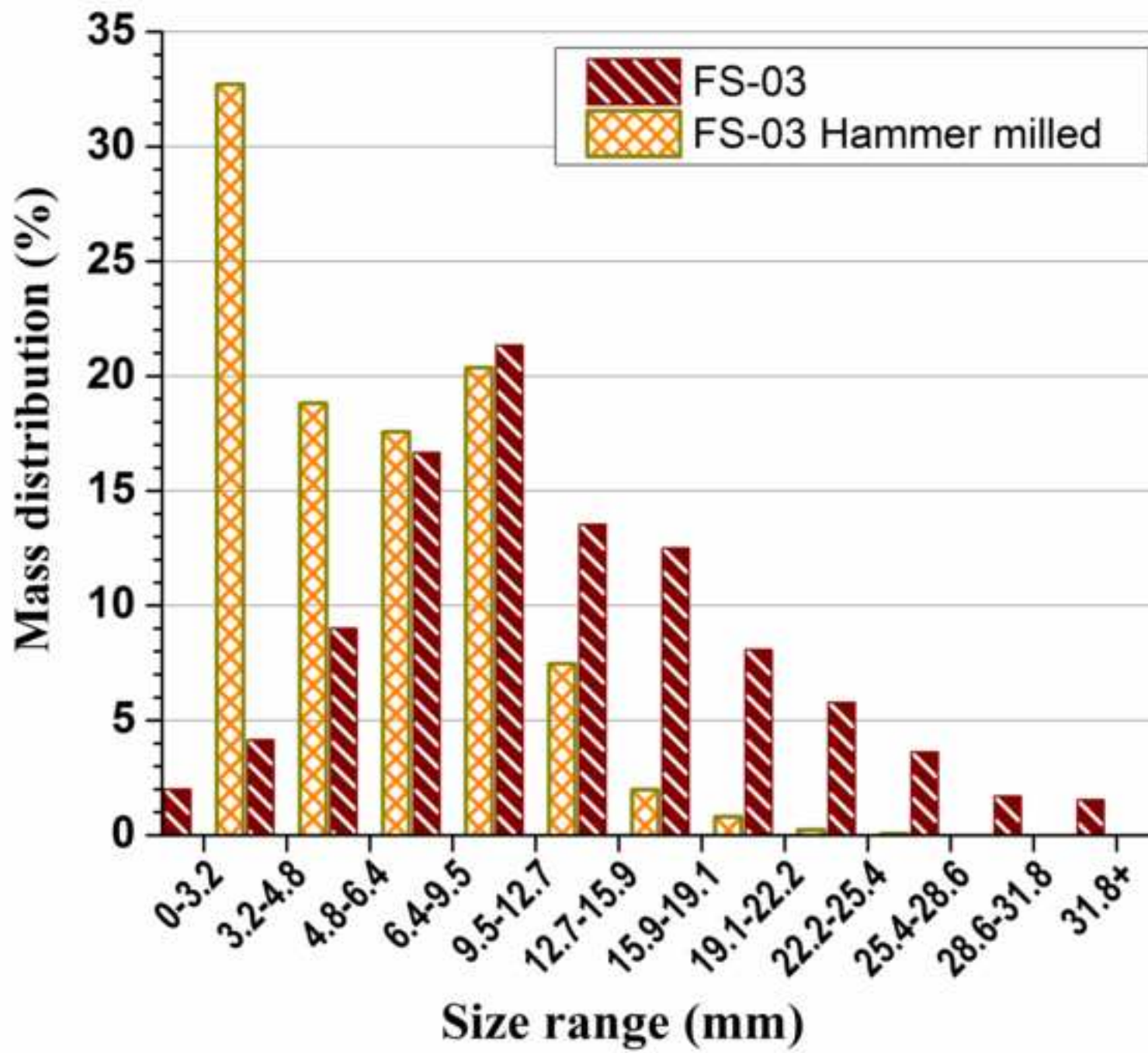
Figure 4a

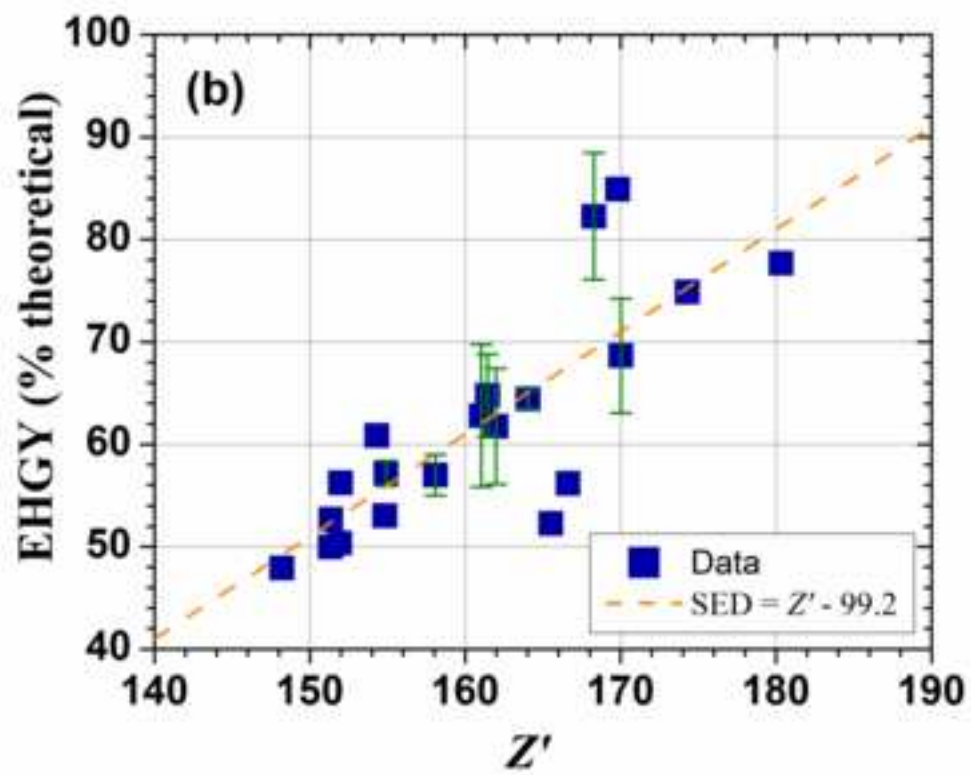(b) $A = 2.21\%$, $t = 75$ min

(c) B = 10%, t = 75 min
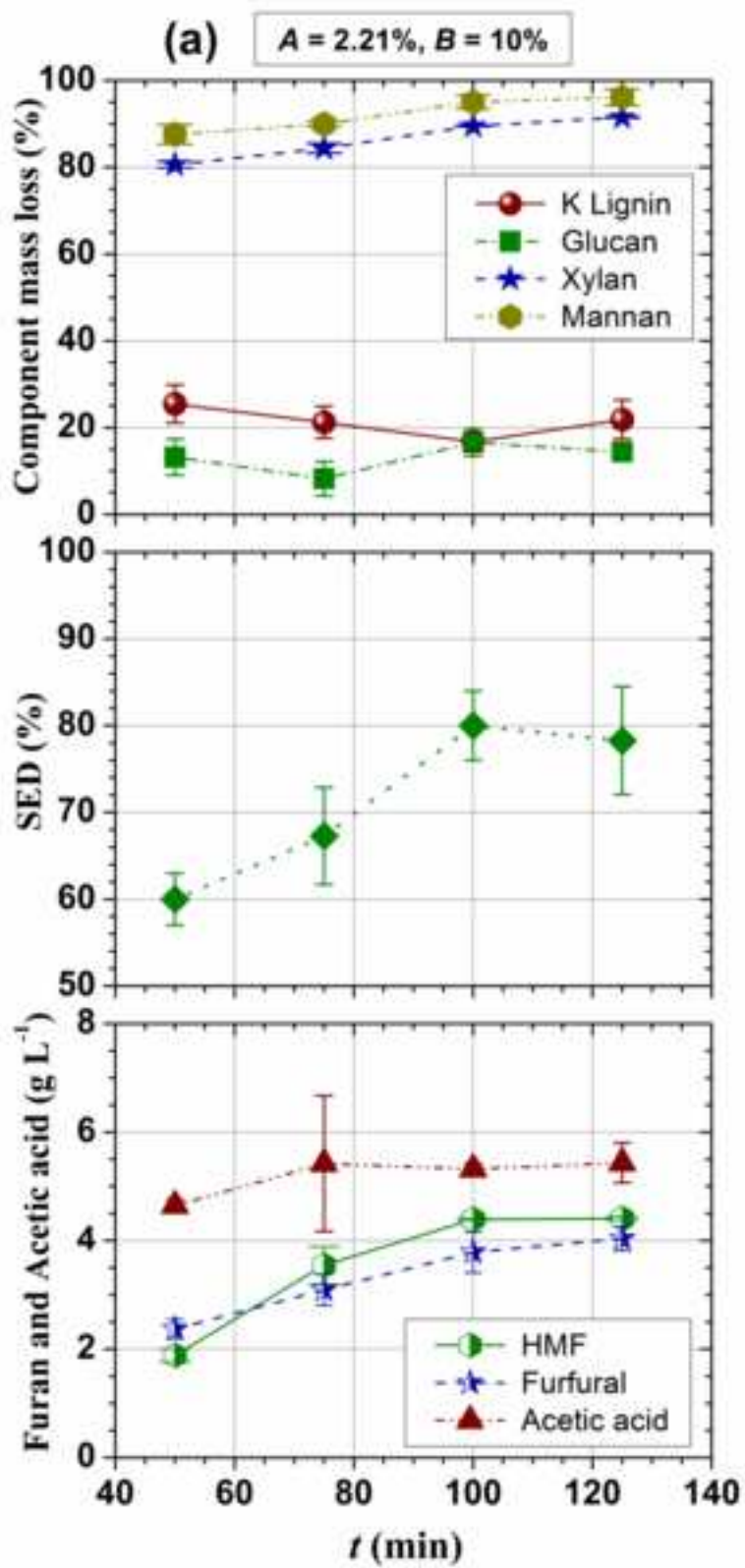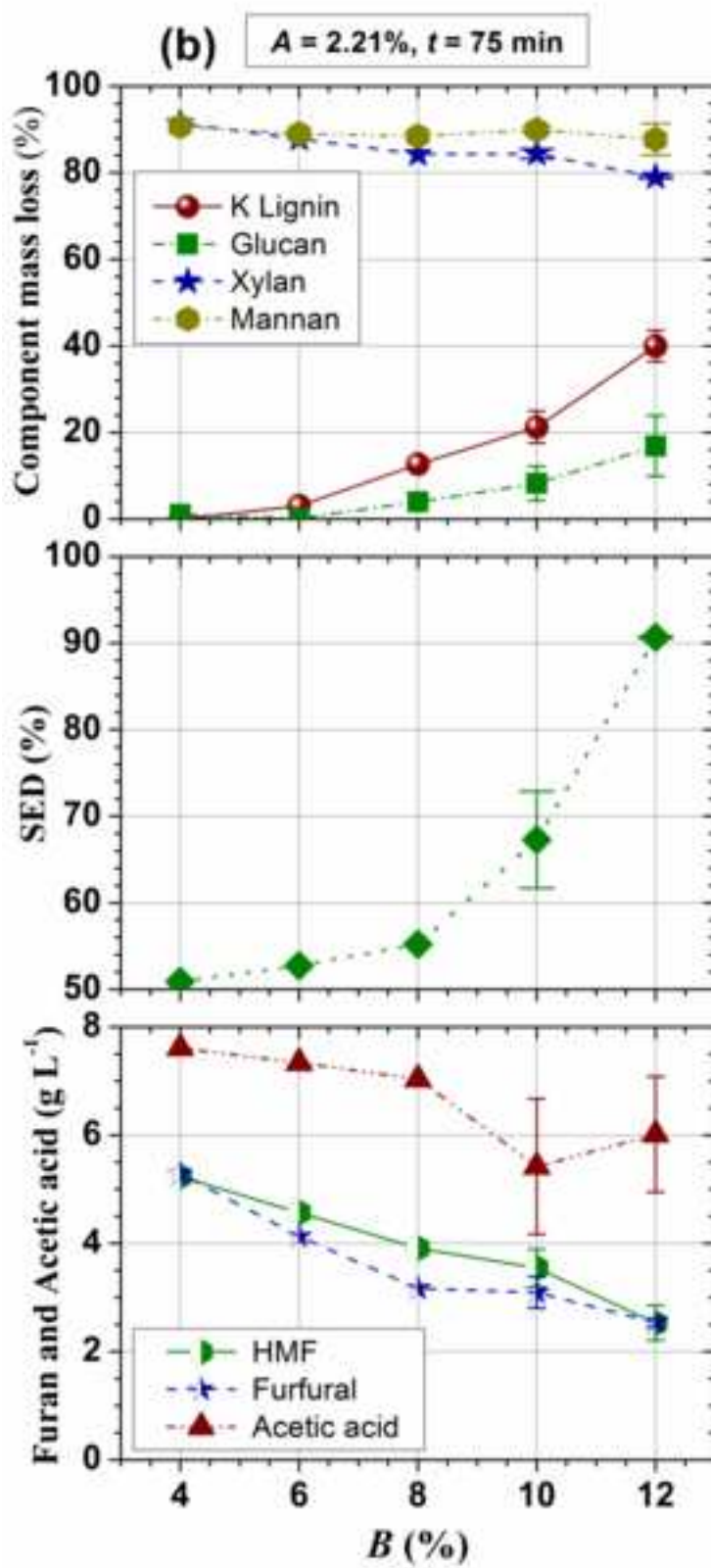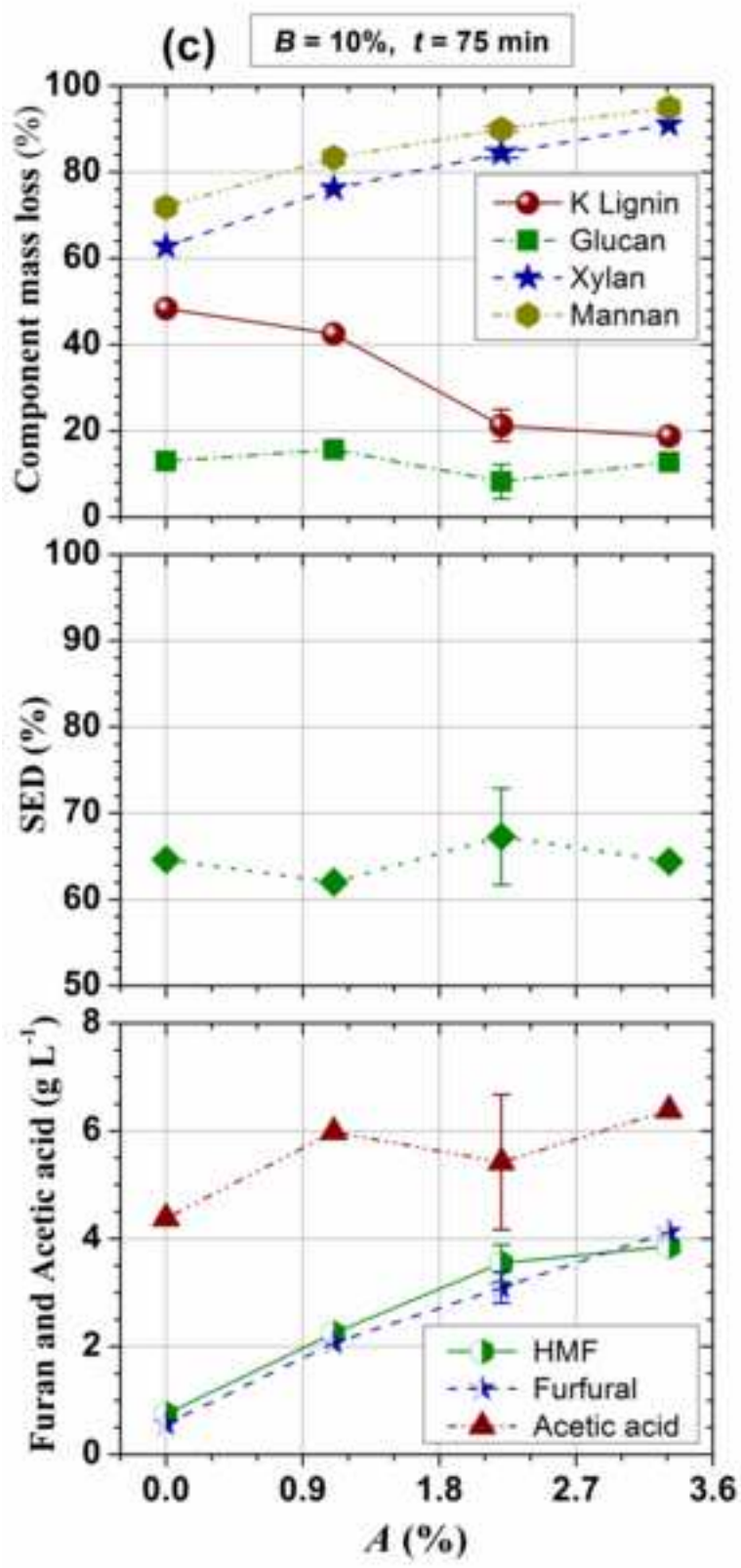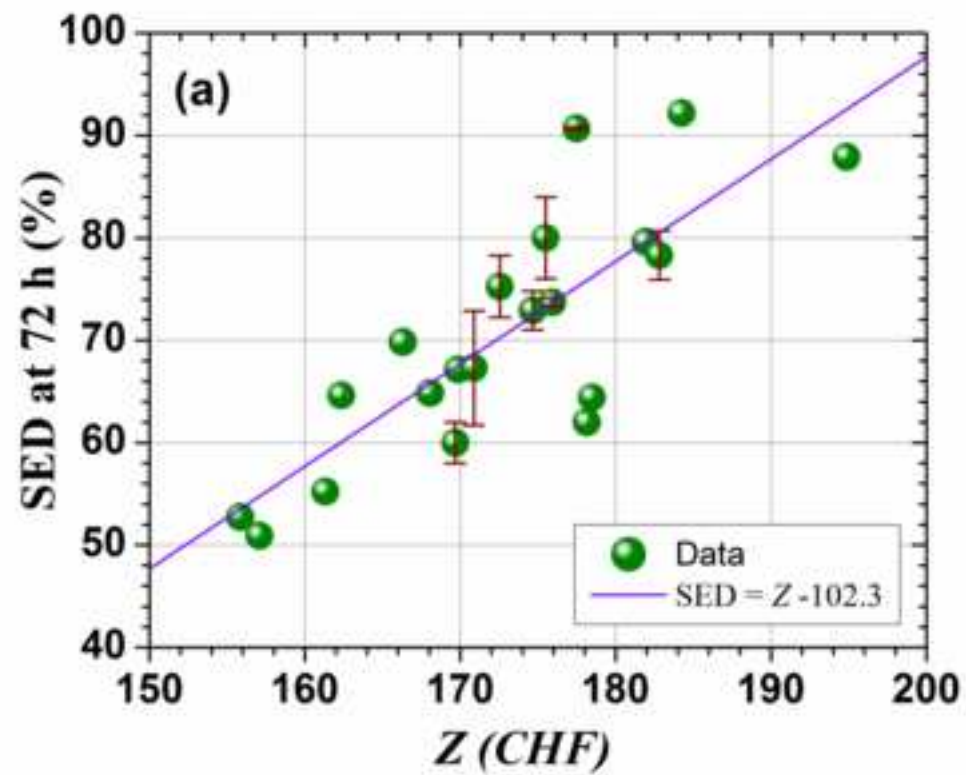
Figure 5
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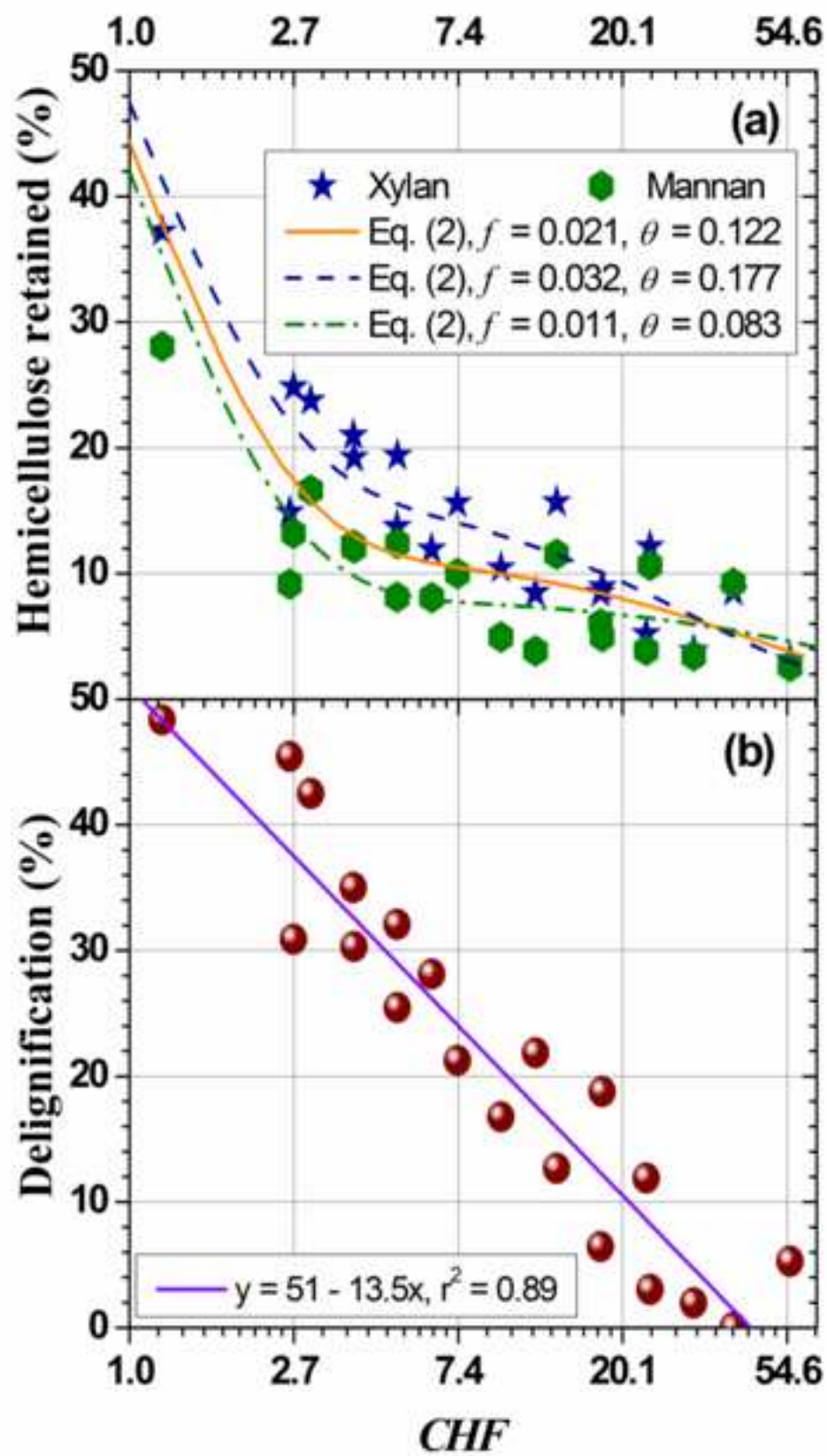
Figure 6

Figure 7
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Figure 7