Size Effects on Acid Bisulfite Pretreatment Efficiency: Multiple Product Yields in Spent Liquor and Enzymatic Digestibility of Pretreated Solids

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ABSTRACT: Currently, feedstock size effects on chemical pretreatment performance were not clear due to the complexity of the pretreatment process and multiple evaluation standards such as the sugar recovery in spent liquor or enzymatic digestibility. In this study, we evaluated the size effects by various ways: the sugar recovery and coproduct yields in spent liquor, the chemical and thermal properties, and enzymatic digestibility of the pretreated solids. Four initial particle sizes were employed and characterized with the geometric mean diameters, which were 10.40, 2.25, 1.49, and 0.84 mm. The results showed that a decrease in the particle size improved the hydrolysis of hemicellulose and cellulose in acid bisulfite pretreatment. Higher concentrations of hydroxymethylfurfural and furfural were produced in the pretreatment of the 0.84 and 10.40 mm particles. The maximum total sugar yield of 72% was achieved with a 2.25 mm particle size at 145 °C.

KEYWORDS: Douglas-fir, Lignosulfonates, Compositional analysis, Crystallinity, Lignin condensation

INTRODUCTION

Oil security and environmental issues have increasingly raised interests in exploring the potential of lignocellulosic materials to produce liquid fuel substitutes. Pretreatment is a necessary step to overcome the recalcitrance of converting lignocellulosic biomass to fermentable sugars.1 The lignocellulosic biomass is comprised of crystalline cellulose, amorphous hemicellulose, and lignin. The removal of lignin and hemicellulose can improve the accessibility of cellulose to enzymes. The lignin and hemicellulose removal process is usually accompanied by the degradation of cellulose.2–6 Understanding the change of these three components due to pretreatment process is of significant importance.

Currently, there are various pretreatment methods for biomass conversion.7,8 Different biomass types (herbaceous and woody) favor different pretreatment methods.9,10 Herbaceous biomass can be adequately pretreated at severities lower than those of woody biomass when using the diluted acid pretreatment process.11,12 Three pretreatment methods, i.e., steam explosion, ionic liquid, and sulfite treatment, were shown to achieve adequate sugar yield with woody biomass.8,13–15

Kim and Lee16 proposed a sulfuric acid diffusion model to explain the relationship between the particle size of the feedstock and chemical reaction rate of the pretreatment. The initial particle size can influence the chemical reaction rate and product profile only when the reactions occur above a specific temperature. Therefore, temperature and particle size effects on pretreatment performance are dependent. Wolfrum17 found that temperature influenced detecting enzymatic digestibility differences. As a matter of size effects, Ballesteros et al.18 evaluated the effect of three hammer-milled particle size fractions of softwood (2–5, 5–8, and 8–12 mm) on the sugar recovery following the steam explosion at 190 and 210 °C for 4 and 8 min. They found that different treatment temperatures favored different particle sizes. Medium size particles (5–8 mm) achieved the highest overall yield of fermentable sugars from the pretreatment at 190 °C, while the large size particles (8–12 mm) favored 210 °C. In contrast, another study19 assessed the influences of two different particle sizes (2–5 and 12–15 mm) on the liquid hot water and steam explosion pretreatments at various temperatures. No significant effects of particle size on overall sugar and ethanol yield were reported.

In addition, the size effects on pretreatment performance varied when employing different types of pulp chip sizes and treatment methods.20 The effect of chip size on sulfur dioxide...
(SO₂) assisted steam explosion (195 °C, 4.5 min) of Douglas-fir (Pseudotsuga menziesii) was evaluated. Results showed that large chips (5 × 5 cm) resulted in maximum sugar yield and hydrolysis rate. Moreover, a higher concentration of furan was formed using fine particle sizes. Monavari also investigated chip thickness (1–2, 3–4, and 5–6 mm in thickness) effects on the SO₂ assisted steam explosion treatment (200 °C, 5 min) of spruce (Picea abies). Overall sugar yield was found to be higher at a chip thickness of 1–2 mm.

The ionic liquid method had also been used for woody biomass. Douglas-fir and forestry residues were treated by 1-ethyl-3-methylimidazolium acetate (EMIMAc) with two particle sizes (unmilled pulp chip and 2 mm Wiley-milled) at 120 and 160 °C. Unmilled Douglas-fir chips obtained higher glucose yield at both 120 and 160 °C, while 2 mm Wiley-milled forest residuals obtained higher yield at 160 °C. Results indicated no statistically significant increase in sugar yield from Wiley-milled particles. Another investigation found that different types of ionic liquid favor different particle sizes during pretreatment.

Additionally, the acid bisulfite method was found to be an efficient method for softwood pretreatment. Previous bisulfite pretreatment investigations have exclusively used pulp chip with thickness of 2–8 mm as the feedstock. Investigating particle size effects on the sulfite treatment can provide additional insights for improving the performance of this pretreatment to increase total sugar recovery.

The objective of this study is to investigate particle size effects on acid bisulfite pretreatment performance. The pretreatment temperature was included in this study to evaluate the optimal condition for identifying particle size effects. To evaluate the pretreatment efficiency under various particle sizes, the sugar recovery in spent liquor, coproducts yields, the compositional analysis of pretreated solids, and total sugar yields from both pretreatment and enzymatic hydrolysis were analyzed.

## MATERIALS AND METHODS

### Material

Douglas-fir wood chips were acquired from a local sawmill (Vaagen Brothers Lumber Inc. located at Colville, Washington). The initial moisture content of chips was 51% (wet mass basis). The received chips were screened into three portions by a screening system at a chip thickness of 1 mm. Large chips (5 × 5 cm) were selected for each formulation. The treatment time (i.e., time at the target temperature following the heating ramp) was fixed at 3 h. In total, there are 12 formulations based on temperature and particle size. Two replicates were performed for each formulation.

A Parr reactor (Parr Bench Top Reactor, 1 L, series No. 4520) was used to carry out the pretreatments. For each, 60 g (oven-dried) hammer-milled wood particles were used for each batch, and 50 g (oven-dried) wood chips were used due to low bulk density. It required 25–45 min to reach the target temperature (135–155 °C) and 15 min to cool to 80 °C. The stirring system was initiated when the temperature reached the set point. After pretreatment, the slurry was separated by vacuum filtration into solid and spent liquor fractions. The pretreated solids were rinsed with 2 L of distilled water to remove entrained soluble sugars. The Parr reactor was also thoroughly washed to collect all the solids in the system. The spent liquor was collected and stored in −20 °C freezer, whereas the washed pretreated solids were stored in 4 °C refrigerator. The wet mass and moisture content of the washed solids were measured to calculate the solid recovery of the pretreatment process.

### Spent Liquor Analysis

The spent liquor was analyzed for sugar, furfural, hydroxymethylfurfural (HMF), acetic acid, and lignosulfonates (LS) contents. The monomeric and oligomeric sugars in the spent liquor were measured according to the NREL standard.

### Pretreated Solid Analysis

The samples for compositional analysis were prepared according to the NREL standard. Two-step acid hydrolysis was carried out based on the NREL standard. Monomeric sugars were measured by high-performance liquid chromatography (HPLC) system (Waters, Milford, MA) equipped with a differential refractive index detector (ERC-5710, ERMA) and a Rezex ROA organic acid column (7.8 mm × 300 mm, Phenomenex, Torrance, CA) eluted with 0.005 N aqueous H₂SO₄ (0.5 mL/min) at 65 °C. LSS was measured with a UV–visible spectrophotometer (PerkinElmer Lambda 35, PerkinElmer, Inc., Waltham, MA) according to the absorbance at the wavelength of 232.5 nm.

### Pretreated Solid Analysis

The samples for compositional analysis were prepared according to the NREL standard. Two-step acid hydrolysis was carried out based on the NREL standard. Monomeric sugars were measured by high-performance liquid chromatography (Dionex ICS-3000). The xylose and mannose cannot be differentiated by this equipment. Therefore, the composition for xylose and xylan are presented together in this paper and is not deemed to be a limitation because xylen content of Douglas-fir is typically in the range of 2–5%. The acid-soluble lignin (ASL) was measured by the UV–visible spectrophotometer.

### Fourier Transform Infrared (FTIR) Analysis

The samples for FTIR analysis were prepared by drying in a convection oven at 45 °C for 24 h and then milled by a Wiley mill. FTIR spectra were carried out with a Thermo Nicolet Avatar 370 spectrometer operating in the attenuated total reflection (ATR) mode (Smart Performance, ZnSe crystal). FTIR results were used to analyze chemical group difference between different treatment conditions.

### X-ray Diffraction (XRD) Analysis

XRD analysis was done. Untreated and pretreated samples were conducted with an X-ray diffractometer (Miniflex 600, Rigaku, Japan) operated at 40 V and 15 mA with Ni-filtered Cu Kα radiation. The untreated samples were dried at 45 °C for 24 h and then milled by Wiley mill with a 60 mesh screen. The samples were scanned with the 2θ range of 10°–40° at a scan speed of 2°/min. Crystallinity index (Cri) (%) of each sample was analyzed using the following equation:

\[
\text{Cri} = 100 \times \frac{I_{002} - I_{am}}{I_{002}}
\]

where \(I_{002}\) is the intensity of the 002 peak at 2θ = 22.4° and \(I_{am}\) is the intensity of the background scatter at 2θ = 18.5°, which is the characteristic peak of cellulose-I. The crystalline structure modification
was analyzed with XRD, and the acid treatment increased the CrI through hemicellulose removal.

**Thermal Gravimetric Analysis (TGA) Analysis.** TGA was performed by a thermal analyzer (SDT Q600, TA Instruments, New Castle, DE) under a nitrogen atmosphere with a purge gas flow of 100 mL/min. The samples were oven-dried and milled with Wiley mill to achieve the proper particle size used elsewhere. A wood sample of 6–10 mg was heated from room temperature to 600 °C at a heating rate of 10 °C/min. Duplicates were tested for each sample.

**Enzymatic Hydrolysis.** The pretreated solids from the acid bisulfitone pretreatment were milled for 10 min with a planetary ball mill (Across International, PQ-N04, two 100 mL steel jars, 100 steel balls with a diameter of 6 mm and 16 steel balls with a diameter of 10 mm in each jar). Cellic CTec2 and HTec2 were complimentarily provided by Novozymes North America (Franklinton, NC). The milled pretreated solids were hydrolyzed for 72 h with a 4% (w/w) CTec2 and 0.4% (w/w) HTec2 (based on oven-dry wood basis) enzyme product loading. The solid loading was 2% (w/v) of a total 50 mL volume. One percent (w/w) sodium azide was used for antibacterial function in hydrolysis slurry. The supernatant was collected and diluted 500-fold for the analysis of the monomer sugars. Total sugar yield was computed as the percentage of the theoretical yield available in the raw material.

**RESULTS AND DISCUSSION**

**Spent Liquor Analysis.** Table 1 shows the concentrations of soluble sugars, degradation products (furfural, HMF, and acetic acid), and LS in the spent liquor. The yields of the sugar degradation products increased with an increase in the pretreatment temperature. It is reasonable to conclude that the increase in sugar degradation products accounted for the darkening of the color of the spent liquor. However, the particle size did not have a commensurate influence on degradation product formation. At 135 °C, no substantial difference of concentrations for the degradation products was found between particle sizes, while at 145 and 155 °C, smaller particle size resulted in a higher concentration of degradation products. This may due to the rapid hydrolysis of polymeric sugars into monomeric sugars and the subsequent monomeric sugar degradation into furfural or HMF. The concentration of LS also increased with a decrease in particle sizes with an exception of the smallest particle size of 0.84 mm under 145 and 155 °C.

The glucan and xylan/mannan recovery in the spent liquor are shown in Figure 1. Increasing temperature and decreasing particle size increased the glucan yield in the spent liquor, especially at 145 °C. However, at 135 °C, no obvious particle size influence was found. The glucan yield dropped when particle size was smaller than 1.49 mm at 155 °C. This can be explained by increasing HMF content, which resulted in lower glucose content. Initial particle size did not have a significant effect on xylose/mannose yield in spent liquor. The yield of xylose/mannose at 145 °C was 5–10% higher than the yield at 135 °C and then decreased to 65% at 155 °C. This can be attributed to the degradation of monomer sugars into furfural and HMF at high temperature.

**Solid Recovery and Compositional Analysis.** As shown in Table 2, solid recovery decreases along with an increase in treatment temperature and a decrease in initial particle size. This tendency is consistent with Socha’s study with ionic liquid pretreatment. Table 2 also shows the compositional analysis of untreated and pretreated solids. The glucan content initially increased and then decreased with a decrease in particle size, while the content of ASL continued to decrease. This can be explained by hemicellulose removal, lignin dissolution in spent liquor, and minor cellulose hydrolysis. The content of acid-insoluble lignin (AIL) had a sudden rise at a particle size of 0.84 mm under 145 and 155 °C. The potential reason for this is lignin condensation at thermochemical conditions. The yields of LS also supported this assumption.

**FTIR Analysis of Pretreated Solids.** Figure 2 shows a comparison of untreated and pretreated solids with various particle sizes at 135 °C. The band 1733 cm$^{-1}$ was assigned to...
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Table 2. Compositional Analysis of the Pretreated Solids (w/w % of the Pretreated Sample)

<table>
<thead>
<tr>
<th>temperature (°C)</th>
<th>size (mm)</th>
<th>CrI (%)</th>
<th>arabinan (%)</th>
<th>galactan (%)</th>
<th>glucan (%)</th>
<th>xyl/mann (%)</th>
<th>ASL (%)</th>
<th>AIL (%)</th>
<th>total (%)</th>
<th>solid recovery (%)</th>
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<td>1.2</td>
<td>2.8</td>
<td>43.3</td>
<td>15.2</td>
<td>2.0</td>
<td>29.7</td>
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<td>135</td>
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<td>49</td>
<td>0.2</td>
<td>0.6</td>
<td>50.5</td>
<td>4.8</td>
<td>5.3</td>
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<td>5.3</td>
<td>3.8</td>
<td>32.3</td>
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<td>93.2</td>
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</tr>
<tr>
<td>145</td>
<td>0.84</td>
<td>61</td>
<td>0.0</td>
<td>0.0</td>
<td>54.8</td>
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<tr>
<td>155</td>
<td>0.84</td>
<td>61</td>
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<td>0.0</td>
<td>51.0</td>
<td>0.8</td>
<td>3.1</td>
<td>40.2</td>
<td>95.2</td>
<td>66.8</td>
</tr>
</tbody>
</table>

Figure 2. FTIR spectra of pretreated solids under (a) 135 °C and (b) 145 °C with a range of 1800–800 cm⁻¹.

carbonyl groups, which mainly derived from acetyl groups in hemicelluloses or lignin. 33,39 This band disappeared after pretreatment without obvious difference among the initial particle sizes, indicating the acetyl groups were rapidly removed regardless of the different particle sizes.

The bands 1509 and 1603 cm⁻¹ are attributed to aromatic skeletal vibration in lignin, while the band at 1267 cm⁻¹ is the C–O stretch from guaiacyl unit of lignin (G-unit). Also, the band 1106 cm⁻¹ is from the aromatic skeletal vibration and C–O stretch in lignin. The band 1055 cm⁻¹ is attributed to C–O vibration in the carbohydrates. 39–41 As shown in Figure 2, no significant difference was found in the spectra of the characteristic band 1509 cm⁻¹ of lignin among various initial particle sizes. The bands 1106 and 1055 cm⁻¹ show difference among different particle sizes at the temperature of 145 and 155 °C.

XRD Analysis of Pretreated Solid. A previous study showed the acid pretreatment slightly increased the crystallinity of lignocellulosic materials. 35 Table 2 shows that the untreated sample had a CrI of 48%, which increased after pretreatment. At 135 °C, the CrI of the pretreated solids was essentially unchanged among initial particle size. When treated at 145 and 155 °C, the CrI increased with a decrease in initial particle size compared to untreated materials. However, the CrI change after acid bisulfite pretreatment among different particle sizes was not dramatic.

TGA Analysis. Thermal decomposition of the untreated Douglas-fir wood powders revealed two main mass loss activities at a temperature range of 250–450 °C, which can be attributed to hemicelluloses, lignin, and cellulose degradation. 21 Figure 3a shows a shoulder peak at 303 °C that exists only for the chip size of 10.8 mm, indicating the presence of residual hemicelluloses in the sample after pretreatment. As shown in Figure 3b, the maximum peaks of three pretreated samples with a particle size of 10.40 mm shift from 366 °C (the untreated sample) to 334 °C (the 155 °C pretreated sample). It can be concluded that the high treatment temperature decreased the thermal stability of Douglas-fir wood materials. The decrease in thermal stability due to lignin and hemicellulose removal is beneficial for the enzyme digestibility. However, the cellulose also degraded during the pretreated process, which laid negative effects on total sugar recovery.

Total Sugar Yield. Table 3 shows the maximum sugar yield of 72% was achieved under 145 °C with the particle size of 2.25 mm. The total sugar yield also reached maximum at the particle size of 2.25 mm under 135 °C, whereas the particle size of 1.49 mm is optimal at 155 °C. Achieving an optimal total sugar yield is a complex endeavor that includes balancing sugar degradation in the pretreatment process and efficiency of enzymatic hydrolysis. The higher treatment temperature of 155 °C resulted in overall lower total sugar yield due to a large amount of monomeric sugar degradation in the pretreatment process. Therefore, investigating the pretreatment process in terms of monomeric sugar production and degradation is of significance for total sugar optimization.

CONCLUSION

A decrease in initial particle size and an increase in temperature were beneficial to the hydrolysis of hemicellulose and cellulose. Higher concentrations of furfural and HMF were produced in the spent liquors in acid bisulfite pretreatment at the particle sizes of 0.84 and 10.40 mm. The maximum total sugar yield of
72% was achieved at a particle size of 2.25 mm under 145 °C. This study is meaningful in identifying size effects and exploring optimal particle size for multiple products.

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**Notes**  
The authors declare no competing financial interest.

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