

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

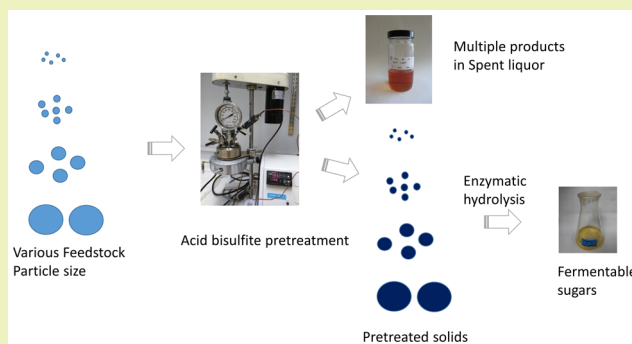
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**ABSTRACT:** Currently, feedstock size effects on chemical pretreatment performance were not clear due to the complexity of the pretreatment process and multiple evaluation standards such as the sugar recovery in spent liquor or enzymatic digestibility. 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.<sup>1</sup> 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.<sup>2–6</sup> Understanding the change of these three components due to pretreatment process is of significant importance.

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

Kim and Lee<sup>16</sup> 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. Wolfrum<sup>17</sup> found that temperature influenced detecting enzymatic digestibility differences. As a matter of size effects, Ballesteros et al.<sup>18</sup> 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 study<sup>19</sup> 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.<sup>20</sup> The effect of chip size on sulfur dioxide

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(SO<sub>2</sub>) assisted steam explosion (195 °C, 4.5 min) of Douglas-fir (*Pseudotsuga menziesii*) was evaluated.<sup>21</sup> 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<sup>22</sup> also investigated chip thickness (1–2, 3–4, and 5–6 mm in thickness) effects on the SO<sub>2</sub> 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.<sup>14</sup> 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.<sup>23</sup>

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.<sup>12,15,24,25</sup> 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 gyratory screen (Model 580, Serial 4095-76, Black Clawson, Everett, WA) with the accepted chips passing a 25.4 mm top deck and retained on a 4.75 mm bottom deck. The accepted chips were air-dried at ambient temperature to a moisture content of approximately 13% and subsequently stored in an environmental moisture conditioner room (at a moisture level of 11%) before use. A hammer mill (Prater, 14.9 kW) was used to grind the accepted chips with three screen sizes (11.11, 6.35, and 1.59 mm).<sup>26</sup> ASABE S318.4 was used to compute the geometric mean diameter of the accepted chips and three types of hammer milled particles,<sup>26</sup> which were computed to be 10.40, 2.25, 1.49, and 0.84 mm, respectively. These four material classes were then used in all subsequent experiments.

**Acid Bisulfite Pretreatment.** The calcium bisulfite cooking liquor was provided and analyzed by Weyerhaeuser Corp. (Federal Way, Washington). The concentrations of calcium bisulfite and free sulfur dioxide were 3.34 and 1.06% (fraction of total mass), respectively. The equivalent sulfur dioxide loading on wood was selected as 6%. Solid to liquid ratio in this pretreatment is 1:4. Three pretreatment temperatures (135, 145, and 155 °C) were employed to determine the optimal temperature under which particle size effects could be discerned. 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.<sup>27</sup> Monomeric sugar concentrations were measured by Dionex ICS-3000 (CarboPac PA20 Guard column: 4 × 50 mm and IonPac AS11-HC analytical column: 4 × 250 mm, ED40 electrochemical detector, AS40 autosampler, degassed E-pure water, 50 and 200 mM NaOH solution as eluent with a flow rate 0.5 mL/min). Oligomeric sugars in the spent liquor were first hydrolyzed into monomer sugars and then measured with Dionex ICS-3000 as the difference of sugar concentrations between the spent liquor and the hydrolyzed spent liquor. The washed soluble sugar was compensated by multiplying the total liquor volume with the measured sugar concentration.

The concentrations of degradation products were measured with a high-performance liquid chromatography (HPLC) system (Waters, Milford, MA) equipped with a differential refractive index detector (ERC-S710, ERMA) and a Rezex ROA organic acid column (7.8 mm × 30 cm, Phenomenex, Torrance, CA) eluted with 0.005 N aqueous H<sub>2</sub>SO<sub>4</sub> (0.5 mL/min) at 65 °C. LS 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.<sup>28,29</sup>

**Pretreated Solid Analysis. Compositional Analysis of Pretreated Solids.** The samples for compositional analysis were prepared according to the NREL standard.<sup>30</sup> Two-step acid hydrolysis was carried out based on the NREL standard.<sup>31</sup> Monomeric sugars were measured by ionic chromatography (Dionex ICS-3000). The xylose and mannose cannot be differentiated by this equipment. Therefore, the composition for xylan and mannan are presented together in this paper and is not deemed to be a limitation because xylan content of Douglas-fir is typically in the range of 2–5%.<sup>14,24</sup> 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 performance, ZnSe crystal). FTIR results were used to analyze chemical group difference between different treatment conditions.<sup>32,33</sup>

**X-ray Diffraction (XRD) Analysis.** XRD analysis of untreated and pretreated samples was conducted with an X-ray diffractometer (Miniflex 600, Rigaku, Japan) operated at 40 V and 15 mA with Ni-filtered Cu K $\alpha$  radiation. The pretreated 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 2 $\theta$  range of 10–40° at a scan speed of 2°/min. Crystallinity index (CrI, %) of each sample was analyzed using the following equation:

$$\text{CrI} = 100 \times \frac{I_{002} - I_{\text{am}}}{I_{002}} \quad (1)$$

where  $I_{002}$  is the intensity of the 002 peak at  $2\theta = 22.4^\circ$  and  $I_{\text{am}}$  is the intensity of the background scatter at  $2\theta = 18.5^\circ$ , which is the characteristic peak of cellulose-I. The crystalline structure modification

was analyzed with XRD,<sup>34</sup> and the acid treatment increased the CrI through hemicellulose removal.<sup>35</sup>

**Thermal Gravimetric Analysis (TGA) Analysis.** TGA was performed by a thermal analyzer (SDT Q600, TA Instruments, New Castle, NE) 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.<sup>36</sup> 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 bisulfite 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 14 steel balls with a diameter of 10 mm in each jar). Cellic CTec2 and HTec2 were complementarily 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 overall sugar recovery in both the spent liquid from the acid bisulfite treatment process and enzymatic hydrolysis of the pretreated solids.

## RESULTS AND DISCUSSION

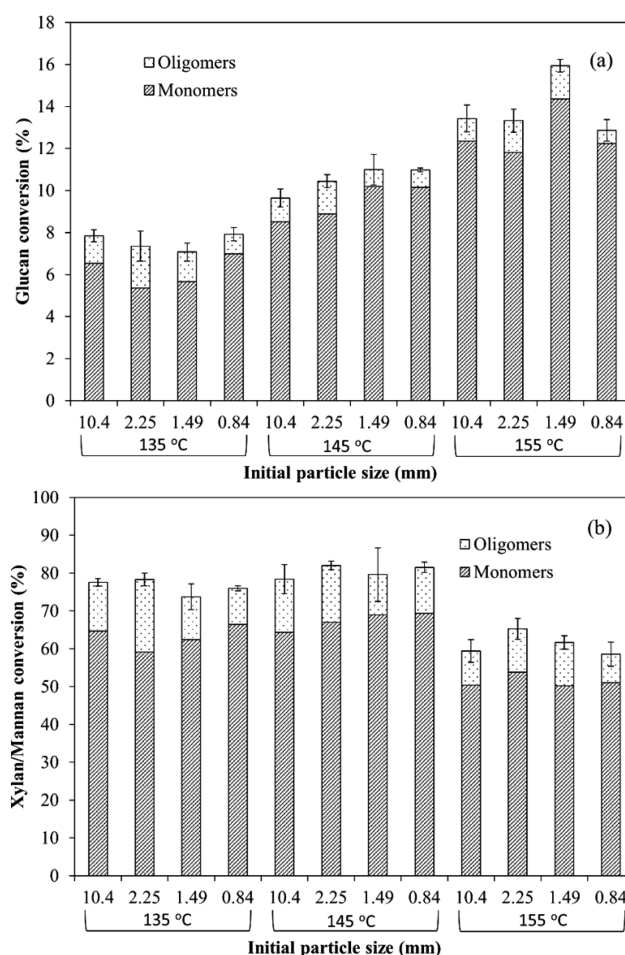
**Spent Liquor Analysis.** Table 1 shows the concentrations of soluble sugars, degradation products (furfural, HMF, and

**Table 1. Soluble Sugars, Degradation Products, and Lignosulfonate Analysis in Spent Liquor**

| temperature (°C) | size (mm) | soluble sugars (g/L) | furfural (g/L) | HMF (g/L) | acetic acid (g/L) | LS (g/L) |
|------------------|-----------|----------------------|----------------|-----------|-------------------|----------|
| 135              | 10.4      | 43.8                 | 0.90           | 0.85      | 1.48              | 17.6     |
| 135              | 2.25      | 45.0                 | 0.91           | 0.86      | 1.54              | 15.2     |
| 135              | 1.49      | 43.3                 | 0.83           | 0.89      | 1.58              | 16.5     |
| 135              | 0.84      | 44.5                 | 1.41           | 0.88      | 1.56              | 15.3     |
| 145              | 10.4      | 47.7                 | 2.20           | 1.11      | 1.91              | 19.9     |
| 145              | 2.25      | 47.0                 | 2.04           | 0.9       | 2.06              | 19.8     |
| 145              | 1.49      | 49.1                 | 2.36           | 1.39      | 1.73              | 21.5     |
| 145              | 0.84      | 48.5                 | 2.68           | 1.38      | 1.82              | 18.1     |
| 155              | 10.4      | 41.9                 | 5.13           | 5.28      | 2.81              | 19.1     |
| 155              | 2.25      | 43.8                 | 3.85           | 2.19      | 3.43              | 22.7     |
| 155              | 1.49      | 42.7                 | 4.42           | 1.92      | 3.85              | 29.3     |
| 155              | 0.84      | 42.2                 | 5.00           | 3.06      | 5.29              | 18.6     |

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



**Figure 1.** Yield of (a) glucose and (b) xylose/mannose in spent liquor, expressed as percent of the theoretical yield available in the raw material.

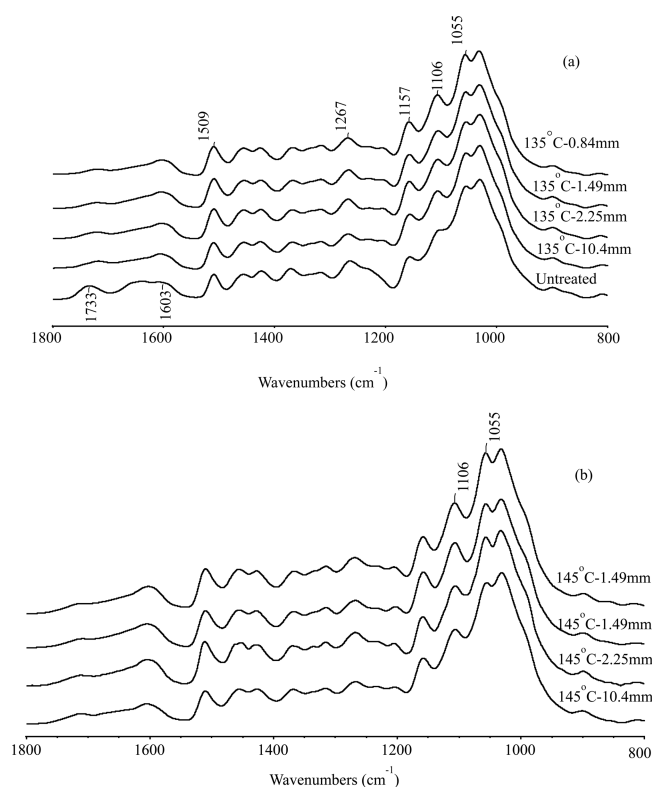
size influence was found. The glucan yield dropped when particle size was smaller than 1.49 mm at 155 °C. This can be explained by increasing HMF content, which resulted in lower glucose content. Initial particle size did not have a significant effect on xylose/mannose yield in spent liquor. The yield 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<sup>14</sup> 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.<sup>37,38</sup> 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<sup>-1</sup> was assigned to

Table 2. Compositional Analysis of the Pretreated Solids (w/w % of the Pretreated Sample)

| temperature (°C) | size (mm) | CrI (%) | arabinan (%) | galactan (%) | glucan (%) | xyl/mann (%) | ASL (%) | AIL (%) | total (%) | solid recovery (%) |
|------------------|-----------|---------|--------------|--------------|------------|--------------|---------|---------|-----------|--------------------|
| untreated wood   |           | 48      | 1.2          | 2.8          | 43.3       | 15.2         | 2.0     | 29.7    | 94.3      |                    |
| 135              | 10.4      | 49      | 0.2          | 0.6          | 50.5       | 4.8          | 5.3     | 32.4    | 93.9      | 76.7               |
| 135              | 2.25      | 50      | 0.0          | 0.1          | 52.5       | 5.3          | 3.8     | 32.3    | 93.9      | 76.4               |
| 135              | 1.49      | 51      | 0.0          | 0.1          | 54.5       | 4.7          | 3.4     | 31.2    | 93.9      | 75.4               |
| 135              | 0.84      | 51      | 0.0          | 0.0          | 54.5       | 4.7          | 3.0     | 32.2    | 94.3      | 73.4               |
| 145              | 10.4      | 51      | 0.1          | 0.5          | 54.8       | 3.4          | 5.1     | 32.2    | 96.1      | 71.7               |
| 145              | 2.25      | 57      | 0.0          | 0.0          | 55.6       | 2.0          | 3.3     | 33.6    | 94.5      | 68.3               |
| 145              | 1.49      | 59      | 0.0          | 0.0          | 54.2       | 2.2          | 3.1     | 33.7    | 93.2      | 68.6               |
| 145              | 0.84      | 61      | 0.0          | 0.0          | 54.8       | 1.6          | 2.3     | 35.4    | 94.1      | 68.2               |
| 155              | 10.4      | 55      | 0.0          | 0.2          | 53.4       | 1.2          | 5.1     | 35.0    | 95.0      | 71.4               |
| 155              | 2.25      | 58      | 0.0          | 0.0          | 55.4       | 1.1          | 2.7     | 36.2    | 95.3      | 67.7               |
| 155              | 1.49      | 62      | 0.0          | 0.0          | 51.2       | 0.8          | 3.9     | 37.3    | 93.1      | 66.7               |
| 155              | 0.84      | 61      | 0.0          | 0.0          | 51.0       | 0.8          | 3.1     | 40.2    | 95.2      | 66.8               |

Figure 2. FTIR spectra of pretreated solids under (a) 135 °C and (b) 145 °C with a range of 1800–800  $\text{cm}^{-1}$ .

carbonyl groups, which mainly derived from acetyl groups in hemicelluloses or lignin.<sup>33,39</sup> 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  $\text{cm}^{-1}$  are attributed to aromatic skeletal vibration in lignin, while the band at 1267  $\text{cm}^{-1}$  is the C–O stretch from guaiacyl unit of lignin (G-unit). Also, the band 1106  $\text{cm}^{-1}$  is from the aromatic skeletal vibration and C–O stretch in lignin. The band 1055  $\text{cm}^{-1}$  is attributed to C–O vibration in the carbohydrates.<sup>39–41</sup> As shown in Figure 2, no significant difference was found in the spectra of the characteristic band 1509  $\text{cm}^{-1}$  of lignin among various initial particle sizes. The bands 1106 and 1055  $\text{cm}^{-1}$  show difference among different particle sizes at the temperature of 145 and 155 °C.

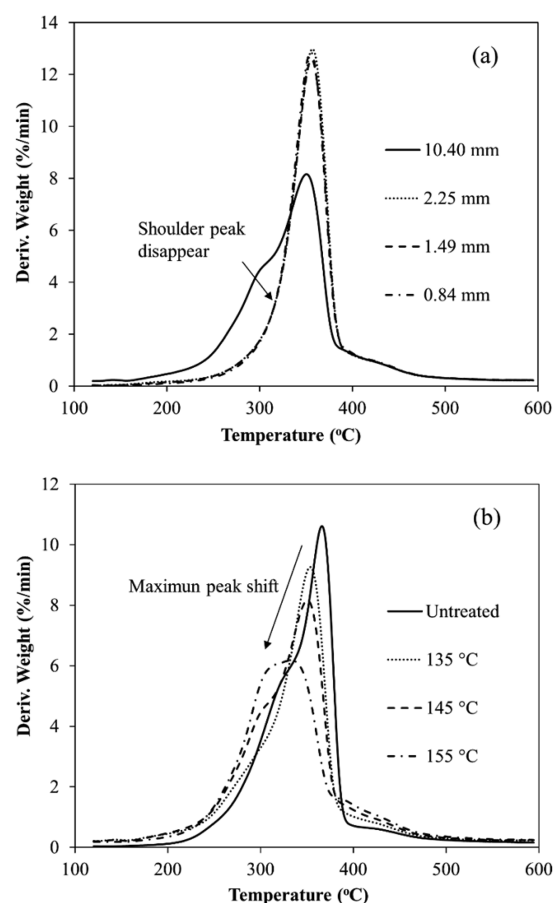
**XRD Analysis of Pretreated Solid.** A previous study showed the acid pretreatment slightly increased the crystallinity of lignocellulosic materials.<sup>35</sup> Table 2 shows that the untreated sample had a CrI of 48%, which increased after pretreatment. At 135 °C, the CrI of the pretreated solids 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.<sup>42</sup> 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 mm and 10.40 mm. The maximum total sugar yield of



**Figure 3.** DTG curves for (a) 4 particle sizes under 145 °C and (b) the untreated and pretreated solids with a particle size of 10.40 mm under 3 temperatures.

**Table 3.** Total Sugar Yield under Various Conditions

| particle size (mm) | total sugar yield (%) |        |        |
|--------------------|-----------------------|--------|--------|
|                    | 135 °C                | 145 °C | 155 °C |
| 10.4               | 59.6                  | 65.9   | 55.4   |
| 2.25               | 66.6                  | 72.0   | 59.3   |
| 1.49               | 60.2                  | 63.1   | 64.4   |
| 0.84               | 61.5                  | 61.5   | 46.6   |

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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