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Elucidating thermochemical pretreatment effectiveness of different particle-size switchgrass for cellulosic ethanol production

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\textsuperscript{1}Jikai Zhao and Yang Yang contributed equally to the experiment
Abstract

Effects of switchgrass particle sizes (< 0.25 mm, 0.5-1.0 mm, and 2.0-4.0 mm) on the effectiveness of \( \text{H}_2\text{SO}_4 \) and \( \text{NaOH} \) pretreatments were investigated. As particle size increased, glucan, xylan, and lignin contents in raw switchgrass augmented from 30.32 to 32.02%, 18.44 to 19.03%, and 14.78 to 15.33%, respectively. Glucan and xylan (58.54 to 60.94% and 18.55 to 20.01%) contents in \( \text{NaOH} \) pretreated switchgrass and their recoveries (91.95 to 94.69% and 47.91 to 52.31%) increased. The highest glucan content (55.76%) and recovery (79.72%) in \( \text{H}_2\text{SO}_4 \) pretreated switchgrass were reached by middle particle size. The lowest (59.39% for \( \text{H}_2\text{SO}_4 \) and 58.99% for \( \text{NaOH} \)) and highest (65.23% for \( \text{H}_2\text{SO}_4 \) and 66.15% for \( \text{NaOH} \)) \( \text{CrI} \) values were obtained from middle and small particle sizes, respectively. SEM images and FTIR spectra showed no visible variations in microstructure and chemical bonds among different particle sizes under the same pretreatment conditions. The highest ethanol concentration and efficiency (based on pretreated switchgrass) were reached by big particle size for untreated (2.61 g/L and 29.56%) and \( \text{H}_2\text{SO}_4 \) pretreated (7.03 g/L and 49.28%) switchgrass, while they were achieved by small particle size for \( \text{NaOH} \) pretreated (11.68 g/L and 72.37%) switchgrass. The highest ethanol yield based on raw switchgrass was attained by big particle size for untreated (29.54%), middle particle size for \( \text{H}_2\text{SO}_4 \) pretreated (30.60%), and small particle size for \( \text{NaOH} \) pretreated (62.36%) switchgrass. These findings indicate that the choice of biomass particle size varies depending on the pretreatment methods and the calculation benchmark for ethanol conversion yield.

Keywords: Lignocellulosic biomass; Pretreatment; Particle size; Ethanol fermentation
1. Introduction

Global demand for coal- and petroleum-based energy sources resulted in the potential depletion of non-renewable fossil fuels [1–3]. Upgrading abundant lignocellulosic biomass to ethanol has been an essential worldwide mission to alleviate overdependence on fossil fuels and collateral environment issues [4–6]. However, the effects have not led to its large-scale worldwide commercial realization yet. The foremost technical challenge is the inherent recalcitrance of lignocellulosic biomass constraining enzymatic accessibility [7–9]. An indispensable step for cellulosic ethanol production is thermochemical pretreatment to render lignocellulosic biomass more acquiescent to enzymes and microbes. Numerous pretreatment approaches such as liquid hot water, acid, alkaline, steam explosion, organosolv, ionic liquid, and deep eutectic solvent have been considerably investigated [10–15]. In this regard, dilute sulfuric acid (H$_2$SO$_4$) pretreatment is preferred by the National Renewable Energy Laboratory to achieve the minimum ethanol selling price of $2.15/gal [16]. Whereas for the emerging ionic liquid pretreatment, the minimum ethanol selling price ranging from $8.8 to $9.4/gge was reported by the Lawrence Berkeley National Laboratory at the pilot scale [17]. This significant variation was mainly associated with the cost of chemical reagents because of excessive washing processes for their recovery [18,19]. This is consistent with our previous report that the advantages of higher lignin and ethanol yields from acetic acid pretreatment were offset by its high chemical cost compared to H$_2$SO$_4$ pretreatment [7]. Therefore, conventional acid-base pretreatment may be the most economically feasible method for cellulosic ethanol production.

Particle size reduction is an initial step to enhance the bulk density and flowability of biomass [20]. Also, the particle size of biomass is of great interest, particularly concerning sugar conversion. It is commonly assumed the smaller the biomass size, the better the sugar hydrolysis
efficiency, due to efficient pretreatment effects such as increased surface area [21]. However, previous studies have conveyed substantial inconsistencies in enzymatic digestibility as a function of biomass particle size [22]. There are two main methods to calculate sugar conversion: either based on the raw biomass (considering the sugar recovery during pretreatment and conversion efficiency during enzymatic hydrolysis) or the pretreated biomass (only counting conversion efficiency during enzymatic hydrolysis) [23]. The variation in calculating sugar conversion yield would result in significant misleading. If one pretreatment method achieves a higher sugar conversion (based on the pretreated biomass) than another, it would be controversial to recommend it without considering the loss of sugar during pretreatment. The same holds for different biomass particle sizes. For example, it is assumed that the H$_2$SO$_4$ pretreatment achieves a sugar recovery of 60% after pretreatment and conversion efficiency of 80% after hydrolysis while the sodium hydroxide (NaOH) pretreatment reaches sugar recovery of 80% after pretreatment and conversion efficiency of 60% using the same particle size, so which pretreatment method is better in terms of total sugar yield? Similarly, under the same pretreatment conditions, 60% sugar recovery after pretreatment and 80% conversion efficiency after hydrolysis are achieved for small particles, whereas 80% sugar recovery after pretreatment and 60% conversion efficiency after hydrolysis are realized for large particles, so which particle size is better regarding total sugar yield? Obviously, their total sugar yields are the same based on raw biomass. In the case of ethanol fermentation, Kumar et al. [24] reported that ethanol yield [based on ethanol concentration (g/L)] of the microbial assisted diluted acid pretreated sesame plant residue increased with a decrease in particle size. Chen et al. [25] found that ethanol yield (based on the pretreated biomass) of microwave-assisted dilute ammonia pretreated sorghum bagasse was achieved by small particles. However, few studies have been conducted to
demonstrate the relationship between ethanol yield (either based on the raw biomass or the pretreated biomass) and biomass particle size in response to H$_2$SO$_4$ and NaOH pretreatments. In addition, comminution is an energy-consuming process [26], so it is critical to choose a suitable biomass particle size for cellulosic ethanol production.

The objective of this work was to clarify the thermochemical pretreatment effectiveness of different particle-size switchgrass for ethanol production. In this regard, the compositional (glucan, xylan, arabinan, and lignin), physicochemical (morphological properties, component recovery, particle size distribution, chemical bonds, crystallinity, and thermal transition), and fermentative characteristics (ethanol conversion efficiency and yield) of switchgrass with different particle sizes were demonstrated in response to H$_2$SO$_4$ and NaOH pretreatments.

2. Material and methods

2.1. Materials

Switchgrass was cultivated at the Kansas State University Operations Center (Manhattan, KS). After harvesting, the biomass samples were cut into sections and dried for several days. The Benshaw Hammer Mill (Benshaw Inc. Pittsburgh, PA) with a sieve of 8 mm was used to grind the switchgrass at 4000 rpm. The ground samples were sieved by RX-29 8 Inch Sieve Shaker (Hogentogler & Co. Inc. Columbia, MD) to obtain three main particle sizes (< 0.25 mm, 0.5-1.0 mm, and 2.0-4.0 mm). Cellulase (Cellic® CTec3, 516 mg protein/mL) and hemicellulase (NS22244, 266 mg protein/mL) provided by Novozymes (Franklinton, NC) were utilized to hydrolyze cellulose and hemicellulose in the biomass, respectively [27].
2.2. Biomass pretreatment

Thermochemical pretreatment of different particle-size switchgrass was conducted in a sand bath (Techne Inc., Princeton, NJ). Concisely, 4 g switchgrass (wet basis) was poured into 75 mL stainless steel reactors (Swagelok, Kansas City Valve & Fitting Co., KS), followed by loading 40 mL of H$_2$SO$_4$ (pH = 1.37, around 0.05 mol/L) and NaOH (pH = 13.52, around 0.3 mol/L) aqueous solution at a solid/liquid ratio of 1:10 (w/v). The reactors were manually shaken to homogenize the slurry and then immersed into the sand bath with a preheated temperature of 190 °C for 40 min. The selection of pretreatment conditions was based on our previous studies [7,27–29]. To quench the further reactions, the reactors were instantly plunged into cold water after pretreatment [30]. The pretreated switchgrass was separated from liquid fraction using a slow drip metal filter and then washed with tap water [9]. The residues were dried at 49 °C overnight and stored in the plastic bag before further analysis.

2.3. Physicochemical characterization

Microstructural characteristics of raw and pretreated switchgrass were exhibited using the FEI Nova NanoSEM 450 (Thermo Fisher Scientific, Waltham, MA). To present the structural changes, switchgrass samples were mounted on conductive adhesive tapes and then coated with a thin metal film coat before observing and capturing pictures [29,31,32]. The particle size distribution of raw and pretreated switchgrass was determined using an LS I3 320 Laser Diffraction Particle Size Analyzer (Beckman Coulter, USA) based on volume distribution. A 400 Fourier Transform Infrared (FTIR) spectrophotometer (PerkinElmer Corp., Shelton, CT) aligned with a Room Temperature Deuterated Lanthanum α Alanine doped TriGlycine Sulphate (RT-DLaTGS) detector was used to identify and analyze the changes in chemical bonds of
switchgrass before and after pretreatment. The procedures in detail can be found in our previous study [32]. The crystallinity of switchgrass was measured by a Siemens D-5000 diffractometer (Bruker, Ettlingen, Germany) with Cu-K radiation created at 40 kV and 40 mA. Diffraction intensities were tested in the range of 5-40° (2θ) with a step rate of 0.1°/second [28]. The differential scanning calorimeter-DSC 204 F1 Phoenix (NETZSCH-Gerätebau GmbH, Selb, Germany) was used to determine thermal properties of switchgrass before and after pretreatment. The temperature range of DSC measurement ranged from 20 to 600 °C with a nitrogen flow rate of 20 mL/min for the analysis.

2.4. Simultaneous saccharification and fermentation

First of all, the culture broth (2% glucose, 0.5% peptone, 0.3% yeast extracts, 0.1% KH₂PO₄, and 0.05 % MgSO₄·7H₂O) was sterilized at 121 °C for 20 min. When the broth was cooled down to room temperature, 1.0 g dry yeast (Ethanol Red, Lesaffre, Milwaukee, WI, USA) was weighed into the broth (19 mL) and incubated at 38 °C at 160 rpm for 25 min. The activated yeast culture had a cell concentration of about 10⁹ cells/mL [32]. The pretreated switchgrass and sodium acetate buffer (50 mM, pH 4.8) were loaded into 125 mL Erlenmeyer flasks to form a 5% (w/v) solid loading. After pH adjusting, Cellic® CTec3 (20 mg/g-cellulose) and NS22244 (12 mg/g-hemicellulose) were added to the flasks. The simultaneous saccharification and fermentation (SSF) process was initiated by adding 0.10 g yeast extracts and inoculating activated yeast culture (about 5×10⁸ cells). The flasks were incubated in an orbital shaker (I2400 Incubator Shaker, New Brunswick, USA) at 38 °C with 150 rpm for 72 h. During SSF, 60 μL of supernatant was pipetted from each flask for compositional analysis.
2.5. Analytical approaches

The chemical composition of raw and pretreated switchgrass was examined following the procedures from National Renewable Energy Laboratory [33,34]. Glucose, xylose, arabinose, and ethanol concentrations were measured by a 1260 HPLC system (Agilent, Santa Clara, CA) equipped with an HPX-87H organic acid column with the mobile phase was 50 mM H$_2$SO$_4$ solution. In this regard, the measurement conditions can be found in our previous study [31]. The component recoveries were calculated based on comparing the final amount of components in pretreated biomass with the initial amount of components in raw biomass [32]. Ethanol conversion efficiency (take the pretreated biomass as the base) was calculated as the amount of ethanol in the slurry divided by the amount of theoretical ethanol from pretreated biomass. Ethanol conversion yield (take the raw biomass as the base) was evaluated as the percentage of the amount of ethanol produced in the slurry compared with the amount of potential ethanol generated in raw biomass.

3. Results and discussion

3.1. Chemical composition of raw switchgrass

Table 1. Chemical composition (% db) of raw switchgrass with different particle sizes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucan</th>
<th>Xylan</th>
<th>Arabinan</th>
<th>Lignin</th>
<th>Extractives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small size</td>
<td>30.32 ± 0.25$^b$</td>
<td>18.44 ± 0.27$^a$</td>
<td>2.16 ± 0.23$^{ab}$</td>
<td>14.78 ± 0.04$^c$</td>
<td>28.81 ± 0.45$^a$</td>
</tr>
<tr>
<td>Middle size</td>
<td>30.79 ± 0.05$^b$</td>
<td>18.62 ± 0.06$^a$</td>
<td>2.38 ± 0.08$^a$</td>
<td>14.86 ± 0.07$^b$</td>
<td>26.33 ± 0.24$^b$</td>
</tr>
<tr>
<td>Big size</td>
<td>32.02 ± 0.05$^a$</td>
<td>19.03 ± 0.08$^a$</td>
<td>1.61 ± 0.04$^b$</td>
<td>15.33 ± 0.05$^a$</td>
<td>25.33 ± 0.18$^c$</td>
</tr>
</tbody>
</table>

Means ± standard deviations. In each column, means with different letters are significantly different at $p < 0.05$.
The composition of lignocellulosic biomass is of great interest, particularly in relation to pretreatment effectiveness and bioproduct conversion performance [35]. To this end, the chemical composition such as glucan, xylan, and lignin profiles in the biomass from different particle size sources has been investigated by many research groups. However, inconsistent reports of compositional results are often found. One party assumed that biomass from different particle size sources had the same composition [21,36–41], and the other party reported that biomass with different particle sizes showed compositional differences [23,42,43]. Table 1 summarizes the chemical composition of raw switchgrass with different particle sizes. As particle size increased, glucan, xylan, and lignin contents amplified slightly from 30.32 to 32.02%, 18.44 to 19.03%, and 14.78 to 15.33%, respectively. Similarly, Yang et al. [23] reported that glucan content increased with the particle sizes of wheat straw increased from < 0.25 mm to 4.00 mm. In contrast, Chundawat et al. [42] observed the incremental trend of xylan content in corn stover from 20.10 to 27.50% as particle sizes increased from < 150 µm to > 850 µm. The increased lignin content indirectly reflects the resistance of switchgrass to hammer mill grinding, but no significant trend was found in the other literature [23,42]. Extractives in the switchgrass increased as a decrease of particle size, which is consistent with the findings that water and ethanol solubles increased with the particle size of corn stover and wheat straw decreasing [23,42]. In addition, middle-size switchgrass contained the highest arabinan content of 2.38%. These results indicate that the parts of switchgrass with high crystallinity and lignin have resistance and maintain large particle size during mechanical grinding.

3.2. Chemical composition of pretreated switchgrass
Table 2. Chemical composition of H$_2$SO$_4$ and NaOH pretreated switchgrass with different particle sizes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucan</th>
<th>Xylan</th>
<th>Arabinan</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$SO$_4$ (small size)</td>
<td>49.52 ± 0.25$^c$</td>
<td>1.44 ± 0.27$^d$</td>
<td>0.20 ± 0.23$^d$</td>
<td>39.95 ± 0.04$^b$</td>
</tr>
<tr>
<td>H$_2$SO$_4$ (middle size)</td>
<td>55.76 ± 0.05$^c$</td>
<td>1.48 ± 0.06$^d$</td>
<td>0.25 ± 0.08$^d$</td>
<td>37.72 ± 0.07$^c$</td>
</tr>
<tr>
<td>H$_2$SO$_4$ (big size)</td>
<td>51.65 ± 0.05$^d$</td>
<td>1.17 ± 0.08$^d$</td>
<td>0.21 ± 0.04$^d$</td>
<td>44.32 ± 0.05$^a$</td>
</tr>
<tr>
<td>NaOH (small size)</td>
<td>58.54 ± 0.25$^b$</td>
<td>18.55 ± 0.27$^c$</td>
<td>1.92 ± 0.23$^c$</td>
<td>13.70 ± 0.04$^d$</td>
</tr>
<tr>
<td>NaOH (middle size)</td>
<td>59.82 ± 0.05$^{ab}$</td>
<td>19.39 ± 0.06$^b$</td>
<td>2.59 ± 0.08$^a$</td>
<td>10.81 ± 0.07$^c$</td>
</tr>
<tr>
<td>NaOH (big size)</td>
<td>60.94 ± 0.05$^a$</td>
<td>20.01 ± 0.08$^a$</td>
<td>2.26 ± 0.04$^b$</td>
<td>11.05 ± 0.05$^f$</td>
</tr>
</tbody>
</table>

Means ± standard deviations. In each column, means with different letters are significantly different at $p < 0.05$.

Table 2 presents the composition of H$_2$SO$_4$ and NaOH pretreated switchgrass. Glucan (58.54-60.94% vs. 49.52-55.76%), xylan (18.55-20.01% vs. 1.17-1.48%), and arabinan (1.92-2.59% vs. 0.20-0.25%) contents in the NaOH pretreated switchgrass were notably higher than those in the H$_2$SO$_4$ pretreated switchgrass, due to the pre-hydrolysis of glycosidic bonds of carbohydrates [32,44,45]. However, the lignin contents (37.72-44.32%) of H$_2$SO$_4$ pretreated switchgrass were three times higher than those (10.81-13.70%) of NaOH pretreated switchgrass, because the acid-soluble lignin content is very low [31]. It was found that glucan and xylan contents in the NaOH pretreated switchgrass gradually increased with the increment of particle size. Especially, NaOH pretreated middle-size switchgrass had higher arabinan and lower lignin contents. However, these findings are inconsistent with the report that NaOH pretreated ultrafine wheat straw owned the highest glucan and lowest hemicellulose contents compared to those of general ground samples [46]. Moreover, Khullar et al. [47] observed that glucan content in the ammonium hydroxide pretreated Miscanthus x giganteus decreased as particle size increased from 0.08 to 6.00 mm. In the case of H$_2$SO$_4$ pretreatment, the highest glucan and lowest lignin contents were reached by middle-size switchgrass. Particularly, H$_2$SO$_4$ pretreated big-size switchgrass had the highest lignin content of 44.32%. In contrast, Khullar et al. [47] reported that...

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glucan and xylan contents in the H$_2$SO$_4$ pretreated Miscanthus x giganteus increased while lignin
content decreased with the increment of particle size. Liu et al. [48] found that lignin content in
steam explosion pretreated corn stover increased as the particle size increased from 5 to 25 mm,
but there was no significant trend in terms of glucan, xylan, and arabinan contents as a function
of particle size. Ballesteros et al. [49] investigated the steam explosion pretreatment of Brassica
carinata biomass under different conditions and found that glucan content in pretreated biomass
increased as the particle size increased from 2 to 12 mm, but the trends of other components
depended on pretreatment time and temperature. These variations among studies can be directly
correlated with biomass types, handling processes of biomass, and thermochemical pretreatment
conditions.

3.3. Sugar, lignin, and solid recoveries of pretreated switchgrass

Some studies have demonstrated that the particle size of lignocellulosic biomass has a
significant influence on component recovery. In the case of steam explosion pretreatment, it was
reported that the larger the particle size used for pretreatment, the higher the cellulose recovery,
but the particle size had no obvious linear relationship with hemicellulose recovery [48,49]. In
terms of H$_2$SO$_4$ pretreatment, Yang et al. [23] demonstrated that glucan, arabinan, and solid
recoveries decreased as the particle size of wheat straw decreased. It is consistent with the result
that arabinan recovery increased from 3.97 to 5.27% as the particle size of switchgrass increased,
but the highest glucan (79.72%) and solid (44.00%) recoveries were obtained by middle-size
switchgrass (Fig. 1a). Moreover, the lowest glucan (65.53%) and xylan (2.49%) recoveries were
reached by big-size switchgrass (Fig. 1a). It has been demonstrated that H$_2$SO$_4$ pretreatment can
cause cross-linking of derivatives to form non-degradable pseudo-lignin [9,50,51]. The
formation of pseudo-lignin was not affected by the particle size of switchgrass, and in particular, big particle size produced more pseudo-lignin (117.48% lignin recovery). Regarding NaOH pretreatment, Qu et al. [46] confirmed that the solubilization level of cellulose, hemicellulose, and lignin into liquid fraction significantly increased with the reduction of wheat straw particle size. This is consistent with the findings that glucan, xylan, and arabinan recoveries gradually increased from 91.95 to 94.69%, 47.91 to 52.31%, and 42.66 to 70.07%, respectively (Fig. 1b). In addition, solid recovery slightly increased from 47.63 to 49.75%. Surprisingly, however, the small particle size achieved the lignin recovery of 44.14% (Fig. 1b), which can be indirectly reflected by its highest lignin content (Table 2). The evaluation of these comparisons indicates that empirical assumptions may differ from actual experimental results.

![Sugar, lignin, and solid recoveries of H₂SO₄ (a) and NaOH (b) pretreated switchgrass with different particle sizes.](https://ssrn.com/abstract=4056867)
3.4. Morphological property of raw and pretreated switchgrass

Two studies have reported the significant variation in morphological properties of the pretreated biomass with different particle sizes [46,48], while it has been revealed that the microstructure of pretreated biomass among different particle sizes was not significant, depending on subjective image selection [23]. It should be highlighted that no significant differences in SEM images were noticed among different particle sizes regardless of raw or pretreated switchgrass in this work. To avoid selection bias, three representative SEM images were presented to show the traces left by pretreatment. It was observed that the external surface of raw switchgrass displayed a rigid and smooth shield, while the inner surface is relatively rough but still intact (Fig. 2a-c). After H$_2$SO$_4$ pretreatment, the vascular bundles and conduit structures were significantly fragmented (Fig. 2d-f), indicating the depolymerization of vulnerable hemicellulose around the cellulose [32]. However, the skeleton of recalcitrant lignin almost remained integral with some recondensed lignin sticking to the surface (Fig. 2f). These residual lignin units might crosslink with enzymes during saccharification and fermentation to prevent cellulose accessibility. In terms of NaOH pretreatment, wave-like structures were exhibited due to the saponification effect (Fig. 2g-i), indicating the swelling of cellulose and the solubilization of amorphous hemicellulose and robust lignin [46].
Fig. 2. Scanning electron microscopy images of raw (a-c) and H$_2$SO$_4$ (d-f) and NaOH (g-i) pretreated switchgrass.

3.5. Particle size distribution of raw and pretreated switchgrass

Due to the use of standard sieves to screen the raw switchgrass, the true switchgrass size, especially the length, should be longer than the represented values (Fig. S1). When detecting big particle size samples, the vacuum pipe of LS I3 320 Laser Diffraction Particle Size Analyzer was often blocked, resulting in inaccurate detection results. For example, the geometric mean
diameters of big particles were smaller than those of middle particles (Table S1). Nevertheless, compared to raw switchgrass with a small size, H$_2$SO$_4$ and NaOH pretreated switchgrass had smaller particle sizes. Interestingly, compared to raw switchgrass with middle and big sizes, H$_2$SO$_4$ and NaOH pretreated switchgrass showed larger particle sizes. The latter was in agreement with the report that compared to that of untreated wheat straw, an increase in particle size was observed in NaOH-pretreated wheat straw attributed to the swelling and aggregation [46].

3.6. FTIR spectra of raw and pretreated switchgrass

Fig. 3. Fourier transform infrared spectrum of raw and H$_2$SO$_4$ and NaOH pretreated switchgrass. FTIR spectra changes are often accompanied by significant changes in the chemical composition of biomass [8,32,52]. For example, compared to those of raw switchgrass, the intensities of cellulose related peaks [3300-3400 cm$^{-1}$ (O-H stretching), 2910-2930 cm$^{-1}$ (C-H
stretching), 1091 cm\(^{-1}\) (C-O vibration), 1160-1170 cm\(^{-1}\) (C-O-C stretching), 1110-1120 cm\(^{-1}\) (C-OH skeletal vibration), 1050-1060 cm\(^{-1}\) (C-O-C pyranose ring skeletal vibration), and 1023 cm\(^{-1}\) (C-O-C pyranose ring skeletal vibration)] in H\(_2\)SO\(_4\) and NaOH pretreated switchgrass increased (Fig. 3). In addition, H\(_2\)SO\(_4\) pretreated switchgrass had stronger lignin related peak intensities [1730-1740 cm\(^{-1}\) (C=O stretching of aldehydes), 1570-1590 cm\(^{-1}\) (C=C stretching of aromatic skeletal vibration), 1514 cm\(^{-1}\) (aromatic skeleton), and 1319 cm\(^{-1}\) (syringyl and guaiacyl lignin units)] than raw switchgrass (Fig. 3). However, there was no visual difference in the response of FTIR spectra to different particle sizes of switchgrass (Fig. 3), which is in accordance with the finding that particle size had an inadequate influence on the FTIR absorption bands [23].

3.7. Crystallinity of raw and pretreated switchgrass

Fig. 4. X-ray diffraction of raw and H\(_2\)SO\(_4\) and NaOH pretreated switchgrass.
The XRD patterns of switchgrass with different particle sizes were shown in Fig. 4. The crystallinity index (CrI) of switchgrass was calculated by comparing the maximum peak intensity (I\(_{002}\)) of lattice diffraction angle with the amorphous (I\(_{am}\)) contribution (Table S2). The CrI values (46.42-49.62%) of raw switchgrass with different particle sizes were relatively lower than those of H\(_2\)SO\(_4\) (59.39-65.23%) and NaOH (58.99-66.15%) pretreated switchgrass. This can be mainly ascribed to the disintegration of the amorphous regions (hemicellulose, lignin, and extractives) and the enhancement of cellulose peak strength during pretreatment [8,28,29,32]. In contrast, Dougherty et al. [36] found that ammonia fiber expansion and ionic liquid pretreatments were capable of reducing the CrI of switchgrass no matter which particle size was used. The reduction in crystallinity was explained by the significant disruption of hydrogen bonds in cellulose fibrils [36]. Under the same particle size of switchgrass, H\(_2\)SO\(_4\) and NaOH pretreatments generated almost comparable CrI values (Table S2). It was also found that the lowest (59.39% for H\(_2\)SO\(_4\) and 58.99% for NaOH) and highest (65.23% for H\(_2\)SO\(_4\) and 66.15% for NaOH) CrI values were obtained from middle and small particle sizes after pretreatment, respectively (Table S2). The former is consistent with the research results from Dougherty et al. [36] who demonstrated that the CrI values of small particle size (32–50 µm) of switchgrass after H\(_2\)SO\(_4\), ammonia fiber expansion, and ionic liquid pretreatments were lower than those of middle (75–100 µm) and big (> 200 µm) particle sizes. This is because the cellulose in small particles is more likely to be depolymerized during pretreatment [53]. However, the latter is different from the previous report where the CrI values of H\(_2\)SO\(_4\), ammonia fiber expansion, and ionic liquid pretreated switchgrass gradually increased as particle size amplified [36]. This variation is likely the result of the different trends of I\(_{002}\) and I\(_{am}\) in response to thermochemical pretreatment (Fig. 4).
3.8. Thermal property of raw and pretreated switchgrass

Fig. 5. Differential scanning calorimeter heat flow of raw and H$_2$SO$_4$ and NaOH pretreated switchgrass.

Thermal transitions of switchgrass with different particle sizes were elucidated by DSC analysis to provide information about the degradation phenomena such as decomposition, cross-linking, crystallization, oxidation, evaporation, and melting [54–57]. The DSC curve was reported to reflect the compositions and structures of biomass [54], which can be indirectly verified by the findings in this work. For example, H$_2$SO$_4$ pretreated switchgrass showed a weak endothermic peak (related to the melting of hemicellulose) at around 250 °C than raw and NaOH pretreated switchgrass (Fig. 5), due to the notable deconstruction of its hemicellulose (Table 2). In addition, H$_2$SO$_4$ pretreated switchgrass presented a stronger endothermic peak (attributed to the melting of lignin) at around 350 °C compared to raw and NaOH pretreated switchgrass (Fig. 5).
which is associated with its high lignin content (Table 2). For raw and H$_2$SO$_4$ pretreated switchgrass, it showed identical DSC patterns among different particle sizes. For NaOH pretreatment, significant variation in heat flow pattern was observed among different particle sizes (Fig. 5). Here, it should be mentioned that the qualitative and quantitative relationships between heat flow peaks and changes in composition and structure of biomass need to be further investigated.

3.9. Ethanol fermentation of raw and pretreated switchgrass

**Fig. 6.** Time courses of bioethanol fermentation of raw and pretreated switchgrass with different particle sizes. Glucose and ethanol concentrations for untreated switchgrass (a), H$_2$SO$_4$ pretreated switchgrass (b), and NaOH pretreated switchgrass (c); ethanol conversion efficiency and yield for raw switchgrass (d), H$_2$SO$_4$ pretreated switchgrass (e), and NaOH pretreated switchgrass (f). The abbreviations are denoted as small size-ethanol concentration (S-E); small size-glucose concentration (S-G); middle size-ethanol concentration (M-E); middle size-glucose concentration (M-G); big size-ethanol concentration (B-E); big size-glucose concentration (B-G).
G); small size-ethanol conversion efficiency (S-CE); small size-ethanol yield (S-EY); middle size-ethanol conversion efficiency (M-CE); middle size-ethanol yield (M-EY); big size-ethanol conversion efficiency (B-CE); and big size-ethanol yield (B-EY).

It is empirically supposed that the pretreatment effectiveness would be beneficial for smaller particle sizes of biomass as the overall destruction of biomass and increased enzymes accessible area would result in the high ethanol yield [21]. However, several studies demonstrated that particle size influences biomass digestibility to a different extent: large [21,36,47,49], middle [37,38,41], and small [42,53,58,59] biomass particle sizes have been reported to achieve the highest sugar conversion yield under a variety of pretreatment methods. The inconsistencies among these studies were partially associated with the calculation method of sugar yield which is based on the pretreated biomass or raw biomass [22]. For example, Semerci and Ersan [39] reported that glucose yields based on the triethylammonium hydrogen sulfate pretreated hornbeam at the solid loadings of 10 and 20% (w/w) was higher than that of untreated hornbeam. However, under the same conditions, glucose yield based on raw hornbeam reversed the result [39]. In this work, the two methods of calculating ethanol conversion yield were distinguished: ethanol conversion efficiency was based on the pretreated switchgrass; ethanol conversion yield was based on raw switchgrass (Fig. 6).

It was found that NaOH pretreated switchgrass reached higher ethanol concentrations (10.86-11.57 g/L) and conversion efficiencies (65.85-72.37%) than H_2SO_4 pretreated (5.55-7.03 g/L and 40.59-49.28%) and untreated (1.71-2.61 g/L and 28.12-29.56%) switchgrass after 72-h fermentation (Fig. 6). The main reason for the low ethanol conversion efficiency from H_2SO_4 pretreatment was that the lignin formed cross-linkages with enzymes via hydrophobic adsorption under the condition of the low amount of enzymes [60]. Moreover, the highest ethanol concentration and conversion efficiency were achieved by big particle size for untreated (Fig. 6a...
and d), big particle size for H$_2$SO$_4$ pretreated (Fig. 6b and e), and small particle size for NaOH pretreated switchgrass (Fig. 6c and f). However, sugar yields from H$_2$SO$_4$ pretreated (27.92-34.29%) and untreated (28.12-29.56%) switchgrass were comparable but were half those of NaOH pretreated (61.58-66.64%) switchgrass (Fig. 6d-f). This is because NaOH pretreatment had higher glucan recoveries (14-30%) and ethanol conversion efficiencies (20-32%) than H$_2$SO$_4$ pretreatment under the same particle sizes (Fig. 2). The highest ethanol conversion yield was achieved by big particle size (29.54%) for untreated (Fig. 6d), middle particle size (30.60%) for H$_2$SO$_4$ pretreated (Fig. 6e), and small particle size (62.36%) for NaOH pretreated switchgrass (Fig. 6f). These results indicate that sugar degradation rate during pretreatment and ethanol conversion efficiency during fermentation should be taken into consideration when screening pretreatment methods. Therefore, the optimal pretreatment method is to ensure both high sugar recovery and ethanol conversion efficiency.

4. Conclusions

The responses of three switchgrass particle sizes to the H$_2$SO$_4$ and NaOH pretreatments were fully revealed. As particle size increased, glucan, xylan, and lignin contents of raw switchgrass slightly increased 0.59-1.70%; glucan and xylan contents in the NaOH pretreated switchgrass increased from 58.54 to 60.94% and 18.55 to 20.01%, respectively. Middle size switchgrass pretreated by H$_2$SO$_4$ had the highest glucan (55.76%) and lowest lignin (37.72%) contents, while big size switchgrass pretreated by H$_2$SO$_4$ had the highest lignin content of 44.32%. There are no visible variations in microstructures among different particle sizes. For NaOH pretreatment, glucan (91.95 to 94.69%), xylan (47.91 to 52.31%), and arabinan (42.66 to 70.07%) recoveries gradually increased as particle size increased. For H$_2$SO$_4$ pretreatment,
arabinan recovery increased from 3.97 to 5.27%, but the highest glucan (79.72%) and solid 
(44.00%) recoveries were obtained by middle-size switchgrass. The particle size had an 
inadequate influence on the chemical bonds of biomass according to FTIR spectra. The lowest 
(59.39% for H$_2$SO$_4$ and 58.99% for NaOH) and highest (65.23% for H$_2$SO$_4$ and 66.15% for 
NaOH) CrI values were gained from middle and small particle sizes, respectively. Raw and 
H$_2$SO$_4$ pretreated switchgrass showed comparable DSC patterns among different particle sizes, 
while notable variation was detected among different particle sizes for NaOH pretreatment. It 
was a big particle size for untreated and H$_2$SO$_4$ pretreated switchgrass to achieve the highest 
ethanol concentration and conversion efficiency, whereas it was a small particle size for NaOH 
pretreated switchgrass. However, the highest ethanol conversion yield was achieved by big 
particle size (29.54%) for untreated, middle particle size (30.60%) for H$_2$SO$_4$ pretreated, and 
small particle size (62.36%) for NaOH pretreated switchgrass.

Author's contributions

Jikai Zhao: experimental design, data interpretation, and manuscript writing-original draft, 
review & editing; Yang Yang: experimental design and implementation; Meng Zhang: review & 
editing; Charles W. Rice: resources; Donghai Wang: supervision, resources, review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References:


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https://doi.org/10.1021/acssuschemeng.1c05169.


J. Zhao, Y. Xu, W. Wang, J. Griffin, D. Wang, High Ethanol Concentration (77 g/L) of Industrial Hemp Biomass Achieved through Optimizing the Relationship between Ethanol Yield/Concentration and Solid Loading, ACS Omega. 5 (2020) 21913–21921. https://doi.org/10.1021/acsomega.0c03135.


S.I. Tsujiyama, A. Miyamori, Assignment of DSC thermograms of wood and its


Electronic copy available at: https://ssrn.com/abstract=4056867