Combined Deacetylation and PFI Refining Pretreatment of Corn Cob for the Improvement of a Two-Stage Enzymatic Hydrolysis

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ABSTRACT: A combined deacetylation and PFI refining pretreatment was applied to corn cob for the improvement of a two-stage enzymatic hydrolysis. In stage 1, the pretreated corn cob was first hydrolyzed by xylanase to produce xylo-oligosaccharides (XOS). In stage 2, the solid residue isolated from stage 1 was further hydrolyzed by cellulase and β-glucosidase. NaOH, Na2CO3, and Ca(OH)2 were tested to remove acetyl groups in the process of deacetylation, and it was found that Ca(OH)2 could be the most suitable alkali for deacetylation in this work. After deacetylation using 0.8 mmol of Ca(OH)2/g of substrate and PFI refining, 50.5% xylan in the raw material could be hydrolyzed into XOS. The corresponding xylan yield of stage 1, the glucan yield of stage 2, and the total sugar yield (all sugars released in the hydrolyzate) after the two-stage enzymatic hydrolysis were 0.306, 0.305, and 0.661 g/g of corn cob, respectively.

KEYWORDS: corn cob, deacetylation, PFI refining, enzymatic hydrolysis, pretreatment, xylo-oligosaccharides (XOS)

INTRODUCTION

Xylo-oligosaccharides (XOS) are widely used as food and feed additives, which are considered soluble dietary fibers that have prebiotic activity, favoring the improvement of bowel and immune functions and having antimicrobial and other health benefits.1 Currently, XOS are usually produced from the separated xylan or pretreated xylan-rich biomass through acidic or enzymatic hydrolysis.2−7 Corn cob is an inexpensive and widely available resource of hemicellulose, which is usually chosen to produce XOS.2−6,6 In comparison to the chemical technologies (e.g., acid hydrolysis), the enzymatic hydrolysis is a better route for the food industries, because no toxic and undesired byproducts, such as furfural, are generated during the enzymatic process.5 However, the enzymatic substrates are mostly prepared by severe processes, which would not be friendly to the environment. Furthermore, the cellulose in the xylan-deprived corn cob residue should be used in a more economical and environmentally benign method.

For enzymatic hydrolysis of biomass, pretreatment is an important technology to alter the structure of cellulosic biomass to make cellulosic/hemicellulose more accessible to enzymes.8 Nowadays, most commercially promising pretreatment processes (e.g., dilute acid/alkali pretreatment,9,10 steam explosion,11 sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) method,12 etc.) are mainly aimed to remove the lignin or hemicellulose through severe chemical reactions or mechanical treatment with respect to the properties of feedstock and the goal of end use. For corn cob, which has a high hemicellulose (32−39%) and low lignin content (9−14%),3,4,6 the acetyl groups on hemicellulose would be the main recalcitrance barrier, because the steric hindrance of acetyl groups could lower the activity of xylanase.13 Thus, the selective removal of acetyl groups from the backbone of xylan in feedstock by deacetylation can significantly improve the xylan digestibility and cellulose enzymatic hydrolysis.14 Acetyl xylan esterase,15,16 hydroxylamine solutions,17 and dilute alkali13 can be used for deacetylation, and the conditions of deacetylation are usually milder compared to the traditional pretreatments, because acetyl groups are more reactive than the other enzymatic hydrolysis hinders (e.g., lignin).18 Furthermore, deacetylation could reduce the acetic acid generated during pretreatment and enzymatic hydrolysis, which could restrain the growth of microbes in the ethanol fermentation.19,20

On the other hand, many mechanical refining technologies that have been widely applied in the pulp industry were used to increase enzymatic hydrolysis of various pretreated biomass. The applied technologies included disk-mill,12,21,22 PFI mill,23−25 food processor blender,22 extruder,22,25,26 Szego mill,23 etc. It was reported that all of the refining technologies could improve the biomass digestibility by 10−20%.22 Among them, the PFI mill was a commonly used laboratory mechanical refiner, which could provide accurate control and induce homogeneous refining effects on the substrate.27 As a lab equipment, the PFI mill could refine a small quantity of biomass and be easily handled. Also, the PFI mill was usually used to simulate a disc refiner, which could be used at a large scale, although the refining of the PFI mill differed from disc and conical refiners (PFI mill imposed a greater proportion of compressive to shear forces). It has been known that the PFI mill is a high-energy and low-intensity refiner and normally uses 10 times the energy typically consumed in industrial refiners.27
Therefore, in this work, to improve the XOS yield and efficiently use cellulose from corn cob, a combined deacetylation and PFI refining pretreatment process was established and a two-stage enzymatic hydrolysis for the pretreated corn cob was carried out. The two-stage enzymatic hydrolysis was also used to evaluate the effectiveness of the combined pretreatment method. In the first stage of enzymatic hydrolysis (stage 1), just xylanase was added to release XOS, while in the second stage of hydrolysis (stage 2), both cellulase and β-glucosidase were used to digest the solid residue obtained from stage 1, aiming for the full utilization of the corn cob.

Table 1. Component of Corn Cob before and after Deacetylation

<table>
<thead>
<tr>
<th>Alkali Charge (mmol/g of od substrate)</th>
<th>C_{glucan} (R_{glucan})</th>
<th>C_{xylan} (R_{xylan})</th>
<th>C_{arabin} (R_{arabin})</th>
<th>C_{acetyl} (R_{acetylation})</th>
<th>C_{lignin} (R_{lignification})</th>
<th>R_{acid} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>44.72 (92.9)</td>
<td>40.98 (84.50)</td>
<td>3.62 (90.81)</td>
<td>0.08 (97.89)</td>
<td>5.06 (63.68)</td>
<td>72.50</td>
</tr>
<tr>
<td>0.6</td>
<td>42.02 (99.44)</td>
<td>39.95 (91.84)</td>
<td>3.15 (90.02)</td>
<td>0.30 (90.99)</td>
<td>8.36 (31.64)</td>
<td>82.59</td>
</tr>
<tr>
<td>0.4</td>
<td>40.06 (100.44)</td>
<td>38.45 (95.68)</td>
<td>3.29 (99.60)</td>
<td>0.62 (80.27)</td>
<td>8.29 (28.19)</td>
<td>87.49</td>
</tr>
<tr>
<td>0.2</td>
<td>39.23 (101.72)</td>
<td>37.49 (96.49)</td>
<td>3.11 (97.38)</td>
<td>1.80 (40.77)</td>
<td>8.90 (20.23)</td>
<td>90.49</td>
</tr>
<tr>
<td>Na_{2}CO_{3}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>39.32 (98.23)</td>
<td>37.81 (93.76)</td>
<td>3.08 (92.92)</td>
<td>0.79 (74.95)</td>
<td>8.66 (25.24)</td>
<td>87.19</td>
</tr>
<tr>
<td>0.6</td>
<td>37.5 (99.71)</td>
<td>36.96 (97.55)</td>
<td>2.79 (89.59)</td>
<td>1.02 (65.58)</td>
<td>8.00 (26.50)</td>
<td>92.80</td>
</tr>
<tr>
<td>0.4</td>
<td>39.33 (101.19)</td>
<td>37.57 (95.94)</td>
<td>3.09 (96.00)</td>
<td>1.76 (42.53)</td>
<td>8.47 (24.70)</td>
<td>89.79</td>
</tr>
<tr>
<td>0.2</td>
<td>38.77 (100.75)</td>
<td>37.1 (95.69)</td>
<td>2.97 (93.20)</td>
<td>2.33 (23.16)</td>
<td>8.76 (21.34)</td>
<td>90.69</td>
</tr>
<tr>
<td>Ca(OH)_{2}</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.8</td>
<td>39.6 (96.76)</td>
<td>37.06 (89.89)</td>
<td>3.36 (99.15)</td>
<td>0.13 (95.97)</td>
<td>7.97 (32.70)</td>
<td>85.28</td>
</tr>
<tr>
<td>0.6</td>
<td>39.44 (97.91)</td>
<td>37.05 (91.30)</td>
<td>2.75 (82.44)</td>
<td>0.57 (82.04)</td>
<td>8.62 (26.02)</td>
<td>86.64</td>
</tr>
<tr>
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<td>39.27 (98.25)</td>
<td>37.26 (92.54)</td>
<td>3.17 (95.78)</td>
<td>0.86 (72.69)</td>
<td>8.63 (25.37)</td>
<td>87.32</td>
</tr>
<tr>
<td>0.2</td>
<td>38.51 (98.60)</td>
<td>36.51 (92.79)</td>
<td>3.11 (96.16)</td>
<td>1.90 (38.26)</td>
<td>8.83 (21.88)</td>
<td>89.36</td>
</tr>
<tr>
<td>Raw material</td>
<td>34.90</td>
<td>35.16</td>
<td>2.89</td>
<td>2.75</td>
<td>10.10</td>
<td></td>
</tr>
</tbody>
</table>

“Measured as Na_{2}O.

Materials. The corn cob was harvested in 2012, in Shandong province (China), further milled by an agriculture use hammer mill, and screened to obtain the fraction with a particle size between the meshes of 20 and 80 (i.e., 0.18–0.85 mm). The screened corn cob was stored in sealed plastic bags at room temperature to be ready for the component analysis and pretreatment. The solid content of the prepared corn cob was 93.9%.

Xylanase was generously provided by Habio Enzyme (Mianyang, Sichuan province, China), and its activity was 38 international units (IU)/mL. Celluclast 1.5 L (cellulase) and Novozyme 188 (β-glucosidase) were bought from Sigma-Aldrich (St. Louis, MO). The activities of cellulase and β-glucosidase were 121 filter paper units (FPU)/mL and 741 IU/mL, respectively. All of the enzymes and chemicals were prepared corn cob was 93.9%,

The crystallinities of corn cobs were measured using a Bruker D8 Advance X-ray diffractometer (Bruker Co., Germany). The crystallinity was prepared by placing approximately 0.1 g of the freeze-dried and milled biomass sample on a glass sample holder and then placing a drop of acetone on the sample to fix it on the holder. The crystallinity was scanned at 2θ/min from 2θ = 10–26° with a step size of 0.05°.
RESULTS AND DISCUSSION

Effect of the Pretreatment on Structural Features of Corn Cob. Table 1 shows the compositions of native and deacetylated corn cobs, as well as the recovery or removal rate of the corresponding component. In most cases, the deacetylation process could not efficiently remove the lignin, which was a main barrier of enzymatic hydrolysis, except using NaOH at an alkali charge of 0.8 mmol/g of substrate. A total of 63% delignification was achieved when using 0.8 mmol of NaOH/g of substrate, while the lignin removal at other conditions was just 20–32%. This was probably due to the fact that the acetyl groups were easier to remove compared to lignin. A lower dosage of NaOH (e.g., 0.6 mmol/g of substrate) could only sufficiently remove acetyl groups, while a higher dosage of NaOH (e.g., 0.8 mmol/g of substrate) could also provide enough alkali to remove much more lignin during deacetylation. Under these relatively mild alkaline conditions, the recovery rates of glucan and xylan after deacetylation were all higher than 92 and 84%, respectively (Table 1), because there was only a small amount of carbohydrates degraded in deacetylation (particularly for glucan). The glucan and xylan compositions of the samples were all improved by the removal of ash, lignin, soluble sugars, and other water-soluble extractives. Also, as shown in Table 2, after deacetylation, there was a slight increase (1–2%) of the crystallinity index of corn cob. This was because of the removal of amorphous components (i.e., acetyl groups and lignin) in deacetylated corn cob. However, the decrease of cellulose crystallinity after deacetylation was due to the increase of the cellulose content in the deacetylated corn cob (Table 1). After PFI refining, both the crystallinity index and cellulose crystallinity of deacetylated corn cob were decreased (Table 2). This was mainly due to the destruction of the crystalline region in cellulose caused by the compression and shear forces during PFI refining.

Unlike the delignification and crystallinity, the deacetylation increased significantly with the increase of alkali charge. For example, when the alkali charge was increased from 0.2 to 0.8 mmol/g of substrate using Na2CO3, the degree of deacetylation was increased from 23 to 74%. For the two weak alkali [Na2CO3 and Ca(OH)2], the effect of Ca(OH)2 on deacetylation was closer to that of NaOH compared to Na2CO3, because of the higher pH derived from Ca(OH)2 at the same alkali charge. For instance, 96% deacetylation could be achieved after Ca(OH)2 treatment with the alkali charge of 0.8 mmol/g of substrate at 70 °C for 3 h, suggesting that the mild process could efficiently remove the acetyl groups in corn cob.

Two-Stage Enzymatic Hydrolysis of Deacetylated Corn Cob. Low solids (2%) enzymatic hydrolysis was performed to test the digestibility of deacetylated corn cob in the near-ideal conditions, in which the mass transfer limitations between enzymes and substrate as well as the inhibition from products could be negligible. Figures 1 and 2 show the effect of the alkali charge on the percentage of enzymatic hydrolysis and the sugar yields of the two-stage hydrolysis of corn cobs, which were deacetylated by NaOH, Na2CO3, and Ca(OH)2, respectively. Both the percentage of enzymatic hydrolysis and the sugar yields were increased with the increase of the alkali charge. When the corn cobs were deacetylated by NaOH, Na2CO3, and Ca(OH)2 at the alkali charge of 0.8 mmol/g of substrate, the hydrolysis percentages of xylan in stage 1 were 93, 71, and 85%, respectively. The hydrolysis percentages of glucan in stage 2 were 86, 58, and 74%, respectively. For the case of Ca(OH)2, although the enzymatic hydrolysis percentage of xylan was lower than that for NaOH, the sugar yield (0.293 g/g of corn cob at 0.8 mmol/g of substrate) was similar to the sugar yield using NaOH (0.300 g/g of corn cob at 0.8 mmol/g of substrate) because of the high recovery of xylan after the deacetylation process could not effectively remove the acetyl groups in corn cob.

Table 2. Crystallinity Index of Corn Cob before and after Deacetylation and PFI Refining

<table>
<thead>
<tr>
<th></th>
<th>raw corn cob</th>
<th>deacetylated corn coba</th>
<th>deacetylated and PFI refined corn cobb</th>
</tr>
</thead>
<tbody>
<tr>
<td>crystallinity index (CrI) (%)</td>
<td>35.7</td>
<td>37.1</td>
<td>34.3</td>
</tr>
<tr>
<td>cellulose crystallinity (CCr) (%)</td>
<td>102.3</td>
<td>94.5</td>
<td>87.3</td>
</tr>
</tbody>
</table>

aDeacetylation condition: Ca(OH)2 of 0.4 mmol/g of substrate alkali charge.

bCrystallinity index and cellulose crystallinity of deacetylated corn cob.24

Figure 1. Percentages of enzymatic hydrolysis ($E_x$ and $E_g$) of the two-stage hydrolysis of corn cob deacetylated by different bases and alkali charges. (A) Percentages of enzymatic hydrolysis of stage 1 (xylanase hydrolysis). (B) Percentages of enzymatic hydrolysis of stage 2 (cellulase and β-glucosidase hydrolysis). Values of 0.8–0.2, samples deacetylated with the alkali charge of 0.8–0.2 mmol/g of substrate (NaOH and Na2CO3 were calculated as Na2O); control, water-treated sample.

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the corn cob could be highly digested in enzymatic hydrolysis with the alkali charge of 0.8 mmol/g of substrate (NaOH and Na2CO3 were calculated as Na2O); control, water-treated sample.

selectivity of xylanase. All of the proport ions of sugars in the hydrolyzate were the glucan in the hydrolyzed solid residue because of the most xylan was separated in stage 1 by xylanase, leaving most of the glucan in the deacetylated corn cob residue. All of the proportions of sugars in the hydrolyzates after stage 1 were similar to the proportion deacetylated by Ca(OH)2 with the alkali charge of 0.8 mmol/g of substrate (Table 3). XOS accounted for about 50% soluble sugar in the hydrolyzate. The comparatively low percentage of XOS could be caused by the long hydrolysis time, which could obtain a higher xylan yield because XOS could be further hydrolyzed into xylose by β-glucosidase contained in some xylanases, which had the ability to hydrolyze xyloligomers into xylose, thus removing the inhibition from xyloligomers during hydrolysis. This was in agreement with the fact that there were no xyloligomers detected in the hydrolyzate after one-stage hydrolysis. On the other hand, in the one-stage hydrolysis, three enzymes (cellulase, β-glucosidase, and xylanase) were added together, and the addition of xylanase may reduce the ineffective adsorption of cellulase to xylan and lignin, thus leading to the relative increase of cellulase activity. Therefore, a higher final sugar yield could be achieved by the one-stage enzymatic hydrolysis. However, in stage 1 of the two-stage enzymatic hydrolysis, used xylanase had a lower ability to hydrolyze xyloligomers to xylose. Thus, many xyloligomers could be removed after the hydrolysis of stage 1. Furthermore, in stage 2, the glucan yield was lower compared to the one-stage hydrolysis. This was possibly due to the relatively increased proportion of lignin in the xylan-deprived corn cob residue. The increased proportion of lignin might increase the ineffective adsorption of cellulase to lignin, thus lowering the glucan yield. However, although the sugar yields from the two-stage hydrolysis were lower than the one-stage hydrolysis, using xylanase and cellulase to “tailor” the deacetylated corn cob in two separated processes could make the component of the product more simplified, which is good for the further microbial or chemical utilizations. In addition, the optimization of enzymatic hydrolysis and the study of the inhibition of the XOS product in the future.

**Impact of Refining on the Enzymatic Hydrolysis.** As shown in Figures 3 and 4, the enzymatic hydrolysis percentages of xylan (E_X) in stage 1, the enzymatic hydrolysis percentages of glucan (E_G) in stage 2, and the total sugar yields (Y_b) were all increased after PFI refining. The increased extents of sugar yields ranged from 0.023 to 0.212 g/g of corn cob (Figure 4), which were comparable to the previous reports. However, the increased extents of hydrolysis percentages and sugar yields for the samples treated at harsh conditions were lower than that for the samples treated with mild conditions. For example, as shown in Figure 4A, 9.7% improvement of the enzymatic hydrolysis percentage of xylan could be obtained for the samples treated with 0.2 g of Ca(OH)2/g of substrate, while only 3.6% improvement could be achieved for the samples treated with 0.8 g of Ca(OH)2/g of substrate. Also, for the yields obtained from the two-stage enzymatic hydrolysis were somewhat lower compared to the traditional one-stage hydrolysis. This was likely because used cellulase and β-glucosidase contained some xylanases, which had the ability to hydrolyze xyloligomers into xylose, thus removing the inhibition from xyloligomers during hydrolysis. In addition, the optimization of enzymatic hydrolysis and the study of the inhibition of the XOS product in high solid loading (≥15%) hydrolysis will be conducted in the future.

### Table 3. Sugar Content of the Hydrolyzate after Xylanase Hydrolysis

<table>
<thead>
<tr>
<th>saccharide</th>
<th>glucose</th>
<th>xylose</th>
<th>X2</th>
<th>X3</th>
<th>X4–X7</th>
<th>arabinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>content</td>
<td>6.25</td>
<td>33.93</td>
<td>41.88</td>
<td>10.12</td>
<td>2.47</td>
<td>5.33</td>
</tr>
<tr>
<td>conversion (%)(b)</td>
<td>5.3</td>
<td>27.7</td>
<td>36.5</td>
<td>9.0</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
</table>

From the PFI refined corn cob [deacetylated by Ca(OH)2 with the alkali charge of 0.8 mmol/g of substrate], X2, xylobiose; X3, xylotriose; X4–X7, xylotetraose, xylopentaose, xylohexaose, and xyloheptaose. On the basis of the total sugar weight in the hydrolyzate. On the basis of xylan or glucan in the raw corn cob.

### Table 4. Comparison of the Sugar Yields Obtained from Different Enzymatic Hydrolysis Processes

<table>
<thead>
<tr>
<th></th>
<th>stage 1 (24 h)</th>
<th>stage 2 (48 h)</th>
<th>total yield</th>
<th>one-stage hydrolysis (48 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y_G1</td>
<td>Y_G2</td>
<td>Y_G</td>
<td>Y_G</td>
</tr>
<tr>
<td></td>
<td>0.293</td>
<td>0.278</td>
<td>0.328</td>
<td>0.336</td>
</tr>
<tr>
<td></td>
<td>0.299</td>
<td>0.321</td>
<td>0.336</td>
<td>0.340</td>
</tr>
</tbody>
</table>

*Hydrolysis substrate: PFI refined corn cob [deacetylated by Ca(OH)2 at the alkali charge of 0.8 mmol/g of substrate]. The total yield of two-stage hydrolysis (Y_G2 and Y_G) was calculated as follows: Y_G = Y_G1 + Y_G2; Y_G = Y_G1 + Y_G2. "Enzyme doses: cellulase, 18 FPU/g of od biomass; β-glucosidase, 9 IU/g of od biomass; and xylanase, 66 IU/g of od biomass.

Figure 2. Sugar yields (Y_G and Y_X) of the two-stage hydrolysis of corn cob deacetylated by different bases and alkali charges. Values of 0.8–0.2, samples deacetylated with the alkali charge of 0.8–0.2 mmol/g of substrate (NaOH and Na2CO3 were calculated as Na2O); control, water-treated sample.
control sample, the total sugar yield was 0.212 g/g of corn cob, while after PFI refining, the total sugar yield could be 2.7 times higher than that of the unrefined sample (Figure 4). This was because the size reduction that resulted from the PFI refining increased the reactive surface area for enzymes, thus leading to an increase of xylan and glucan yields.\(^{18}\) On the other hand, the decrease of crystallinity of cellulose (Table 2) caused by the compression and shear force of the PFI mill could also make cellulose more digestible.\(^{13}\) In addition, as presented in Figure 4, a total sugar yield of 0.661 g/g of corn cob could be obtained from the samples treated by 0.8 mmol of Ca(OH)\(_2\)/g of substrate, which was comparable to the yield (0.688 g/g of corn cob) obtained from the sample treated by NaOH with the same alkali charge. This was due to the fact that higher sugar recovery could be achieved in the deacetylation process by the use of Ca(OH)\(_2\). Therefore, it can be concluded that Ca(OH)\(_2\) is the more appropriate alkali for deacetylation and the suitable alkali charge is 0.8 mmol/g of substrate based on the results obtained in this study. Other benefits using Ca(OH)\(_2\) are that (1) Ca(OH)\(_2\) is an inexpensive and milder base compared to NaOH and (2) Ca(OH)\(_2\) can be easily recovered. Although some calcium ions may precipitate as calcium salts, precipitated calcium could be removed by sufficient washing (e.g., using vacuum washing equipment at large-scale production) after deacetylation. Another possible solution to avoid the precipitation of calcium is to add a small amount of chelating agent (e.g., EDTA) before deacetylation, so that the released calcium ions could be chelated and then removed during washing.

Mass Balance Analysis. The overall process and mass balance for the combined deacetylation [on the basis of Ca(OH)\(_2\)] and PFI refining pretreatment of corn cob plus the two-stage enzymatic hydrolysis are presented in Figure 5. For theoretical calculation, the sugar loss in PFI refining was neglected. As demonstrated in Figure 5, after Ca(OH)\(_2\) deacetylation (with the alkali charge of 0.8 mmol/g of substrate) and PFI refining, 79.8% xylan was hydrolyzed in...
stage 1 of enzymatic hydrolysis and 50.5% hemicellulose was converted to XOS. The cellulose in the solid residue separated from stage 1 could be highly digested by cellulase and β-glucosidase in stage 2. In total, 86.8% xylan and 86.1% glucan in the raw corn cob were hydrolyzed in the two-stage enzymatic hydrolysis, indicating that the combined Ca(OH)₂ deacetylation and PFI refining pretreatment process was a relatively effective method to improve the enzymatic digestion of corn cob. In addition, in comparison to the traditional one-stage enzymatic hydrolysis, the two-stage hydrolysis can make the resulting sugar products simpler and easier to be used in the downstream processes.

**ASSOCIATED CONTENT**

#### Supporting Information

X-ray diffraction (XRD) pattern of raw corn cob (Figure S1) and detailed calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

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*Technological Research and Development Program (QINGHAI) (JQ201305), and the National High Technology Research and Development Program (SPTOL) of China (2012AA022301).*

**Notes**

The authors declare no competing financial interest.

**ABBREVIATIONS USED**

XOS, xylo-oligosaccharides; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; NREL, National Renewable Energy Laboratory; HPLC, high-performance liquid chromatography; R̄_solid, recovery rate of solid after deacetylation; R̄_glucan, recovery rate of glucan; R̄_xylan, recovery rate of xylan; R̄_arabin, recovery rate of arabinan; D̄_deacetylation, de格unication percentage after deacetylation; D̄_degradation, de格unication percentage after deacetylation; Ē_X0, enzymatic hydrolysis percentage of xylan; Ē_Y0, enzymatic hydrolysis percentage of glucan; Ȳ_x, xylan yield; Ȳ_G, glucan yield; Ȳ_G, total sugar yield.

**REFERENCES**


