Effects of Mechanical Pretreatments on Enzymatic Hydrolysis of Mixed Lignocellulosic Substrates for Biorefineries

Yingying Hu, Bing Wang, Wei Liu, Ruijie Wu, and Qingxi Hou *

The enzymatic hydrolysis of lignocellulosic biomass into fermentable sugars remains a critical challenge for producing bio-based products due to complexity and recalcitrance of the biomass. The effects of mechanical pretreatments (i.e., wet grinding for corncob residues and PFI refining for wood fibers) on the enzymatic hydrolysis of mixed substrates of corncob residues (CRs) and wood fibers (WFs) were investigated. The results showed that the applied combined mechanical pretreatments noticeably enhanced the enzymatic digestibility of the mixed substrates. A 99.8% conversion yield of cellulose to glucose and 19.2 g/L glucose concentration were obtained when the mixed substrates (composed of the refined WFs and the ground CRs) were enzymatically hydrolyzed for 46 h. The improvements, including swelling ability, specific surface area, and morphology, were mainly attributed to the combined mechanical pretreatments.

Keywords: Corncob residues; Mixed substrates; Wet grinding pretreatment; Enzymatic hydrolysis

Contact information: Tianjin Key Laboratory of Pulp & Paper, Tianjin University of Science & Technology, Tianjin 300457, P.R. China; *Yingying Hu and Bing Wang contributed equally to this work; *Corresponding authors: wei.liu2009@hotmail.com; qingxihou@tust.edu.cn

INTRODUCTION

Lignocellulosic biomass is regarded as an ideal renewable resource to replace fossil fuels due to its abundance, wide availability, renewability, low cost, and low impact on the environment (Kataria et al. 2017). Typically, lignocellulosic biomass can be classified into virgin biomass (woody and herbaceous plants), waste biomass (forestry residues; agricultural residues such as corn stover, corncob, and wheat straw; and industrial and municipal solid wastes), and energy crops (e.g., switchgrass) (Xu et al. 2016). Corncobs, one of the many kinds of agricultural residues, are coproducts of corn production and have a global distribution. According to the United States Department of Agriculture (USDA), a total of 1098.95 million tons of global corns were produced in the period from 2018 to 2019 (USDA 2018). Because corncobs account for approximately 25% to 30% of the corn plant by mass (Potumarthi et al. 2012), it was estimated that 87.8 million to 105.3 million tons of corncobs were obtained in the USA. Meanwhile, it was reported that nearly 215 million tons of corn were generated in China in 2013, and approximately 34.4 million to 38.7 million tons of corncobs can be obtained (Du et al. 2015). Corncobs have been widely used to produce various industrial products such as furfural, oligosaccharides, xylose, xylo-oligomers, xylitol, lactic acid, and so on (Fang et al. 2010). In addition, corncobs have many distinctive advantages, e.g., large abundance, high cellulose content, low lignin content, low bulk density, high energy density, and small particle size (Chang et al. 2017). A large amount of corncob residues (CRs) was generated after the pre-extraction of...
hemicelluloses to produce hemicellulose-based derivatives in the above industrial processes (Bai et al. 2016). Thus, CRs have been conventionally burned as one kind of solid wastes (Bu et al. 2012), while a series of environmental problems such as atmospheric pollution and acid rain are simultaneously caused. It was previously reported that CRs have many advantages for enzymatic hydrolysis, e.g., CRs have a high cellulose content but a low lignin content, and a unique structure that can be readily disrupted (Tang et al. 2011). CRs will become porous due to the removal of hemicelluloses (Liu et al. 2016); thus it is expected that the specific surface area of CRs will easily increase and exhibit a high adsorption capacity. These unique characteristics could promote the binding ability and accessibility of CR substrates to enzymes, and thereby facilitate the subsequent enzymatic hydrolysis. Therefore, CRs could potentially be used as one ideal raw material to produce fermentable sugars.

In this study, wet grinding and PFI refining were applied on corn cob residues and wood fibers, respectively. The pretreated substrates were then mixed together for the subsequent enzymatic hydrolysis. This study investigates the enzymatic hydrolysis performance of the mixed substrate of WFs and CRs. It is expected that the combination of mechanical pretreatment and mixing different substrates can be potentially adopted in an integrated biorefinery.

EXPERIMENTAL

Materials

In the mill located in Henan Province, China, the corn cobs were pre-extracted with sulfuric acid to obtain xylose for producing xylitol. The CRs were air-dried, mechanically crushed, and sieved with a 40-mesh screen. The sieved CR powder was then collected and regarded as one substrate. Fibers from a bleached hardwood kraft pulp (WFs) with an initial beating degree of 12 °SR were chosen as the other substrate. The chemical compositions of both the CRs and WFs were measured according to the appropriate NREL standards NREL/TP-510-42618 (Sluiter et al. 2012) and NREL/TP-510-42619 (Sluiter et al. 2008).

Cellulase was provided by Vlan Biotech Group (Qingdao, China). The cellulase activity (138 FPU/mL) was determined according to the standard NREL/TP-510-42628 (Adney and Baker 2008). The D-(+)-glucose used as the standard was purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). Other chemical reagents used in this work were analytical grade.

Mechanical pretreatments

The CRs were ground using a basket mill (RT-LMO.75; Shanghai Root Mechanical and Electrical Equipment Co., Ltd., Shanghai, China) at a solid to liquid ratio of 1:10 (w/v) at 1000 rpm for 30 min at room temperature. The obtained CRs were named ground corn cob residues (GCRs). The WFs were refined to different beating degrees using a PFI mill (P40110.E000; PTI, Vorchdorf, Austria) following the standard TAPPI T248 sp-00 (2000).

Methods

Enzymatic hydrolysis

The enzymatic hydrolysis was performed in a 100-mL conical flask with 50 mL of reaction volume under the following conditions: 9 FPU/g substrates of cellulase loading,
2.0% (w/w) solids content, pH of 4.8, 50 °C, and 180 rpm for 96 h. During this period, 0.5 mL of enzymatic hydrolysates were collected in a 1.5-mL Eppendorf tubes at regular intervals. The removed enzymatic hydrolysates were promptly deactivated (by immersing the tubes in boiling water for 5 min) and centrifuged at 10,000 rpm for 5 min. The glucose concentration of the test samples was measured using the high performance liquid chromatography method reported by the authors’ previous work (Liu et al. 2015). The conversion yield of cellulose to glucose was calculated according to Eq. 1,

\[
\text{Conversion yield of cellulose to glucose (\%) = } \frac{G}{G_s} \times 100
\]

where \(G_s\) is the amount of glucose in pulp substrates (g), and \(G\) is the amount of glucose in test samples (g).

**Analysis of swelling ability**

The water retention value (WRV) was measured to indicate the swelling ability of the substrates according to previous literature (Meng et al. 2014). First, 0.15 g (equivalent to oven-dried weight) of test substrates was dispersed in 300 mL water, transferred to a centrifuge tube, filtered into a pulp cake by vacuum pump, and centrifuged at 3000 g for 15 min using a centrifugal machine (D-37520; SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). The WRV was calculated using Eq. 2,

\[
\text{WRV (\%) = } \frac{m_1 - m_2}{m_2} \times 100
\]

where \(m_1\) is the weight (g) of samples after centrifugation and \(m_2\) is the weight (g) of oven-dried samples.

**Analysis of specific surface area**

The specific surface area (SSA) of the substrates was measured by using the dye adsorption procedure with Chicago sky blue 6B (Alfa Aesar Chemical Co., Ltd., Shanghai, China) (Liu et al. 2016). First, a certain weight of the test substrates was added into the dye solution at a solid/liquid ratio of 1/100 (w/v). The dosage of NaCl added into the suspension was 20% (of oven-dried weight of test substrates). The mixture was kept at 60 °C for 28 h using a water bath. The concentration of Chicago sky blue 6B was calculated by the standard curve of the relationship between the solution’s absorbance at 600 nm and concentration. The SSA of the test substrates was calculated according to the method described in the authors’ previous work (Liu et al. 2016), and was calculated using the Eq. 3,

\[
A_{\text{cell}} = 6.024 \times 10^{23}[S]_f A_d / M
\]

where \(A_{\text{cell}}\) is the SSA (m²/g), \(A_d\) is the area occupied by each dye molecule (240 \times 10^{-20} m²), \(M\) is the relative molecular weight of dye molecules (999.79), \([S]_f\) is the saturation value of fiber adsorption (g/kg), which can be obtained from the following equation (Hall and Perkins 1971),

\[
1 / [D]_f = 1 / (K[S]_f \times [D]_i) + 1 / [S]_f
\]

where \([D]_f\) is the dye amount adsorbed on fiber (g/kg), \([D]_i\) is the initial dye concentration (g/L), and \(K\) is a constant.

All of the above experiments were conducted in triplicate, and the results were analyzed and reported at a 95% confidence interval. All data was processed with Origin 9.1 software (OriginLab Corporation, Wellesley Hills, MA, USA).
Analysis of the substrate’s surface morphology

A scanning electron microscope (SEM SU1510; Hitachi Ltd., Tokyo, Japan) was used to analyze the surface morphology of test substrates. First, the test substrates were kept in a vacuum oven (VOS–30A; Shanghai STIK Co., Ltd., Shanghai, China) at 50 °C for 24 h. Next, a sputter coating was conducted with gold palladium on an auto fine coater (JEC-3000FC; JEOL Ltd., Tokyo, Japan). An optical microscope (706272; Nikon Corporation, Tokyo, Japan) was also used to observe the morphology and mixing performance of substrates.

RESULTS AND DISCUSSION

Chemical Compositions of Substrates

The chemical compositions of CRs and WFs are summarized in Table 1. The results showed that glucan was the main chemical constituent of CRs (57.3%) and WFs (75.3%). Due to the sulfuric acid pre-extraction of corncob in the mill, the xylan and araban contents of the CRs were low at 4.2% and 0.04%, respectively. Most of the hemicelluloses were removed and solubilized into the prehydrolysates during the acid treatment, which consequently reduces the enzyme demand for the hydrolysis of hemicelluloses (Agrawal et al. 2016). The total lignin content of CRs (17.7%) was much higher than that of WFs (2.8%). It is reported that lignin inhibits saccharification by the non-productive bonding of cellulase, acting as a physical barrier blocking enzymes from carbohydrates, and by deactivating enzymes via lignin-derived inhibitors (Yu et al. 2016), and that hydrolysis was remarkably improved as the removal of lignin was increased up to 30% (Sun et al. 2011). Therefore, it was expected that the enzymatic digestibility of CRs would be lower than that of WFs. Because the mechanical pretreatments have little effect on the chemical compositions of raw materials (Zhang et al. 2016), the chemical compositions of GCRs and refined WFs were considered to be the same as those of the CRs and WFs, respectively. In the present work, the conversion yield of cellulose to glucose was chosen to represent the total sugar conversion yield because the xylan content in both substrates was very low.

<table>
<thead>
<tr>
<th>Raw Materials</th>
<th>CRs</th>
<th>WFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucan (%)</td>
<td>57.3 ± 0.4</td>
<td>75.3 ± 0.2</td>
</tr>
<tr>
<td>Xylan (%)</td>
<td>4.2 ± 0.1</td>
<td>13.5 ± 0.1</td>
</tr>
<tr>
<td>Araban (%)</td>
<td>0.04 ± 0.01</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Acid insoluble lignin (%)</td>
<td>17.0 ± 0.2</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>Acid soluble lignin (%)</td>
<td>0.6 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Total lignin (%)</td>
<td>17.7 ± 0.2</td>
<td>2.8 ± 0.0</td>
</tr>
<tr>
<td>Ethanol extractives (%)</td>
<td>10.6 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.9 ± 0.1</td>
<td>0.6 ± 0.0</td>
</tr>
</tbody>
</table>

Separate Enzymatic Hydrolysis of Substrates

Both CRs and WFs were enzymatically hydrolyzed separately, and their enzymatic hydrolysis performances are shown in Fig. 1. The results showed that the enzymatic digestibility of the WFs was much higher than that of CRs. The glucose concentration of WFs reached approximately 14.0 g/L after 59 h of enzymatic hydrolysis, while that of CRs was only 10.0 g/L after 75 h of enzymatic hydrolysis, as shown in Fig. 1a. In addition, the
conversion yield of cellulose to glucose of WFs was much higher than that of the CRs. After 59 h of enzymatic hydrolysis, the conversion yields of cellulose to glucose for WFs and CRs were 86.1% and 75.8%, respectively, as shown in Fig. 1b.

![Graph](image_url)

**Fig. 1.** Enzymatic hydrolysis performance of CRs, GCRs, and WFs

The CRs were subjected to a wet grinding pretreatment with a basket mill prior to enzymatic hydrolysis to improve enzymatic digestibility. The results revealed that the glucose concentration and conversion yield of cellulose to glucose for the GCRs increased 26.6% and 26.3%, respectively, indicating that the enzymatic digestibility of the GCRs was remarkably enhanced by the wet grinding treatment. In addition, it was found that the enzymatic hydrolysis rate of the GCRs was much faster than that of the WFs and CRs during the initial enzymatic hydrolysis stage, as shown in Fig. 1. After an enzymatic hydrolysis time of 46 h, the conversion yield of cellulose to glucose of the GCRs was 97.9%, while that of the CRs and WFs were 73.8% and 82.6%, respectively. The improved enzymatic digestibility of the GCRs was mainly attributable to the altered characteristics.
of the CRs after the wet grinding treatment. Therefore, these characteristics (SSA and WRV) of the WFs, CRs, and GCRs were also analyzed, and the results are shown in Fig. 2. The SSA has been considered to be one major factor correlating with the enzymatic hydrolysis of pretreated biomass, as it affects the accessibility of cellulose to enzymes (Zhang et al. 2015); improved SSAs can positively increase the accessibility of enzymes to cellulose in substrates (Fernandes et al. 2015). It was also reported that the enzymatic hydrolysis was obviously increased with improved mechanical refining intensity as measured by the WRV (Jones et al. 2013). Indeed, the results showed that the WRV and SSA of the GCRs were much higher than those of the CRs and WFs, which would be favorable for the absorption of enzymes on substrates and would increase the accessibility of cellulose to enzymes (Duan et al. 2016).

![Fig. 2. WRV and SSA of CRs, GCRs, and WFs](image)

**Fig. 2.** WRV and SSA of CRs, GCRs, and WFs

![Fig. 3. Substrate morphologies as observed by SEM (a- CRs; b- GCRs) and optical microscope (c- CRs; d- GCRs; e- mixed substrates of WFs and CRs; f- mixed substrates of WFs and GCRs)](image)

**Fig. 3.** Substrate morphologies as observed by SEM (a- CRs; b- GCRs) and optical microscope (c- CRs; d- GCRs; e- mixed substrates of WFs and CRs; f- mixed substrates of WFs and GCRs)
Moreover, the GCRs showed a rough surface and obvious fibrillation after basket mill grinding, which made them further amenable to enzymatic hydrolysis \cite{Miao et al. 2015}, as shown in Fig. 3. Consequently, the conversion yield of cellulose to glucose of GCRs was higher than that of WFs and CRs. However, the glucose concentration of the GCRs was lower than that of WFs because the glucan content of the GCRs was lower than that of WFs, as shown in Table 1. Additionally, it can be clearly seen that there was much more GCR adsorbed on WFs than on CRs, as shown in Fig. 3e and Fig. 3f. Good adsorption capacity of GCRs would promote the accessibility between the enzymes and substrates by increasing the exposure between these two. Therefore, considering good enzymatic digestibility of GCRs and high glucan content of WFs, a novel enzymatic hydrolysis method of mixing different substrates of GCRs and WFs and further assisting by appropriate mechanical pretreatments was proposed to obtain a good enzymatic hydrolysis performance. The enzymatic hydrolysis performance of the mixed substrates (consisting of WFs and GCRs) is investigated in the following sections.

**Enzymatic Hydrolysis of Mixed Substrates**

Fig. 4. Characteristics and enzymatic hydrolysis efficiency of mixed substrates of WFs and GCRs

Figures 4a and 4b show that the SSA and WRV of the mixed substrates were much higher than those of WFs and that the SSA and WRV continually increased with increasing percentages of GCRs. This indicated that the mixed substrates would exhibit a good enzymatic digestibility, which was confirmed by the enzymatic hydrolysis results. As
presented in Fig. 4c, the glucose concentration of the mixed substrates was much higher after adding GCRs, and it gradually increased with increasing percentages of GCRs. When the percent GCR was increased from 0% to 20% (of the oven-dried weight of WFs), the glucose concentration increased sharply from 14.0 g/L to 17.2 g/L after a 96 h enzymatic hydrolysis. The additional glucose content was mainly provided by the GCRs in the mixed substrates. It was assumed that, if these two substrates of CRs and WFs were separately hydrolyzed without any interactions between each other, the theoretical glucose concentration and conversion yield of cellulose to glucose could be calculated, as shown in Table 2. The results showed that the experimental results, including glucose concentration and conversion yield of cellulose to glucose, were much higher than the theoretical values, which proved that adding the GCRs enhanced the enzymatic hydrolysis efficiency. The addition of GCRs may have had bridging effects between the substrates and enzymes, carrying more enzymes by GCRs to react with WFs and, consequently, increasing the enzymatic hydrolysis. When 20% GCR was added to the mixed substrates, the glucose concentration of the mixed substrate increased 21%, which will have great significance on subsequent sugar fermentation processes.

### Table 2. Theoretical and Actual Enzymatic Hydrolysis Results

<table>
<thead>
<tr>
<th>Samples</th>
<th>Theoretical Glucose Concentration (g/L)</th>
<th>Actual Glucose Concentration (g/L)</th>
<th>Theoretical Conversion Yield of Cellulose to Glucose (%)</th>
<th>Actual Conversion Yield of Cellulose to Glucose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFs + 10% GCRs</td>
<td>14.3</td>
<td>15.9</td>
<td>87.3</td>
<td>88.3</td>
</tr>
<tr>
<td>WFs + 20% GCRs</td>
<td>14.2</td>
<td>17.2</td>
<td>88.1</td>
<td>89.1</td>
</tr>
<tr>
<td>WFs + 40% GCRs</td>
<td>13.9</td>
<td>16.3</td>
<td>89.4</td>
<td>79.5</td>
</tr>
</tbody>
</table>

Note: Theoretical glucose concentrations and conversion yields of cellulose to glucose were obtained when the amount of glucan in WFs and GCRs were converted into glucose separately, without any interaction between the two, under the equilibrium status.

However, the conversion yield of cellulose to glucose of the mixed substrates remained almost constant as the percentage of GCRs in the mixed substrates was increased, as shown in Fig. 4d. In the initial stage, with increasing GCR loadings, the trend of the slope of the conversion rate became steep, which suggested that GCRs enhanced the enzymatic hydrolysis efficiency. However, the benefit of mixing different substrates for enzymatic hydrolysis was not as obvious as was originally expected. Moreover, it was interesting to note that a lower conversion yield of cellulose to glucose of the mixed substrates was obtained when the GCR percentage was further increased from 20% to 40% (of the oven-dried weight of WFs), which was likely due to the high lignin content in the GCRs. At 20% GCR (of the oven-dried weight of WFs) and after 59 h of enzymatic hydrolysis, the glucose concentration and the conversion yield of cellulose to glucose of the mixed substrates were much higher than that obtained under other conditions, i.e., 17.2 g/L and 89.0%, respectively. Therefore, the optimized percentage of the GCRs was fixed as 20% (of the oven-dried weight of WFs).
Enhanced Enzymatic Hydrolysis of Mixed Substrates by Mechanical Pretreatment

In previous works it was found that PFI refining can significantly enhance the enzymatic hydrolysis efficiency of wood-fiber substrates (Jones et al. 2013; Liu et al. 2017). It was also reported that the enzymatic hydrolysis efficiency was closely related to the substrates’ beating degree (Xu et al. 2014). Thus, mechanical refining with a PFI mill was applied on WFs in the present work. To further enhance the enzymatic hydrolysis efficiency of the mixed substrates, the WFs were refined to different beating degrees via a PFI mill, and, then the GCRs were mixed well with the refined WFs to a percentage of 20% (of the oven-dried WFs weight). These samples then underwent enzymatic hydrolysis, and the results are shown in Table 3 and Fig. 5. As shown in Fig. 5a and 5b, it was found that the enzymatic hydrolysis efficiency of WFs was increased notably through increasing the degree of beating and that the addition of GCRs enhanced the enzymatic hydrolysis efficiency of the mixed substrates. When the beating degree of WFs was 12 °SR, the glucose concentration of the mixed substrates (WFs + 20% GCR) was 22.9%, which was much higher than that of 100% WFs. When the beating degree of the WFs in the mixed substrates were increased from 12 °SR to 21 °SR, the glucose concentration of the mixed substrates (WFs + 20% GCR) increased from 16.4 g/L to 19.2 g/L, and the conversion yield of cellulose to glucose improved from 84.8% to 99.8% at an enzymatic hydrolysis time of 46 h. However, the enzymatic hydrolysis efficiency was not further increased when the beating degree was increased from 21 °SR to 40 °SR in this study. In the authors’ previous work, it was also found that the enzymatic hydrolysis efficiency of hardwood bleached kraft pulp fibers did not increase with increasing PFI refining intensities (Liu et al. 2016). The present work used a combination of pretreatment methods of mechanical refining and mixing different substrates of CRs and WFs for subsequent enzymatic hydrolysis (instead of chemical pretreatments), and the results had already indicated an obvious superiority.
Fig. 5. Enhanced enzymatic hydrolysis efficiency of substrates mixed with refined WFs and GCRs

Table 3. Characteristics of Mixed Substrates of Refined WFs and GCRs

<table>
<thead>
<tr>
<th>Samples</th>
<th>WRV (%)</th>
<th>SSA (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCRs</td>
<td>201.5 ± 3.1</td>
<td>328.0 ± 3.8</td>
</tr>
<tr>
<td>WFs (12 °SR)</td>
<td>80.1 ± 1.4</td>
<td>85.2 ± 4.3</td>
</tr>
<tr>
<td>WFs (12 °SR) + 20% GCR</td>
<td>97.3 ± 2.1</td>
<td>133.2 ± 6.7</td>
</tr>
<tr>
<td>WFs (21 °SR)</td>
<td>133.8 ± 3.3</td>
<td>99.7 ± 5.3</td>
</tr>
<tr>
<td>WFs (21 °SR) + 20% GCR</td>
<td>153.1 ± 0.5</td>
<td>210.8 ± 10.5</td>
</tr>
<tr>
<td>WFs (40 °SR)</td>
<td>168.2 ± 1.2</td>
<td>148.6 ± 6.9</td>
</tr>
<tr>
<td>WFs (40 °SR) + 20% GCR</td>
<td>181.1 ± 1.1</td>
<td>209.0 ± 10.5</td>
</tr>
</tbody>
</table>

The WRV and SSA of the mixed substrates were analyzed, and the results are shown in Table 3. The results showed that the WRV and SSA of the mixed substrates were much higher than those of WFs but lower than those of GCRs, and that they increased with increasing beating degrees of WFs. When the beating degree of WFs was 12 °SR, the WRV
and SSA of the mixed substrates (WFs + 20% GCR) were 97.2% and 133.2 m²/g, whereas those of the WFs were only 80.1% and 85.2 m²/g, respectively. For a WFs beating degree of 21 °SR, the SSA of the mixed substrates (WFs + 20% GCR) was 210.8 m²/g, and the WRV was 153.1%. The improved SSA and WRV of the mixed substrates were mainly caused by the adsorption between the GCRs and the WFs, and further promoted the contact accessibility between enzymes and the substrates. These results were consistent with the enzymatic hydrolysis performance of the mixed substrates, i.e., the conversion yield of cellulose to glucose for WFs or the mixed substrates (WFs + 20% GCR) was not changed obviously through an increase in the beating degree of the WFs from 21 °SR to 40 °SR, as shown in Fig. 5a. Therefore, considering the enzymatic hydrolysis performance and the refining energy input, the mechanical refining with a certain intensity was sufficient and appropriate for the enzymatic hydrolysis of WFs.

CONCLUSIONS

1. The addition of ground corn cob residues (GCRs) enhanced the enzymatic hydrolysis of the mixed substrates of GCRs and WFs, especially on the improvement of glucose concentration. Mechanical refining of WFs further enhanced the enzymatic hydrolysis performance of the mixed substrates.

2. The mechanical pretreatments changed the characteristics of the substrates, i.e., their SSA, WRV, and morphology. When the GCRs were mixed (20% GCR of the oven-dried weight of WFs) with refined WFs (beating degree of 21 °SR), a high enzymatic hydrolysis efficiency of the mixed substrates was obtained, i.e., 99.8% conversion yield of cellulose to glucose and 19.2 g/L of glucose concentration after 46 h of enzymatic hydrolysis.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 31770634 and 31300491), the Natural Science Foundation of Tianjin City (17JCYBJC20600).

REFERENCES CITED


Article submitted: November 27, 2018; Peer review completed: April 7, 2019; Revisions received: April 21, 2019; Revisions accepted: April 22, 2019; Published: April 24, 2019. DOI: 10.15376/biores.14.2.4639-4652