Strategy to Utilize the High Ash Content Biomass Feedstock for Fermentable Sugars

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A prewashing step was used to remove ash from straw pulping solid residue (waste wheat straw, WWS) prior to pretreatment and enzymatic hydrolysis. The effects of prewashing on the effectiveness of liquid hot water pretreatment (LHWP) and dilute acid pretreatment (DAP) were investigated. Prewashing effectively removed the ash in raw WWS. However, a certain amount of polysaccharides was also removed. The decreased pH of hydrolyzate after pretreatment suggested that both the prewashing and DAP could destroy the buffering effect of ash and thus improve the pretreatment efficiency. Consequently, the highest enzymatic hydrolysis yields of 79.4% and 76.1% could be obtained for LHWP and DAP, respectively. In addition, the LHWP resulted in the highest sugar recovery of 84.4% at 180 °C, and a sugar recovery of 86.8% was also reached by DAP at 160 °C. LHWP combined with prewashing strongly facilitated the enzymatic digestibility of WWS pretreated at 180 °C. However, for pretreatment at low temperature, DAP was more suitable.

Keywords: Waste wheat straw; Prewashing; Liquid hot water pretreatment; Dilute acid pretreatment; Enzymatic hydrolysis yield

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INTRODUCTION

The pulp and paper industry is an important industrial sector in China. It is estimated that more than 50 Mt paper has been consumed every year in the past decade, and the amount still grows with an annual rate of 10.2% (Zhang et al. 2012). Traditionally, hardwood and softwood are used as the raw materials for paper making. In China, a leading agricultural country, the wheat straw is usually used as an alternative feedstock for pulp making. Prior to the pulping process, the harvested crude wheat straw has to be screened and sorted to remove the contained wheat ears, leaves, and straw scraps, which have too low a quantity for the pulp production. This separating process generates a large amount of solid residues, which has been named waste wheat straw (WWS) in this study. It is estimated that an annual output of WWS is more than 2×10^5 tons in a straw pulp mill in China. Generally, the residues are simply burned without effective application, in addition to causing a serious environmental problem. Thus, exploring an efficient and environmentally friendly way of utilizing those residues should be put forward.

During the past decades, the reserves of the global fossil fuel have continued to decrease due to the expanding demand from humans and industry. Recently, much research has been devoted to exploring some efficient and renewable alternatives. Bio-ethanol has long been regarded as one of the most potential renewable energies because of the proven technique by which it is derived (Cannatelli and Ragauskas 2016). The high price of the...
raw material, however, has been a key obstacle towards the large-scale application of lignocellulosic ethanol. For example, the price of corn stover is approximate 83 dollar/ton, which indicates the cost in feedstock will reach >4×10^7 dollars for a bio-ethanol plant with an annual output of 1×10^5 tons (based on 5 ton biomass producing 1 ton of ethanol) (Kazi et al. 2010). Such a huge cost in raw material highlights the advantages of using WWS as feedstock for the production of bio-ethanol, because its cost for a functioning pulping mill is zero. To the authors’ knowledge, papers about bio-refinery seldom include these solid residues from the pulping industry. Hence, using the WWS for bio-ethanol production is a creative work that merits further investigation.

Technically, bio-ethanol production from lignocellulosic biomass includes pretreatment, enzymatic hydrolysis, fermentation, and ethanol distillation, in which pretreatment is the first and most important step. The pretreatment process aims to break down the recalcitrant structure of lignocellulosic materials and facilitate the following enzymatic hydrolysis (Hendriks and Zeeman 2009). Various pretreatment technologies have been investigated, which can be characterized as physical, chemical, biological, or in combination (Knappert et al. 1981; Chang et al. 1998; Park et al. 2016). All of the reported pretreatment technologies either dissolve the lignin or degrade the hemicellulose, or both, to enhance the accessibility of cellulose to enzymes. In addition, it has been reported that the removal of hemicellulose shows a better improvement in the enzymatic digestibility of the pretreated solid, compared with lignin removal (Meng et al. 2015). Liquid hot water pretreatment (LHWP) and dilute acid pretreatment (DAP), the two typical pretreatments that can cause the degradation of hemicellulose, were therefore chosen for study in this research. Liquid hot water pretreatment has the advantage of having no chemicals as well as being environmentally friendly (Huang et al. 2016a). The DAP process has the potential to achieve the highest sugar yield compared with other pretreatments. Although LHWP and DAP have been extensively studied on many feedstocks such as corn stover, wheat straw, and wood (Teramoto et al. 2008; Rajan and Carrier 2014; Geng et al. 2015), their effects on the straw pulping solid residue have never been investigated. The WWS has an ash content as high as 29.5%, which was much higher than the content in conventional lignocellulosic biomass, such as wood (Huang et al. 2017). He et al. (2014) has reported that the ash in material has a negative effect not only on the enzymatic hydrolysis but also on the pretreatment efficiency. In consideration of that, some de-ashing treatments prior to pretreatment can be beneficial.

Previous work by the authors has established that the ash exerted a buffering effect through its cation exchange capacity (Huang et al. 2016b). The prewashing effectively removed the ash and thus improved the pretreatment efficiency. However, the main problem of the previous work was the high water consumption (as high as 500 g/g-WWS) which was unpractical for large-scale application. Moreover, the previous work mainly considered the enzymatic hydrolysis yield of the liquid hot water pretreated solid without any further information. In this study, the water dosage in the prewashing step was decreased and sulfuric acid was introduced to the pretreatment system. Meanwhile, the effects of LHWP and DAP on the prewashed WWS were compared for the first time on the basis of pretreatment hydrolyzate, enzymatic hydrolysis, microstructure, and mass balance.
EXPERIMENTAL

Materials
Waste wheat straw was provided by a straw pulp mill in Liaocheng City, Shandong Province, China. The raw material was air-dried in a dark room before prewashing and pretreatment.

Methods

Prewashing
Prewashing of WWS was conducted in a bucket with water dosages of 50 and 100 g/g-WWS to remove the free ash. In detail, 200 g of WWS was soaked in the water for 10 min with agitation. The suspended WWS was then collected and filtered by a cloth bag to remove the water and ash. The collected solid of prewashed WWS was stored at 4 °C to balance the moisture before pretreatment.

Pretreatment
Liquid hot water pretreatment (LHWP) and dilute acid pretreatment (DAP) were conducted in a laboratory oil bath with 10 stainless steel tubes (1 L). The pretreatments were carried out at the temperature of 160 and 180 °C for 40 min with the solid to liquid ratio of 1:10. The dilute acid pretreatment was conducted with the sulfuric acid charge of 0.4% and 0.8% (w/v). Briefly, 50 g raw or prewashed WWS (dry weight) was mixed with distilled water or acid solution and impregnated at 60 °C for 30 min. After sufficient impregnation, the temperature was raised to the target temperature at a rate of 1 °C/min and maintained for 40 min. After pretreatment, the tubes were cooled down by putting them into a cold water bath. Then the prehydrolyzate and solid were separated with a cloth bag. The separated solid was washed with 500 mL of distilled water three times and stored at 4 °C for subsequent experiments. The prehydrolyzate was collected for the analysis of the pH and the concentrations of sugars and inhibitors.

Enzymatic hydrolysis
Cellulase (Cellic® CTec2), kindly provided by Novozymes (Franklinton, NC), was used for enzymatic hydrolysis in this study. Enzymatic hydrolysis was performed in 150 mL flasks with a working volume of 50 mL at substrate loading of 5%. In detail, 2.5 g of each sample (dry weight) was mixed with cellulase at the loading of 25 g/g-cellulose. Next, 2.5 mL of 1 M acetate buffer was added to adjust the pH to around 4.8. Tetracycline solution (0.2 g/L) was used in the enzymatic hydrolysis system to inhibit the growth of microorganisms. After supplementing to 50 mL with distilled water, the flasks were put in a shaking incubator at 50 °C and 150 rpm for 48 h. After enzymatic hydrolysis, 1 mL of sample was withdrawn for centrifugation (10000 rpm for 10 min), and the supernatant was diluted for sugar analysis by HPLC. All experiments were carried out in duplicate, and the results were presented by considering the average value of the two replicated tests.

Analytical methods
A scanning electron microscope (SEM, FEI Quanta 400, Hitachi, Japan) at 25 kV was used to analyze the microstructure of the pretreated WWS. Prior to tests, each sample was sputter coated with gold using a SC7640 automatic/manual high-resolution sputter coater (Quorum Technologies, Newhaven, UK.)
The chemical compositions of all the samples in this study were analyzed according to a standard analysis procedure provided by National Renewable Energy Laboratory (NREL), including cellulose, xylan, arabinan, total lignin, and ash (Ehrman 1994, 1996; Templeton and Ehrman 1995; Ruiz and Ehrman 1996). To determine the amount of sugars in prehydrolyzate, a post-hydrolysis with 4% (w/w) H$_2$SO$_4$ at 121 °C for 1 h was performed to hydrolyze the oligosaccharides into monosaccharides.

All the monomeric sugars and inhibitors in this study were measured by high performance liquid chromatography (HPLC). The HPLC system (Agilent 1260 series, Agilent Technologies, Santa Clara, CA, USA) was equipped with a refractive index detector. A Bio-Rad Aminex HPX-87H column maintained at 55 °C was used to realize the separation of different components with 5 mM H$_2$SO$_4$ as eluent at the flow rate of 0.6 mL/min.

The enzymatic hydrolysis yield of pretreated samples was calculated as follows:

$$\text{Enzymatic hydrolysis yield} \left(\%\right) = \left(\frac{\text{glucose in enzymatic hydrolyzate (g)}}{\text{initial glucose in substrate (g)}}\right) \times 100\%$$  \hspace{1cm} (1)

RESULTS AND DISCUSSION

Effects of Prewashing on the Composition Recovery of Raw WWS

In the pulping industry, the WWS is screened and sorted from the harvested crude wheat straw; thus the ash content in WWS is extremely high. This part of ash is mainly composed of soil and dust, which are introduced during the feedstock collection process. It is reported that the ash has a negative effect on the efficiency of pretreatment, and further decreases the enzymatic digestibility of the pretreated solid (Huang et al. 2016b). Hence, in this work, prewashing was adopted as a de-ashing method prior to pretreatment, and the effects of prewashing on the recovery amount of compositions are shown in Fig. 1. As shown, it contained 29.5 g of ash per 100 g WWS, which was even higher than the amounts of cellulose and hemicellulose (sum of xylan and arabinan).

![Fig. 1. The recovery amount of components in WWS after prewashing (based on 100 g raw WWS)](image-url)
Figure 1 reveals that the prewashing process could effectively remove the ash from the WWS. Specifically, the ash content was decreased from 29.5 g (unwashed WWS) to 14.5 g and 9.8 g, respectively, with the water dosages increased to 50 and 100 g/g-WWS. However, it can be noted that a considerable amount of carbohydrates were also removed during the prewashing process. When prewashed with a water dosage of 50 g/g-WWS, the recovered amount of cellulose and hemicellulose decreased from 26.5 g and 18.8 g to 22.7 g and 15.3 g, respectively. Further increase of the water dosage to 100 g/g-WWS resulted in only 20.4 g of cellulose and 14.0 g of hemicellulose recovered. The decreased recovery of carbohydrates was mainly caused by the loss of fine feedstock particles in WWS during the prewashing process. These results are important, because all the previous studies dealing with prewashing were conducted at high water consumption (Yang and Chen 2010; Huang et al. 2017). The present results demonstrated that the high water dosage during prewashing may not only waste lots of water but also remove a certain amount of carbohydrates. Thus, reducing the water usage during the prewashing process was advantageous in order to obtain a high solid recovery yield.

Recovery Amounts of Solid and Composition After Pretreatments

Almost all the previous reports about prewashing have focused on the analysis of the pretreated solid, and few of them have studied the compositions changes during the pretreatment process. In this study, the raw and prewashed WWS with different ash contents were subjected to LHWP and DAP at 160 °C and 180 °C. The DAP was performed with the acid concentrations of 0.4% and 0.8% (w/v), and the LHWP was conducted with the water as the only medium (acid concentration of 0%). The recovery amounts of the total solid and various components after pretreatments are shown in Fig. 2.

From Fig. 2, it can be seen that the total solid recovery of WWS pretreated at 160 °C was obviously higher than that at 180 °C. In particular, the solid recovery of the pretreated WWS ranged from 50.9 g to 75.0 g at 160 °C, while it ranged from 45.4 g to 62.5 g at 180 °C.
In addition, solid recovery of WWS-0 (raw WWS), WWS-50 (prewashed with water dosage of 50 g/g-WWS) and WWS-100 (prewashed with water dosage of 100 g/g-WWS) decreased as the acid charge increased from 0 to 0.8%, at both 160 °C and 180 °C. Taking WWS-0 for example, when conducting pretreatment at 160 °C, the recovered solid decreased from 65.31 to 58.04 and 52.93 g with acid charges of 0, 0.4%, and 0.8%. These reduced recovery amounts of the total solid were primarily due to the removal of hemicellulose (as can be seen in Fig. 2), which is liable to degrade at increased temperature and acid charge because of its low degree of polymerization, higher branched structure, and more amorphous nature (Redding et al. 2011; Gu et al. 2013).

Different from hemicellulose, for the three tested samples, the cellulose seemed to be non-degradable at the low temperature of 160 °C, corresponding to the constant cellulose recovery at the varied acid charges (Fig. 2a). However, when conducting pretreatments at 180 °C, obvious decreases in cellulose recovery were observed with increasing the acid concentration (Fig. 2b). Specifically, when the acid charge was increased from 0 to 0.8%, the cellulose recovery amount decreased from 24.9 g (WWS-0), 31.0 g (WWS-50), and 31.4 g (WWS-100) to 23.5 g, 29.2 g, and 27.5 g, respectively. The results indicated the cellulose also degraded under pretreatment with severe cooking conditions. Beyond these, the lignin and ash recoveries in three tested samples were almost invariable during all the pretreatment conditions (as increasing temperature and acid charge). The results indicated that the lignin and ash are stable during the LHWP and DAP.

**Prehydrolyzate Analysis**

The authors’ previous study reported that the ash in the WWS can buffer the hydronium ions formed and thus decrease the pretreatment efficiency (Huang et al. 2016b). In this study, the prewashing and sulfuric acid were regarded as methods to eliminate the ash’s negative effect. The pH values of the pretreatment hydrolyzate at various conditions are shown in Fig. 3. It was found that prewashing could effectively decrease the pH of LHWP system (red dashed line). In detail, the pH decreased from 6.4 to 5.6 at 160 °C and from 5.9 to 4.2 at 180 °C, when the water dosage increased from 0 to 100 g/g-WWS. The buffering behavior of the ash was caused by an ion-exchange reaction between the metal cations in the ash and the hydronium ions in the prehydrolyzate. The prewashing treatment removed the exchangeable cations and thus reduced the buffering capacity of the WWS (Huang et al. 2016b). Furthermore, the LHWP prehydrolyzate pH of the highest water dosage prewashed WWS (5.57 and 4.26 at 160 °C and 180 °C, respectively) was similar to the value of the conventional wheat straw (Ertas et al. 2014), which indicated that the prewashing was efficient in enhancing the LHWP efficiency.

In addition, Fig. 3 also shows that, compared with LHWP (with prewashed WWS), the DAP was much more efficient in decreasing the prehydrolyzate pH (black solid line), with the lowest pH obtained at the acid charge of 0.8% for different samples. This decrease in pH values indicated the DAP could also break the buffer system of the ash and further improve pretreatment efficiency.

However, it should be noted that the pH value of the DAP hydrolyzate (especially at 0.8% sulfuric acid) was quite low, which may do harm to the environment if discharged into the river without any further treatment. The main advantage of the LHWP is the use of water as the only medium and thus made the prehydrolyzate almost non-polluting.
Generally, the byproducts of HMF, acetic acid, and furfural can be degraded from xylose and glucose during acidic pretreatment (Chu et al. 2013). These compounds are regarded as the inhibitors, and as such they have potential inhibitory effects on the enzymatic hydrolysis and fermentation processes (Arora et al. 2012; Ren et al. 2016). In this work, the amounts of inhibitors in prehydrolyzate were analyzed and summarized in Fig. 4. It is obvious that the inhibitor concentration of the LHWP hydrolyzate was much lower than that in the DAP hydrolyzate, which indicated that the LHWP is a clean pretreatment technology without causing much pollution to the environment. Furthermore, for samples pretreated at the same condition (acid charge and temperature), increasing prewashing water dosage only resulted in a limited increment in the inhibitor concentration. For example, the total inhibitors in the LHWP prehydrolyzate at 180 °C increased from 1.39 to 2.07 and 2.55 g/L with the water dosage of 0-, 50-, and 100-fold the weight of raw WWS (Fig. 4b). Meanwhile, a sharp increase in the inhibitors was detected in prehydrolyzate of DAP compared with that of LHWP, with a highest concentration of 9.21 g/L produced from pretreatment at 180 °C and 0.8% acid charge. Additionally, it can be known that, for the prehydrolyzate of DAP, the furfural was dominant among the three inhibitors. However, for LHWP, the acetic acid was the most dominant. As reported by Palmqvist and Hahn-
Hägerdal (2000), the DAP can degrade xylan into xylose by acid hydrolysis, and further generate furfural by dehydration. However, LHWP has been reported as a moderate pretreatment technology, which mainly causes the deacetylation reaction of hemicellulose and forms the acetic acid in the prehydrolyzate. The resulted acetic acid can degrade a portion of hemicellulose into oligosaccharides and monosaccharides without further degradation (Garrote et al. 2002; Xiao et al. 2013). This may explain the increasing amount of furfural in the prehydrolyzate of DAP. The large amount of inhibitors in the DAP hydrolyzate will not only affect the fermentation behavior of applied microorganism but also make the downstream wastewater treatment become more onerous.

**Microstructure of the Pretreated Solids**

The microstructure of the pretreated samples was analyzed with scanning electron microscopy (SEM), and results are shown in Fig. 5. From the images, it can be seen that there are many particles in the surface of WWS, even the prewashed WWS. This result indicated that although the prewashing removed most of the free ash, there was still some firmly attached ash that could not be easily washed at the water dosage utilized. In addition, the sample with LHWP showed a relatively smooth surface (Fig. 5a and 5c), which indicated that the LHWP is a mild pretreatment technology, causing minimal degradation of the feedstock.

![SEM images of the solid pretreated at 160 °C. a: LHWP with raw WWS (1000×), b: DAP with prewashed WWS (1000×); c: LHWP with raw WWS (3000×); d: DAP with prewashed WWS (3000×)](image)
In contrast to LHWP, the images of the acid pretreated WWS is completely different. After DAP, the structure of the WWS became rough and corrugated (Fig. 5b and 5d). Moreover, there are many micropores in the acid pretreated WWS (as shown in Fig. 5d). These pores are the features of the DAP and is mainly caused by the acid etching and hemicellulose degradation (Zhang et al. 2017). According to Rollin et al. (2011), the corrugated surface and existed micropores will offer more adsorption sites for cellulase and then enhance the enzymatic digestibility efficiency.

**Effect of Prewashing on the Enzymatic Digestibility of Pretreated Solids**

An ideal pretreatment technology can realize the separation of cellulose, hemicellulose, and lignin, and then facilitate the enzymatic digestibility of the residual cellulose (Mahdy et al. 2014). To evaluate the effects of prewashing on the LHWP and DAP, the enzymatic hydrolysis of pretreated solids was conducted. Results are shown in Fig. 6. As shown, prewashing greatly improved the enzymatic hydrolysis of LHWP solid at a high temperature of 180 °C (red dashed line in Fig. 6), and the enzymatic hydrolysis yield increased from 50.0% to 74.5% and 79.4% with the water dosage of 0, 50, and 100 g/g-WWS, respectively. The increased enzymatic digestibility was mainly caused by the improved pretreatment efficiency due to the ash removal. However, when conducting LHWP at 160 °C, only a 4.2% increment in enzymatic hydrolysis yield was achieved with the increased water dosage (black dashed line).

![Fig. 6. Enzymatic hydrolysis yield of different samples](image-url)

Different from LHWP, DAP seemed to be more suitable for pretreatments at a low temperature of 160 °C (black solid line). In particular, the increments of hydrolysis yield of 29.2%, 29.4%, and 33.5% were achieved for WWS-0, WWS-50, and WWS-100, respectively, by increasing the acid charge from 0 to 0.8%. The results indicated that the sulfuric acid effectively destroyed the ash’s buffering effect and improved the pretreatment efficiency. An interesting phenomenon in this research is that while conducting DAP at 180 °C, a lowered enzymatic efficiency was observed, especially for the prewashed WWS (red solid line). The enzymatic hydrolysis yield decreased from 74.5% (WWS-50) and 79.4% (WWS-100) to 68.2% and 67.7%, with the acid charge increasing from 0 to 0.8%, respectively. The similar phenomenon has also been found by other researchers, who reported that DAP at severe conditions (high temperature and acid charge) may repress the cellulose enzymatic digestibility (Jensen et al. 2010). This drop in enzymatic efficiency
could be attributed to a repolymerization reaction between the polysaccharides degradation products and the lignin, and this reaction can form a lignin-like material termed pseudo lignin (Kumar et al. 2013). The existing pseudo lignin in the pretreated sample could have inhibited the activity of cellulose degrading enzymes, as well as caused non-productive binding between enzymes and lignin-like material (Sannigrahi et al. 2011), and thus decreased the enzymatic digestibility of substrate.

The successful application of prewashing has been widely reported based on the high temperature at which it occurred. However, the results in this paper established that prewashing could only work at the high temperature. If conducting the pretreatment at a low temperature, the DAP was more efficient in improving the enzymatic hydrolysis efficiency of the high ash content feedstock.

Material Balance

Aside from the high enzymatic hydrolysis efficiency, a good pretreatment technology should also recover more fermentable sugars from both the enzymatic hydrolysis and pretreatment hydrolyzate. In this study, all the sugars during the bio-refinery process were determined, and the material balances of LHWP and DAP at 160 °C and 180 °C are shown in Table 1 and Table 2, respectively. As shown in Table 1, the total sugars in enzymatic hydrolyzate were increased with increasing acid charge at 160 °C, with the maximum value of 29.4 g released from the WWS-100 (DAP with 0.8% acid charge). However, when investigating pretreatments at 180 °C (Table 2), the sugars in the enzymatic hydrolyzate showed a decreased trend when increasing the acid charge, revealing a maximum amount of 32.3 g from the WWS-100 through LHWP. Except for the sugars in enzymatic hydrolyzate, a certain amount of sugars can be found in the prehydrolyzate, especially xylose. This part of sugars can also be used for ethanol fermentation (Ertas et al. 2014). Compared with LHWP, DAP can generate a higher amount of sugars in prehydrolyzate at 160 °C. While conducting pretreatments at 180 °C, the sugar in prehydrolyzate decreased with the increased acid charge, and the LHWP combined with prewashing (WWS-100) recovered a maximum value of 15.9 g.

Table 1. Material Balance from LHWP and DAP at 160 °C Followed by Enzymatic Hydrolysis

<table>
<thead>
<tr>
<th>Water dosage (g/g-WWS)</th>
<th>Acid charge (%)</th>
<th>Enzymatic hydrolyzate (g)</th>
<th>Sugar in prehydrolyzate (g)</th>
<th>Sugar recovery (g)</th>
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<tr>
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<td>G&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
<td>T&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>26.9</td>
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<sup>a</sup>G: released glucose  
<sup>b</sup>X: released xylose  
<sup>c</sup>T: total sugars  
<sup>d</sup>Sum of sugars in enzymatic hydrolyzate and prehydrolyzate  
<sup>e</sup>Calculated by (sugar recovery(g)/sugar in the unpretreated WWS(g))
### Table 2. Material Balance from LHWP and DAP at 180 °C Followed by Enzymatic Hydrolysis

<table>
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<tr>
<th>Water dosage (g/g-WWS)</th>
<th>Acid charge (%)</th>
<th>Enzymatic hydrolyzate (g)</th>
<th>Sugar in prehydrolyzate (g)</th>
<th>Sugar recovery (g)</th>
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<tr>
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<td>7.9</td>
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</table>

<sup>a</sup>G: released glucose  
<sup>b</sup>X: released xylose  
<sup>c</sup>T: total sugars  
<sup>d</sup>Sum of sugars in enzymatic hydrolyzate and prehydrolyzate  
<sup>e</sup>Calculated by (sugar recovery(g)/sugar in the unpretreated WWS(g))

For the total sugar recovery (sugars in enzymatic hydrolyzate and prehydrolyzate), a maximum recovery yield of 86.8% was achieved for WWS-100 pretreated by DAP at 160 °C and 0.4% acid charge. And a sugar recovery as high as 84.4% could also be recovered from WWS-100 with LHWP for the pretreatment at 180 °C. The results of the material balance were in correspondence with the enzymatic hydrolysis yield, which indicated that the LHWP combined with prewashing was more effective at high temperatures. On the contrary, the DAP showed the better performance at a relatively low temperature. Finally, it can be noted that the highest sugar recoveries at these temperatures were always achieved from the prewashed sample, which demonstrated that prewashing step was an indispensable technology in the bio-refinery of WWS.

### CONCLUSIONS

1. Prewashing effectively removed the redundant ash in WWS, and also removed a certain amount of carbohydrates.

2. For LHWP at 180 °C, prewashing greatly increased the enzymatic hydrolysis yield of pretreated WWS from 50.0% to 79.4%. Moreover, LHWP was superior to DAP due to the mild pH and lower inhibitors in prehydrolyzate.

3. The DAP may be more suitable at the relatively low temperature on the basis of its high enzymatic hydrolysis yield, total sugar recovery, and low energy consumption.

4. The results indicated that prewashing is an advantageous process for the bio-refinery of high ash content WWS to achieve a high total sugar recovery.

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