Enhanced Bioethanol Production from Industrial Xylose Residue Using Efficient Delignification

Tian Zhou,[a] Liming Zhang,[a] Hong Zhao,[b] Feng Xu,[a,c,*] and Guihua Yang [c]

Xylose residue (XR), the abundant industrial residue from commercial xylose production, was delignified using alkali as a substrate for ethanol production *via* simultaneous saccharification and fermentation (SSF). It was found that pretreatment with 1.5% (w/v) NaOH at 140 °C for 1.5 h was optimal for delignification efficiency (72.2%) and low cellulose loss (7.1%). The physical changes in samples after alkaline pretreatment were characterized for crystallinity and imaged using scanning electron microscopy (SEM), which demonstrated that the surface of samples became coarser with lignin removal. There were rather significant changes in cellulose crystallinity. The widespread accessibility of cellulose in XR favored enzymatic hydrolysis and achieved considerable bioconversion (98.8% with 15 PFU/g substrate). The maximum for ethanol concentration using SSF bioconversion reached 16.3 g/L, which was about four times more than that of the untreated sample. XR treated using the processes of alkaline pretreatment and SSF was an excellent substrate for bioconversion.

Keywords: Bioethanol; Xylose residue; Alkaline pretreatment; Simultaneous saccharification and fermentation

*Contact information: a: Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, China; b: College of Light Chemistry and Textile Engineering, Qiqihar University, Qiqihar 161006, China; c: Shandong Key Laboratory of Paper Science & Technology, Qilu University of Technology, Jinan 250353, China; *Corresponding author: xfx315@bjfu.edu.cn*

INTRODUCTION

After the energy crisis of the mid-1970s, the perceived risk of running out of conventional fossil fuels led to crash programs in developing new renewable and sustainable energy sources (Turner 1999). Second-generation bio-ethanol, which is produced from biomass materials *via* enzymatic hydrolysis and fermentation (Limayem and Ricke 2012), is one such viable alternative to fossil fuels (Rajkumar *et al.* 2013). Its technology is one of the most feasible and economical technologies in the world for decreasing net carbon emission, adding energy efficiency, and reducing energy dependency, potentially overcoming the limitations of first-generation bioethanol (Mohammed *et al.* 2013; O'Keefe *et al.* 2014).

Normally, in the process of commercial xylose production, xylose is produced by the acidic hydrolysis of hemicellulose with a mineral acid (sulfuric acid 1.5%), and the solid residue is considered a waste byproduct and is mainly used for heat generation currently. However, XR is a promising potential lignocellulosic source for bioethanol production because it is enriched in cellulose and almost free of hemicellulose, which is hydrolyzed during xylose production. The lignin in XR can be more easily removed or altered because of deploymerization and re-polymerization reactions (Wang *et al.* 2012). China is already one of the world's largest xylose producers, generating millions of tonnes of XR annually (Fan *et al.* 2014). Hence, making good use of this rich resource is

vitally important for China in its attempt to improve environmental quality, increase the economics of biomass utilization, and boost rural incomes.

However, there are still issues hindering the bioconversion of XR into ethanol. The lignin sheath limits the accessibility of the enzymes to the cellulose. For lignin, its physicochemical features of low molecular weight and hydrophobicity restrict the swelling of cellulose and, therefore, negatively affect the accessible surface area (Donohoe *et al.* 2008; Arantes and Saddler 2010). Additionally, cellulase can be irreversibly adsorbed on lignin *via* hydrophobic interactions, ionic bond interactions, and hydrogen bond interactions, consequently reducing the efficiency of saccharification. Inhibitors generated in xylose production have negative effects on fermentation (Monlau *et al.* 2015). Organic acids, which are soluble in the lipids of the cell membrane and dissociate inside the yeast cells at physiological pH, are the main inhibitors generated in xylose production (Chandel *et al*. 2013). These acids could potentially prolong the lag phase of fermentation, and they are expected to reduce the effect on enzymes associated with glycolysis (Kim *et al.* 2008; Parawira and Tekere 2011). Therefore, pretreatment is a necessary step towards solving these issues. Significant prior research has been conducted on a suite of pretreatment processes for bioethanol processes, such as ammonia fiber explosion (Jackson *et al.* 2011), acid hydrolysis (Ji *et al.* 2015), alkaline pretreatment (Ji *et al.* 2014), and biological treatment (Abubackar *et al.* 2011). Of these, the use of alkali in the pretreatment of XR has received the least attention. Alkaline pretreatment removes lignin without having major impacts on the cellulose component. It also breaks open the polymeric structures of lignin and hemicellulose, and enhances the accessibility of enzymes to the solid substrate during enzymatic hydrolysis (McMillan 1997; Saha *et al.* 2011). The removal of inhibitors is also a factor in evaluating pretreatment performance. The inhibition of these organic acids could be easily offset by alkaline pretreatment (Pirzadah *et al.* 2014). The solvent pretreatment based on alkali has potential advantages on improving the efficiency of enzymatic hydrolysis.

The primary goal of this study was to improve ethanol productivity by alkaline pretreatment and simultaneous saccharification and fermentation (SSF). Alkaline pretreatment were optimized as an effective means of delignification and removal of inhibitors from XR under different temperatures (from 80 °C to 160 °C) and sodium hydroxide concentrations (from 0% to 2%). The substrate features were evaluated with regard to sugar composition, crystal structure, surface morphology, and chemical structure to define the advantages of alkaline pretreatment. The appropriate enzyme dosage was chosen to enhance the transformation of XR cellulose into high yields of glucose and ethanol using enzymatic hydrolysis and SSF, respectively.

EXPERIMENTAL

Materials

XR was kindly provided by the Shandong Lujian Biotechnology Co. Ltd. (Shandong, China). Before pretreatment, the sample was passed through a 0.9-mm screen to remove impurities such as large particles and stones. On the basis of dry weight, it was composed of 46.1% cellulose, 6.4% hemicellulose, and 32.2% lignin. The concentration of lactic acid and acetic acid (organic acids) in raw XR was 0.21 g/g solid and 0.06 g/g solid, respectively.

Alkaline Pretreatment

The pretreatment was performed in a sealed, Teflon-lined, stainless-steel autoclave containing 3 g of solid and 30 mL of reaction solution at concentrations of 0, 1, 1.5, and 2% NaOH (w/v). Samples were treated for 1.5 h. Treatments were performed at temperatures of 80, 100, 120, 140, and 160 °C in an oil bath. After pretreatment, the solid phase and liquid phase were separated by filtration under vacuum with a 400-mesh filter cloth. The solid residue was rinsed with water until it reached neutral pH, oven-dried (105 °C), and stored for further chemical analysis and bioconversion. The vacuum-filtered liquid was collected, and lignin in the liquid phase was determined using a separation procedure (Sun *et al.* 2000). The untreated sample was labeled Z_0, and the treated samples were labeled Z_1, Z_2, Z_3, Z_4, Z_5, Z_6, and Z_7 (Table 1). All assays were conducted in triplicate.

Table 1. Crystallinity Indexes (CrI) of the Untreated Sample and Cellulosic Residues after Pretreatment

Sample	NaOH Concentration (w/v)	Reaction Temperature(°C)	CrI (%)
Z_0	-	-	43.1
Z_1	1	80	44.1
Z_2	1	100	44.4
Z_3	1	120	45.2
Z_4	1	140	47.9
Z_5	1	160	45.6
Z_6	1.5	140	50.9
Z_7	2	140	45.1

Enzyme and Yeast

The filter paper activity of cellulase was 145 FPU/g (Adney and Baker 1996). The cellulase was purchased from Shanghai Youtell Biochemical Co. Ltd. (Shanghai, China). *Saccharomyces cerevisiae* was purchased from the Angel Yeast Co. Ltd. (Hubei, China). Dry yeast was activated at 40 °C for 30 min in 2% glucose solution and then at 30 °C for 2 h.

Enzymatic Hydrolysis

The enzymatic hydrolysis experiments were performed in 25-mL stoppered conical flasks containing 1 g of solid residue after pretreatment, enzyme solution, different enzyme loadings (5, 10, 15, and 20 FPU cellulose/g substrate), and 10 mL of acetate buffer (pH = 4.8). The flasks were incubated at 48 °C and stirred in a rotary shaker at 150 rpm. Supernatant samples of 0.1 mL were taken from the reaction mixture at 4, 8, 12, 24, 30, 36, 48, and 72 h. The released monosaccharides of centrifugal samples were analyzed using a high-performance anion exchange chromatography (HPAEC) system (Dionex ICS-3000, ThermoScientific, Waltham, USA) equipped with a pulse amperometric detector (PAD) and a PA-20 (4 × 250 mm). The eluent 4 mM H_2SO_4 was employed at a flow rate of 0.4 mL/min at 50 °C. All enzymatic hydrolysis experiments were performed in triplicate. The rate of enzymatic digestion was calculated using the following formula,

$$Y_{\text{Enzymatic digestibility}}\,(\%) = \frac{\text{Glucose (g)} \times 100}{\text{Cellulose (g glucose equivalent)} \times 1.1} \quad (1)$$

where cellulose represents the amounts of cellulose in the substrate after pretreatment and glucose represents the glucose in the enzymatic hydrolysates after different times of enzymatic hydrolysis.

Simultaneous Saccharification and Fermentation (SSF)

The SSF experiments were performed under non-sterile conditions. Initially, 1 g of cellulosic residue, 10 g/L yeast extract, and 20 g/L peptone containing 16 mL of sodium acetate buffer (pH 4.8) were added to a 50-mL Erlenmeyer flask sealed with a rubber stopper and fitted with a one-way air valve to maintain an anaerobic environment. The flask was sterilized at 120 °C for 20 min. After cooling, cellulase (Youtell, 15 FPU/g substrate) and *S. cerevisiae* (3.75 g/L) were activated and added to the reaction system. The system was incubated in a shaker at 40 °C and 120 rpm for 48 h. All measurements were performed in triplicate. The concentrations of ethanol, glucose, and xylose were analyzed using the HPLC system. The ethanol production yield was calculated using the following formula,

$$Y_{\text{Ethanol}}\ (\%) = \frac{\text{Ethanol (g)} \times 100}{\text{Cellulose (g)} \times 0.568} \quad (2)$$

where ethanol (g) is the ethanol acquired after fermentation.

Analytical Methods

The surface morphology of untreated and pretreated XR was observed through an S-3400N scanning electron microscope (SEM) (Hitachi, Japan) operated at 10 kv acceleration voltage. Prior to the analyses, all samples were coated with gold (E-1010, Hitachi, Japan) to prevent static charge build-up. The composition of pretreated samples was estimated using analytical methods for biomass (Sluiter *et al.* 2008). The chemical structures of untreated and pretreated samples were characterized using a ThermoScientific Nicolet iN10-MX FT-IR chemical imaging microscope (Madison, USA). All solid samples were dried and mixed with potassium bromide (KBr) before being pressed into discs. The samples were scanned in the range of 4000-500 cm^{-1} with a resolution of 4 cm^{-1}. XRD measurements were performed with Ni-filtered CuKq radiation (λ = 1.54 Å) using an XRD 6000 instrument (D8 ADVANCE, Bruker AXS, Karlsruhe, Germany). The operating voltage and current were 40.0 kV and 40.0 mA, respectively. The crystallinity indexes (CrI) of the samples were calculated according to Segal *et al.* (1959),

$$\text{CrI}\ (\%) = \frac{I_{002} - I_{\text{Amorphous}}}{I_{002}} \times 100 \quad (3)$$

where I_{002} and $I_{\text{Amorphous}}$ represent the maximum and minimum intensity of diffraction at 2θ of 22.4 to 22.5° and 18.0 to 19.0°, respectively.

RESULTS AND DISCUSSION

Composition Analysis

The composition of samples that were pretreated with NaOH were comparable to those of raw materials. The results in Fig. 1 clearly show the influence of an increase in temperature on the contents of lignin and hemicelluloses in the samples at a given NaOH loading. The contents of lignin and hemicelluloses in the pretreated samples decreased with increasing temperature. At a loading of 1% NaOH (w/v), the content of lignin decreased from 25.1% at 80 °C (Z_1) to 11.5% at 160 °C (Z_5), and the content of the hemicelluloses decreased from 3.6% at 80 °C (Z_1) to 1.1% at 160 °C (Z_5). While increasing pretreatment temperature clearly promoted the removal of lignin and hemicelluloses, increases in NaOH loading also had a major effect. At 140 °C and 1% NaOH (w/v) (Z_4), the pretreated solid exhibited a composition of 13.5% lignin and 1.3% hemicelluloses by weight. Increasing the loading of NaOH to 2% (Z_7) decreased the lignin and hemicellulose content to 8.5% and 0.7%, respectively. After pretreatment with 1.5% NaOH (w/v) at 140 °C, the lignin and hemicellulose content dropped to 8.9% and 0.75%, respectively, indicating that 72.2% of lignin was removed. The lignin removal in sample Z_6 was highly comparable to other pretreatment methods at optimal conditions. The removal of 22.4% and 69.2% lignin were obtained with dilute acid and ionic liquid pretreatments of switchgrass (Li *et al.* 2010). The remaining lignin and hemicellulose contents of samples Z_5 and Z_7, which were treated at a more severe condition of 160 °C or 2% NaOH, was only slightly decreased compared with Z_6. Lignin is the main recalcitrant component affecting bioconversion from XR to ethanol. Hence, alkaline pretreatment is a crucial step towards overcoming this recalcitrance by dissolving lignin and hemicellulose.

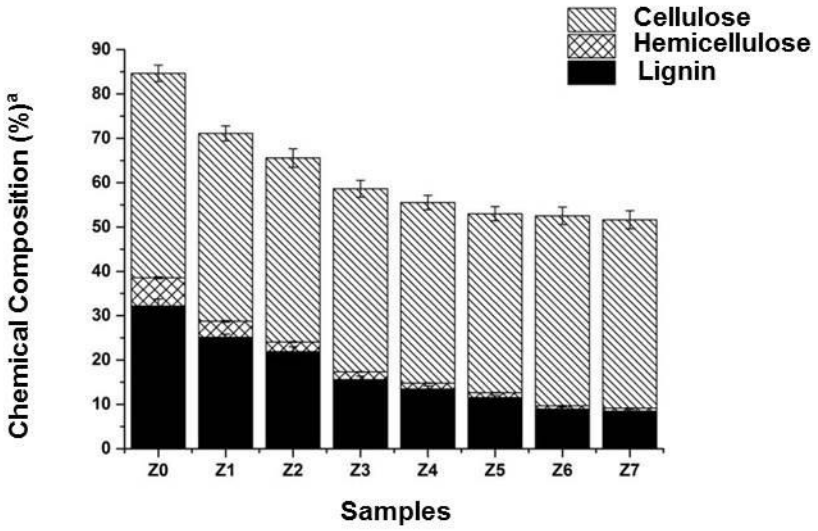

Fig. 1. The relative composition of XR after alkaline pretreatment compared with the untreated sample (Z_0)

Alkaline pretreatment breaks the ester bonds cross-linking lignin and xylan, swells the fibers, and disrupts lignin structure (Sun *et al.* 2000). The effective removal of lignin makes the polysaccharides more amenable to digestion using cellulolytic enzymes (Eggeman and Elander 2005; Himmel *et al.* 2007; Wyman *et al.* 2009; Ding *et al.* 2012;

Karp *et al*. 2014). The content of cellulose also decreased, accompanied by the gradual removal of hemicelluloses and lignin. The cellulose content decreased from 46.1% (Z_0) to 42.4% (Z_7), corresponding 7.7% mass loss, and the cellulose loss of sample Z_6 (7.1%) was lower compared with Z_7. Thus, considering lignin removal, content of cellulose, and energy and chemical savings, pretreatment with 1.5% NaOH (w/v) at 140 °C (Z_6), which reduced lignin content to 8.9% and increased cellulose content to 42.9%, was an appropriate method for alkaline delignification.

Physical Changes after Alkaline Delignification

The residual solids remaining after alkaline pretreatment were characterized for crystallinity index (CrI; Table 1) and imaged using SEM to reveal physical changes in the carbohydrate portions of the biomass. The changes in crystallinity were relatively low as pretreatment severity was increased. The CrI increased after pretreatment; this increase was most apparent in the pretreatment at 140 °C and 1.5% NaOH (w/v) (Z_6). The main reasons for this were the microstructure change and simultaneous removal of multiple fractions of XR. The treatment of cellulose samples with NaOH caused the polymorphous lattice to change from cellulose I to II *via* Na-cellulose, which is a mercerization reaction (Gupta *et al*. 2013), and the conversion from cellulose I to II decreased the CrI (Kong and Eichhorn 2005). However, alkaline pretreatment breaks cellulose hydrogen bonds, and the cellulose removed was mostly amorphous or para-crystalline cellulose (Wang *et al*. 2013), as amorphous cellulose has a greater accessible surface and is more susceptible to attack. Amorphous cellulose is destroyed first during alkaline pretreatment (Yoon *et al*. 2011). And the destruction of amorphous cellulose is critical for the change of CrI. The results shown in Table 1 confirms this change.

In terms of surface characteristics, alkali delignification loosened the structure of the samples (Fig. 2). SEM images of the untreated and pretreated samples show dramatic changes in the microstructure. The surface of the raw sample (Fig. 2a) was very flat and smooth. After pretreatment, the surface became porous and coarse. The sample Z_6 (Fig. 2h) exhibited the roughest surface structure and had the largest quantity of holes. These holes reflected the removal of lignin and hemicellulose dissolution. They made the sample more accessible to enzymes and, thus, enhanced the saccharification process.

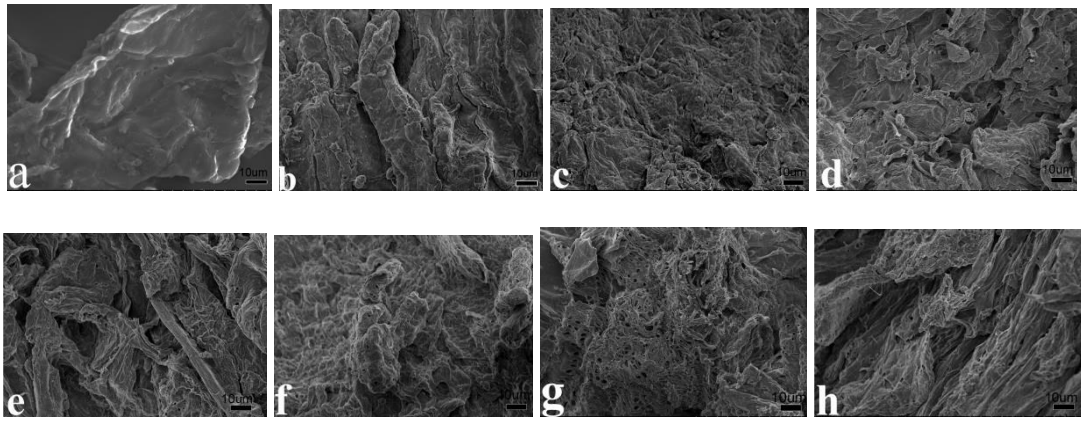

Fig. 2. Scanning electron micrographs of sample surfaces before (a) and after (b, c, d, e, f, g, and h) alkaline pretreatment. The samples were (b) Z_1, (c) Z_2, (d) Z_3, (e) Z_4, (f) Z_5, (g) Z_6, and (h) Z_7.

FTIR Analysis

The chemical structures of both untreated and pretreated XR were investigated using FTIR (Fig. 3). The cellulose structure did not change significantly during the alkaline pretreatment. However, there were changes in certain functional groups and linkages of cellulose after alkaline pretreatment. The band at 897 cm^{-1} characterized the C-O-C stretching at the β-1,4-glycosidic linkage, which is strong and sharp if cellulose II or amorphous cellulose is present (Xu *et al.* 2010). The band at 1425 cm^{-1} represented CH_2 scissoring motion, which is more apparent in crystalline cellulose (Kuo and Lee 2009). The absorbance at 897 cm^{-1} was more intense in untreated than in pretreated samples, and the lowest absorbance among the samples appeared at Z_6. Conversely, the intensity of the absorption band at 1425 cm^{-1} in pretreated samples was stronger than in untreated samples. Hence, the untreated XR sample contained more amorphous cellulose than that in pretreated XR samples. Alkaline pretreatment also removed lignin from XR (Fig. 3). The peaks at 1514 cm^{-1} characterized the aromatic skeletal modes of lignin, whereas the peak at 1330 cm^{-1} was associated with the aromatic hydroxyl groups generated by the cleavage of ether bonds within lignin (Hsu *et al.* 2010). The intensity of these bands weakened after pretreatment, which indicated that the biomass was delignified effectively during pretreatment. Additionally, the absorption peak located at 1733 cm^{-1} was weak; it was associated with the alkyl ester of the acetyl groups in hemicellulose. The intensity of the band at 1053 cm^{-1} due to C-O stretching in cellulose and hemicellulose (Lei *et al.* 2013) was also reduced in alkali-pretreated bagasse. This result supports previous findings that hemicellulose is destroyed during the pretreatment.

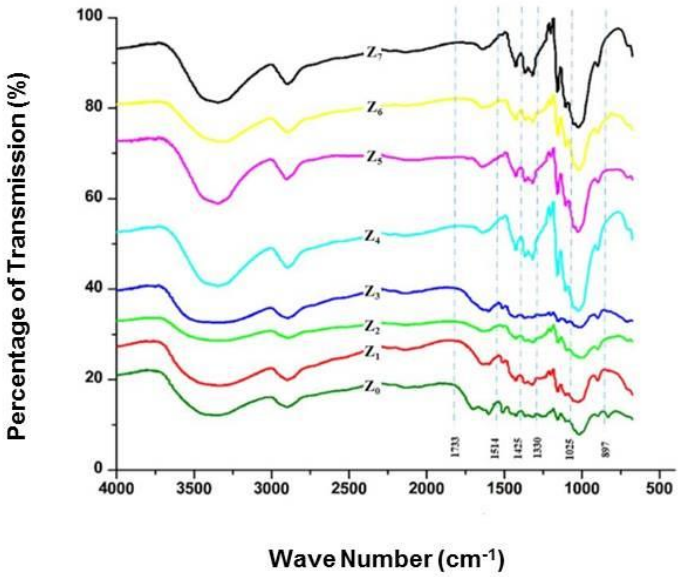

Fig. 3. FTIR spectra of untreated and pretreated XR

Effects of Pretreatment Conditions on Enzymatic Hydrolysis

Both the content and complex structure of residual lignin associated with cellulose after pretreatment have significant influence on the subsequent enzymatic hydrolysis (Mohamed *et al.* 2015). As shown in Fig. 4a, enzymatic saccharification of untreated XR (at 72 h) led to a glucose yield of 25.6%. After alkaline pretreatment, faster saccharification rates and higher sugar yields were achieved. For enzymatic saccharification at a pretreatment temperature of 160 °C, the highest enzymatic

digestibility achieved with the pretreated solid approached 92.5% with the addition of 15 FPU cellulase/g substrate. At cellulase loadings of 5 and 20 FPU cellulase/g substrate, less glucose was obtained in the pretreated samples than that at a cellulase loading of 15 FPU cellulase/g substrate. At a given cellulase loading of 15 FPU cellulase/g substrate, the highest enzymatic saccharification was observed at 140 °C and 1.5% NaOH (w/v). Alkaline pretreatment substantially enhanced saccharification performance, as illustrated by the highest enzymatic digestion of 98.8% with 1.5% NaOH (w/v) and 15 FPU/g substrate at 140 °C. However, the enzymatic digestion of other samples was reduced with more severe pretreatment and more enzyme dosages. This phenomenon showed that the lignin have a negative effect on enzymatic hydrolysis. It is reasonable to assume that the removal of lignin enhanced the efficiency of cellulose hydrolysis (Selig *et al.* 2007). This mechanism reflects the fact that lignin is a major limitation in the digestion of lignocellulosic materials due to its restriction of the fiber swelling, irreversible inactivation of cellulase, and reduction of cellulose accessibility (Yoon *et al.* 2011). The relatively low lignin content reduced non-productive binding between lignin and cellulose (Xu *et al.* 2015), and the structures of samples were "loosened" by this action, which greatly increased the available surface area for enzymatic attack (Fig. 2). Another phenomenon worth noting was that the conversion showed a small trend of increase after 48 h incubation. This result seemed to be due to the decreased cellulose content, which led to the unsaturated status of active cellulase.

In this study, enzymatic digestibility reached 98.8% under the appropriate conditions. However, organic solvent-treated poplar that was fermented in the same solid-liquid ratio only had 80% enzymatic digestibility (Wang *et al.* 2012). Hydrothermal treated corncobs had 97.8% enzymatic digestibility (Sun *et al.* 2014). An enzymatic digestibility of 88.2% can be obtained by optimizing alkaline hydrogen peroxide pretreatment conditions from poplar as previously described (Zhang *et al.* 2014). Hence, enhanced enzymatic hydrolysis following alkaline pretreatment and the appropriate cellulase loading is an effective method for the conversion of XR cellulose into glucose.

Simultaneous Saccharification and Fermentation (SSF)

SSF was conducted to evaluate the ethanol production from alkaline-pretreated XR samples. In Fig. 5, the untreated sample Z_0 showed a low ethanol yield of 4.8 g/L (equivalent to 16.9%), while the highest ethanol production was 15.9 g/L (equivalent to 55.9%) with 1% NaOH (w/v) at various temperatures. Sample Z_6 with 1.5% NaOH (w/v) at 140 °C had the highest ethanol production of 16.3 g/L (equivalent to 57.4%). These results showed that under the highest glucose conversion condition, the ethanol content was also highest. Furthermore, as shown in Figs. 3 and 4, the conversion rate of glucose and xylose contents increased rapidly during the first 32 h, but later, the glucose and xylose conversion was flat. Hence, the conversion during the first 32 h was more effective than in the following hours.

In a previous study, acid-pretreated rice straw had a lower ethanol production of 10 g/L (Belal 2013). An ethanol concentration of 3.8 g/L was obtained from microalgae by optimizing AP pretreatment conditions and the SSF approach (Harun *et al.* 2010). Under acid treatment, the ethanol concentration could reach 7.2 g/L (Harun and Danquah 2011). Rocha *et al.* (2013) suggested the agro-industrial biomass can obtain only an 11.7 g/L ethanol concentration using crude enzyme complex *Aspergillus niger* at 35 °C. However, Li *et al.* (2014) pointed out that under alkaline pretreatment with 12% NaOH (w/v), the final ethanol concentration reached as high as 40.59 g/L corresponding to

74.2% of the theoretical maximum from the xylan-removed sugarcane bagasse (XRSB). In conclusion, XR treated by alkaline pretreatment and SSF is a promising potential source of high ethanol content.

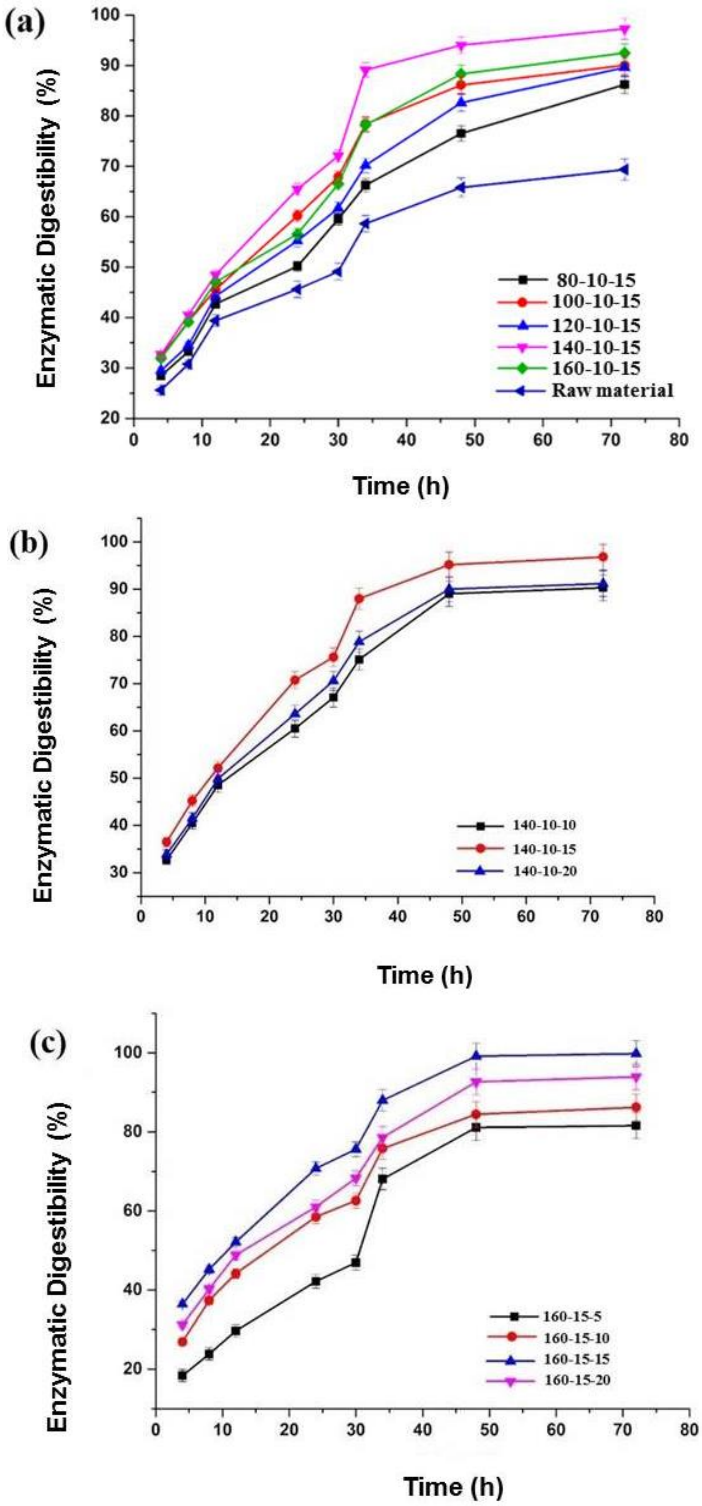

Fig. 4. The effect of temperature (a), NaOH concentration (b), and enzyme dosage, (c) on enzymatic hydrolysis; Values represent conversions of glucan present in the residual pretreated solids

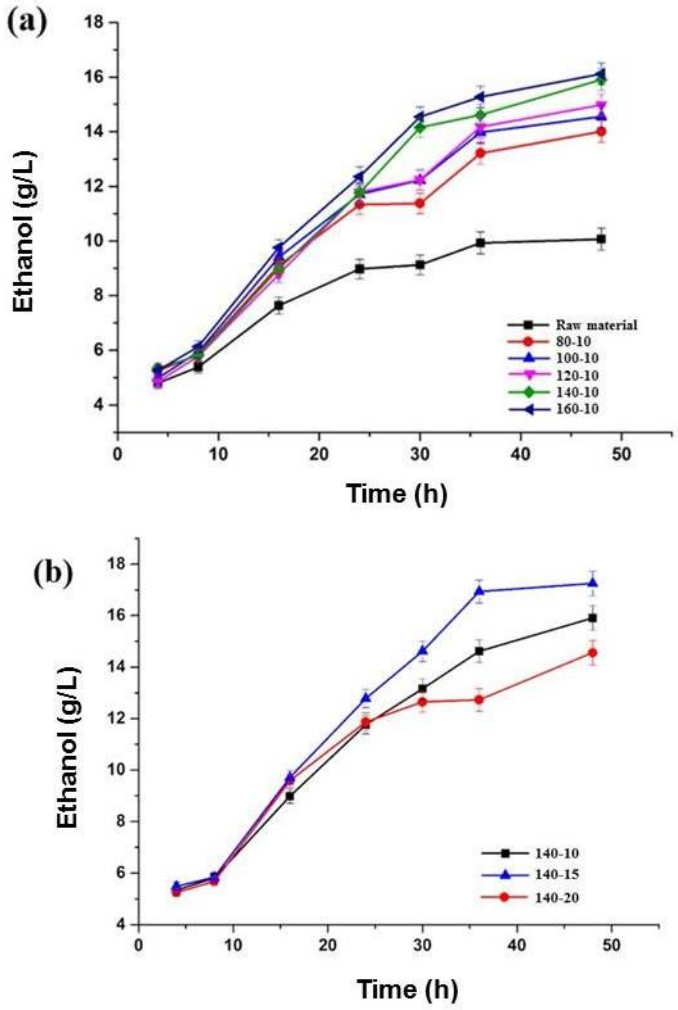

Fig. 5. The effect of temperature (a) and NaOH concentration (b) on ethanol generated by SSF

Overall Mass Balance

Figure 6 shows the complete process from pretreatment to SSF. The overall mass balance started at 100 g of XR with Z_6 (pretreated with 1.5% NaOH (w/v) at 145 °C for 1.5 h). After pretreatment, the liquid and solid phases were separated by filtration. The pretreatment yield was 87.3 g of pretreated solid. Considering the enzymatic hydrolysis, 71.9 g of glucose was obtained from 100 g of XR. A total of 4.5 g of lignin was precipitated from the liquid phase with 6 N HCl. The bioprocess using SSF resulted in 28.4 g of ethanol.

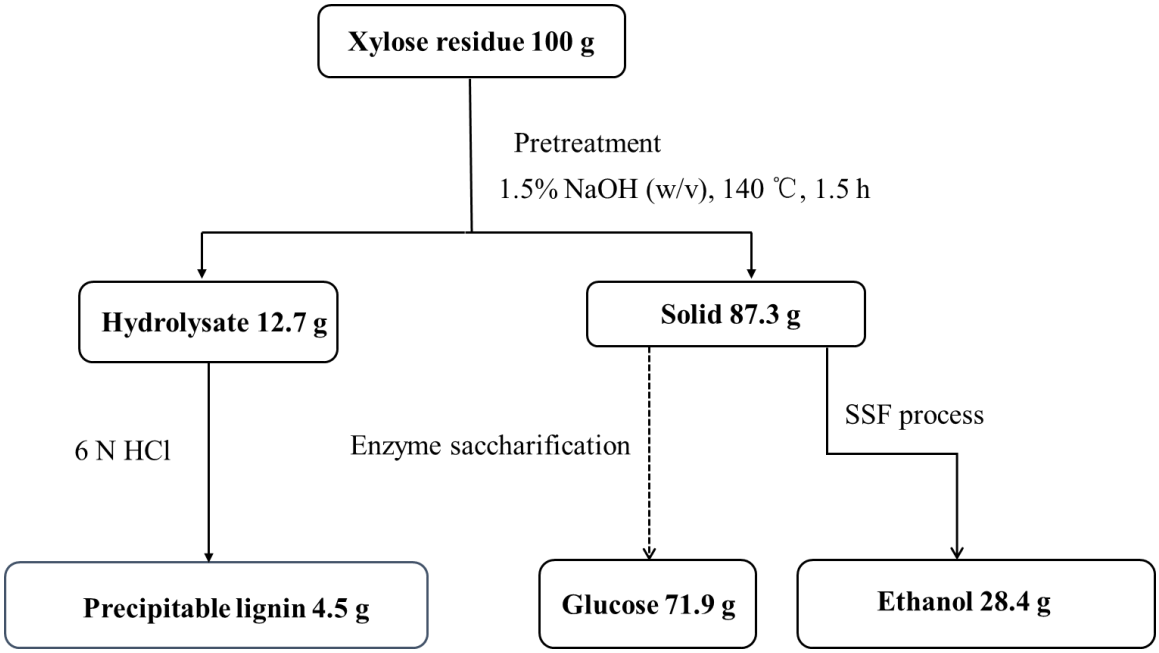

Fig. 6. Mass balance for pretreatment and SSF using XR as feedstock

CONCLUSIONS

1. Industrial xylose residue (XR) was found to be an appropriate material for bioethanol production.
2 Using the optimal conditions of 1.5% NaOH (w/v) at 145 °C for 1.5 h, up to 72.2% of the lignin in XR was efficiently degraded.
3. The sugar yields after enzymatic hydrolysis were further enhanced by lignin removal and physical changes. The enzymatic digestion ranged from 25.6% (Z_0) to 98.8% (Z_6).
4. The maximum ethanol concentration (16.3 g/L) was obtained by simultaneous saccharification and fermentation with an enzyme dose of 15 FPU/g substrate.

ACKNOWLEDGMENTS

This work was supported by the Chinese Ministry of Education (113014A), the Excellent Beijing Doctorial Dissertations Project for Adviser (20131002201), and the State Key Laboratory of Pulp and Paper Engineering (201254).

REFERENCES CITED

Abubackar, H. N., Veiga, M. C., and Kennes, C. (2011). "Biological conversion of carbon monoxide: Rich syngas or waste gases to bioethanol," *Biofuel Bioprod. Bior.* 5(1), 93-114. DOI: 10.1002/bbb.256

Adney, B., and Baker, J. (1996). *Measurement of Cellulase Activities* (NREL/TP-510-42628), National Renewable Energy Laboratory, Golden, CO, USA

Arantes, V., and Saddler, J. N. (2010). "Access to cellulose limits the efficiency of enzymatic hydrolysis: The role of amorphogenesis," *Biotechnol. Biofuels* (3), 1-11. DOI: 10.1186/1754-6834-3-4

Belal, E. B. (2013). "Bioethanol production from rice straw residues," *Braz. J. Microbio.* 44(1), 225-234. DOI: 10.1590/S1517-83822013000100033

Chandel, A. K., Da Silva, S. S., and Singh, O.V. (2013). "Detoxification of lignocellulose hydrolysates: Biochemical and metabolic engineering toward white biotechnology," *BioEnerg. Res.* 6(1), 388-401. DOI: 0.1007/s12155-012-9241-z

Ding, S.-Y., Liu, Y.-S., Zeng, Y., Himmel, M. E., Baker, J. O., and Bayer, E. A. (2012). "How does plant cell wall nanoscale architecture correlate with enzymatic digestibility?," *Science* 338(6110), 1055-1060. DOI: 10.1126/science.1227491

Donohoe, B. S., Decker, S. R., Tucker, M. P., Himmel, M. E., and Vinzant, T. B. (2008). "Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment," *Biotechnol. Bioeng.* 101(5), 913-925. DOI: 10.1002/bit.21959

Eggeman, T., and Elander, R. T. (2005). "Process and economic analysis of pretreatment technologies," *Bioresource Technol.* 96(18), 2019-2025. DOI: 10.1016/j.biortech.2005.01.017

Fan, X., Cheng, G., Zhang, H., Li, M., Wang, S., and Yuan, Q. (2014). "Effects of acid impregnated steam explosion process on xylose recovery and enzymatic conversion of cellulose in corncob," *Carbohydr. Polym.* 114, 21-26. DOI: 10.1016/j.carbpol.2014.07.051

Gupta, P., Uniyal, V., and Naithani, S. (2013). "Polymorphic transformation of cellulose I to cellulose II by alkali pretreatment and urea as an additive," *Carbohyd. Polym.* 94(2), 843-849. DOI: 10.1016/j.carbpol.2013.02.012

Harun, R., and Danquah, M. K. (2011). "Influence of acid pre-treatment on microalgal biomass for bioethanol production," *Process Biochem.* 46(1), 304-309. DOI: 10.1016/j.procbio.2010.08.027

Harun, R., Danquah, M. K., and Forde, G. M. (2010). "Microalgal biomass as a fermentation feedstock for bioethanol production," *J. Chem. Techno. Biot.* 85(2), 199-203. DOI: 10.1002/jctb.2287

Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., and Foust, T. D. (2007). "Biomass recalcitrance: Engineering plants and enzymes for biofuels production," *Science* 315(5813), 804-807. DOI: 10.1126/science.1137016

Hsu, T.-C., Guo, G.-L., Chen, W.-H., and Hwang, W.-S. (2010). "Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis," *Bioresource Technol.* 101, 4907-4913. DOI: 10.1016/j.biortech.2009.10.009

Jackson de Moraes Rocha, G., Martin, C., Soares, I. B., Souto Maior, A. M., Baudel, H. M., and Moraes de Abreu, C. A. (2011). "Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production," *Biomass Bioenergy* 35(1), 663-670. DOI: 10.1016/j.biombioe.2010.10.018

Ji, W., Shen, Z., and Wen, Y. (2015). "Hydrolysis of wheat straw by dilute sulfuric acid in a continuous mode," *Chem. Eng. J.* 260, 20-27. DOI: 10.1016/j.cej.2014.08.089

Ji, Z., Ling, Z., Zhang, X., Yang, G.-H., and Xu, F. (2014). "Impact of alkali pretreatment on the chemical component distribution and ultrastructure of poplar cell walls," *BioResources* 9(3), 4159-4172. DOI: 10.15376/biores.9.3.4159-4172

Karp, E. M., Donohoe, B. S., O'Brien, M. H., Ciesielski, P. N., Mittal, A., Biddy, M. J., and Beckham, G. T. (2014). "Alkaline pretreatment of corn stover: Bench-scale

fractionation and stream characterization," *ACS Sustain. Chem. Eng.* 2(6), 1481-1491. DOI: 10.1021/sc500126u

Kim, Y., Mosier, N., and Ladisch, M. R. (2008). "Process simulation of modified dry grind ethanol plant with recycle of pretreated and enzymatically hydrolyzed distillers' grains," *Bioresource Technol.* 99(12), 5177-5192. DOI: 10.1016/j.biortech.2007.09.035

Kong, K., and Eichhorn, S. (2005). "The influence of hydrogen bonding on the deformation micromechanics of cellulose fibers," *J. Macromol. Sci. Part B: Physics* 44(6), 1123-1136. DOI: 10.1080/00222340500324597

Kuo, C.-H., and Lee, C.-K. (2009). "Enhancement of enzymatic saccharification of cellulose by cellulose dissolution pretreatments," *Carbohydr. Polym.* 77(1), 41-46. DOI: 10.1016/j.carbpol.2008.12.003

Lei, L., Chen, S., and Li, Y. (2013). "Effect of biological treatment on characteristics of soluble organic compounds in hardwood KP bleaching effluent," *BioResources* 8(3), 4349-4358. DOI: 10.15376/biores.8.3.4349-4358

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H. V., Auer, M., Vogel, K. P., Simmons, B. A., and Singh, S. (2010). "Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification," *Bioresource Technol.* 101(13), 4900-4906. DOI: 10.1016/j.biortech.2009.10.066

Li, J., Zhou, P., Liu, H., Wu, K., Xiao, W., Gong, Y., Lin, J., and Liu, Z. (2014). "Ethanol production from xylan-removed sugarcane bagasse using low loading of commercial cellulase," *Bioresour. Technol*. 163, 390-394. DOI:10.1016/j.biortech.2014.04.106

Limayem, A., and Ricke, S.C. (2012). "Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects," *Prog. Energ. Combus.* 38(4), 449-467. DOI: 10.1016/j.pecs.2012.03.002

McMillan, J. D. (1997). "Bioethanol production: Status and prospects," *Renew. Energ.* 10(2-3), 295-302. DOI: 10.1016/0960-1481(96)00081-X

Mohammed, Y., Mokhtar, A. S., Bashir, N., and Saidur, R. (2013). "An overview of agricultural biomass for decentralized rural energy in Ghana," *Renew. Sust. Energ. Rev.* 20, 15-25. DOI: 10.1016/j.rser.2012.11.047

Mohamed, M., Salleh, W., Jaafar, J., Asri, S., and Ismail, A. (2015). "Physicochemical properties of "green" nanocrystalline cellulose isolated from recycled newspaper," *RSC Adv.* 5(38), 29842-29849. DOI: 10.1039/C4RA17020B

Monlau, F., Kaparaju, P., Trably, E., Steyer, J., and Carrere, H. (2015). "Alkaline pretreatment to enhance one-stage CH_4 and two-stage H_2/CH_4 production from sunflower stalks: Mass, energy and economical balances," *Chem. Eng. J.* 260, 377-385. DOI: 10.1016/j.cej.2014.08.108

O'Keefe, K., Springer, C. J., Grennell, J., and Davis, S. C. (2014). "Biofuel development from cellulosic sources," in: *Ecology and the Environment*, R. K. Monson (ed.), Springer, New York, USA. pp. 601-629. DOI: 10.1007/978-1-4614-7612-2_9-1

Parawira, W., and Tekere, M. (2011). "Biotechnological strategies to overcome inhibitors in lignocellulose hydrolysates for ethanol production: Review," *Crit. Rev. Biotechnol.* 31(1), 20-31. DOI: 10.3109/07388551003757816

Pirzadah, T. B., Malik, B., Kumar, M., and Rehman, R. U. (2014). "Lignocellulosic biomass: As future alternative for bioethanol production," in: *Biomass Bioenergy*, Springer, pp. 145-163. DOI: 10.1007/978-3-319-07578-5_8

Rajkumar, R., Yaakob, Z., and Takriff, M. S. (2013). "Potential of micro and macro algae for biofuel production: A brief review," *BioResources* 9(1), 1606-1633. DOI: 10.15376/biores.9.1.1606-1633

Rocha, N. R. de A. F., Barros, M. A., Fischer, J., Coutinho Filho, U., and Cardoso, V. L. (2013). "Ethanol production from agroindustrial biomass using a crude enzyme complex produced by *Aspergillus niger*," *Renew. Energ.* 57, 432-435. DOI: 10.1016/j.renene.2013.01.053

Saha, B. C., Nichols, N. N., and Cotta, M. A. (2011). "Ethanol production from wheat straw by recombinant *Escherichia coli* strain FBR5 at high solid loading," *Bioresource Technol*. 102(23), 10892-10897. DOI: 10.1016/j.biortech.2011.09.041

Segal, L., Creely, J., Martin, A., and Conrad, C. (1959). "An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer," *Text. Res. J.* 29(10), 786-794. DOI: 10.1177/004051755902901003

Selig, M. J., Viamajala, S., Decker, S. R., Tucker, M. P., Himmel, M. E., and Vinzant, T. B. (2007). "Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose," *Biotechnol. Progr.* 23(6), 1333-1339. DOI: 10.1021/bp0702018

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass* (NREL/TP-510-42618), National Renewable Energy Laboratory, Golden, USA.

Sun, R., Tomkinson, J., Wang, S., and Zhu, W. (2000). "Characterization of lignins from wheat straw by alkaline peroxide treatment," *Polym. Degrad. Stab*. 67(1), 101-109. DOI: 10.1016/S0141-3910(99)00099-3

Sun, T.-S., Wang, K., Yang, G., Yang, H.-Y., and Xu, F. (2014). "Hydrothermal treatment and enzymatic saccharification of corncobs," *BioResources* 9, 3000-3013. DOI: 10.15376/biores.9.1.3000-3013

Turner, J. A. (1999). "A realizable renewable energy future," *Science* 285(5428), 687-689. DOI: 10.1126/science.285.5428.687

Wang, K., Yang, H., Chen, Q., and Sun, R.-C. (2013). "Influence of delignification efficiency with alkaline peroxide on the digestibility of furfural residues for bioethanol production," *Bioresource Technol.* 146, 208-214. DOI: 10.1016/j.biortech.2013.07.008

Wang, K., Yang, H., Guo, S., Tang, Y., Jiang, J., Xu, F., and Sun, R.-C. (2012). "Organosolv fractionation process with various catalysts for improving bioconversion of triploid poplar," *Process Biochem.* 47(10), 1503-1509. DOI: 10.1016/j.procbio.2012.06.002

Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., Lee, Y., Mitchinson, C., and Saddler, J. N. (2009). "Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies," *Biotechnol. Progr.* 25(2), 333-339. DOI: 10.1002/btpr.142

Xu, C., Ma, F., Zhang, X., and Chen, S. (2010). "Biological pretreatment of corn stover by *Irpex lacteus* for enzymatic hydrolysis," *J. Agric. Food Chem.* 58(20), 10893-10898. DOI: 10.1021/jf1021187

Xu, J.-K., Sun, Y.-C., and Sun, R.-C. (2015). "Synergistic effects of ionic liquid plus alkaline pretreatments on eucalyptus: Lignin structure and cellulose hydrolysis," *Process Biochem.* 50(6), 955-965. DOI: 10.1016/j.procbio.2015.03.014

Yoon, L. W., Ngoh, G. C., Chua, M., Seak, A., and Hashim, M. (2011). "Comparison of ionic liquid, acid and alkali pretreatments for sugarcane bagasse enzymatic saccharification," *J. Chem. Technol. Biot.* 86(10), 1342-1348. DOI: 10.1002/jctb.2651

Zhang, L., You, T., Zhang, L., Yang, H., and Xu, F. (2014). "Enhanced fermentability of poplar by combination of alkaline peroxide pretreatment and semi-simultaneous saccharification and fermentation," *Bioresource Technol.* 164, 292-298. DOI: 10.1016/j.biortech.2014.04.075

Article submitted: April 18, 2016; Peer review completed: June 12, 2016; Revised version received: July 7, 2016; Accepted: July 11, 2016; Published: July 21, 2016.
DOI: 10.15376/biores.11.3.7551-7565