

Evaluation of Lignocellulose Degradation and Ethanol Production *via* Dilute Acid and Alkali Pretreatment of Hybrid *Pennisetum*

Fengqin Gao,^{a,#} Haoran Wang,^a Feng Li,^a Ying De,^a Yusha Ma,^a Heng Jiang,^a Yuanyuan Jing,^{a,#} Hui Qu,^{a,*} and Ping Li^{b,*}

Chemically pretreating lignocellulose has been widely used in the production of clean energy. An energy grass called hybrid *Pennisetum* was pretreated with different H₂SO₄ and NaOH concentrations to analyse the effects of an acid and alkali pretreatment on the degradation of hybrid *Pennisetum* lignocellulose. The chemical composition, hydrolyzed sugar yield, and microstructure were determined. The results showed that a NaOH pretreatment retained more cellulose and removed more lignins, while a H₂SO₄ pretreatment significantly inhibited the removal of lignins and increased hemicellulose degradation. X-ray diffraction analysis indicated that increasing the concentration of NaOH or H₂SO₄ could intensify the cellulose polymerization; this effect was stronger with NaOH compared to H₂SO₄. Scanning electron microscopy images showed that the cellulose bundles pretreated with H₂SO₄ were arranged tightly, while the cellulose bundles pretreated with NaOH were loose. Fourier transform infrared spectroscopy exhibited that the ether/ester bond between lignins and carbohydrates was broken by the H₂SO₄ pretreatment. The NaOH pretreatment increased the number of carbonyl and hydroxyl groups, resulting in more hydrophilic lignins, enabling greater facile degradation. Although the two methods are different in terms of destroying the structure of lignocellulose, the NaOH pretreatment can retain more cellulose, which is beneficial to subsequent ethanol production.

DOI: 10.15376/biores.17.3.4517-4531

Keywords: Hybrid *Pennisetum*; Chemical pretreatment; Composition change; Structural characteristics

Contact information: a: Grassland Research Institute, Chinese Academy of Agricultural Sciences, Hohhot 010010 P. R. China; b: Sichuan Academy of Grassland Sciences, Chengdu 611431 P. R. China; c: Hohhot Agriculture and Animal Husbandry Bureau, Hohhot 010020 P. R. China; #: These authors contributed equally to this paper; *Corresponding author: quhui@caas.cn (H. Qu); lpyzm@sina.cn (P. Li)

INTRODUCTION

The discovery and development of clean energy has become a trend in the advancements of global energy. The development prospect of using lignocellulose to produce renewable clean energy has attracted much attention over the years (Ward *et al.* 2008; Gao *et al.* 2014; Richa *et al.* 2019). Lignocellulose consists of cellulose, hemicellulose, and lignins and is the most abundant biomass in the world (Ji 2016; Hosseini *et al.* 2018). The natural structure of lignocellulose is complex and stable, making it difficult to convert into a clean fuel, so pretreatment is required to improve its degradation efficiency. Among the current pretreatment methods, chemical pretreatments are still the most important. They are commonly used due to their high efficiency and economic nature,

especially H₂SO₄ and NaOH (Himmel 2010; Behera *et al.* 2014; Chen *et al.* 2020; Wang *et al.* 2020). Although biomass pretreatment for depolymerizing lignocellulose has been studied for nearly 200 years (Rabemanolontsoa and Saka 2016), the structure of lignocelluloses in various plants is complex and different, leading to a diversity of pretreatment conditions. Among various lignocellulosic biomasses, perennial grasses are considered to be an ideal material for producing biomass energy. Hybrid *Pennisetum* is a perennial grass in the Gramineae family and is a triploid cross between *Pennisetum purpureum* and *Pennisetum americanum*. In recent years, hybrid *Pennisetum* has been listed as a potential material for the preparation of bioenergy and chemicals, due to its quality advantages, *e.g.*, good regeneration, rapid growth, high biomass, and extensive planting (Wang *et al.* 2018a,b). A dilute acid pretreatment can remove a portion of the lignins, degrade most of the hemicelluloses, and improve the enzymatic hydrolysis rate of cellulose. However, the dilute alkali pretreatment is primarily used to degrade lignins (Himmel 2010). Therefore, there is a need to study optimal techniques for the pretreatment of this plant for usage in ethanol production.

In this study, hybrid *Pennisetum* was pretreated with different concentrations of H₂SO₄ or NaOH to investigate their effects on lignocellulose degradation, and the optimal concentrations of H₂SO₄ and NaOH were selected to degrade the lignocelluloses of hybrid *Pennisetum*. Then, the mechanism of the acid and alkali degradation of the lignocellulose structure in hybrid *Pennisetum* was elucidated by observing cell wall compositional changes and structural characteristics after the acid and alkali pretreatment. Finally, further evaluation was conducted for ethanol production of the residue by different concentrations of dilute acid and alkali pretreatments. Through double evaluation of the dilute acid and alkali pretreatments and fermentation, the reliability and application value was strengthened. In addition, it provided a novel study on the ethanol production ability of hybrid *Pennisetum*.

EXPERIMENTAL

Materials

Hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum*) was collected from the Xiaotang Mountain grassland of the Beijing Academy of Agricultural and Forestry sciences in October 2018. The samples were air-dried in the field, cut into 2 to 4 cm lengths with a rubbing filament machine and mixed evenly, packed into plastic bags, and brought back to the laboratory for preservation. The dry matter was determined using a drying determination method at a temperature of 105 °C (according to the Association of Official Analytical Chemists, AOAC) in an oven (DHG-9240A, Jinghong Lab Instrument Co. Ltd, Shanghai, China), to ensure a constant weight.

Methods

Pretreatment

Six single factor experiments were conducted to evaluate changes in the H₂SO₄ (1.0%, 2.0%, and 3.0%, w/w) and NaOH (1.0%, 2.0%, and 3.0%, w/w) concentration for 3 replicates of the 3 levels of each factor. Approximately 10 g of the 2 to 4 cm samples of hybrid *Pennisetum*, along with the corresponding concentration of H₂SO₄ or NaOH, according to a solid to liquid ratio of 1 to 8, were put into 500 mL triangular bottles, and then sealed with aluminum foil. The filled bottles were placed in a vertical high-pressure

steam sterilizer for 30 mins at a temperature of 120 °C. After pretreatment, the filter residues were separated through gauze, washed with tap water until the pH was neutral and dried in an oven at a temperature of 40 to 60 °C to determine the cellulose, hemicellulose, and lignin content. Then, 50 mL of the filtrate solution was stored in centrifugal tubes at a temperature of -20 °C for the determination of the glucose and xylose concentrations.

Chemical constituents

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin contents were determined by a method described by Soest *et al.* (1991), using a ANKOM semi-automatic cellulose analyzer (A2000i, Ankom Technology Co., Macedon, NY). The ash content was determined using a muffle furnace (at a temperature of 550 °C for 30 min) (KL-SWCK6-10S smart muffle furnace, Hebi Keli Measurement and Control Technology Co., Ltd., Henan, China). The chemical constituents of the hybrid *Pennisetum* are shown in Table 1. The concentrations of glucose and xylose were determined *via* high performance liquid chromatography (LC 1200-HPLC, Agilent Technologies Co. Ltd., Santa Clara, CA). The analytical conditions were as follows: an Agilent sugar column at a temperature of 30 °C, a mobile phase with an acetonitrile to water ratio of 80 to 20, a refractive index detector at a temperature of 25 °C, a pressure of 40 MPa, and an injection volume of 10 µL, with a flow rate of 1 mL·min⁻¹.

Table 1. Chemical Constituents of Hybrid *Pennisetum*

Item	Content (%DM)
Cellulose	35.57
Hemicellulose	27.65
Lignin	11.23
Neutral detergent fiber (NDF)	48.36
Acid detergent fiber (ADF)	76.45
Ash	3.70
Dry matter	95.20

X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR) analysis

The X-ray diffraction (XRD) was conducted with an X'Pert Pro MPD (X'Pert Pro MPD, PANalytical B.V. Co, Almelo, Netherlands), and the crystallinity of the hybrid *Pennisetum* was reported relative to the 50% crystallinity of SiO₂. Scanning electron microscopy (SEM) images were obtained using a S-530 scanning electron microscope (Hitachi S-530, Hitachi manufacturing Co, Tokyo, Japan). The Fourier transform infrared (FTIR) readings were obtained using a Tensor 27 Fourier transform infrared spectroscopy analyzer (Tensor 27, Bruker Optik GmbH, Ettlingen, Germany) (You *et al.* 2016).

Enzymatic hydrolysis and fermentation

After pretreatment, enzymatic hydrolysis, and fermentation experiments were conducted. First, 3 replicates of the 10 g samples, *i.e.*, the residue after pretreatment, of the pretreated grass were put into 100 mL serum glass bottles. Then, a 3% peptone solution was added, and the serum bottles were transferred to a superclean bench until the liquid sample temperature decreased to 30 °C. Next, temperature-resistant active dry-Angel yeast at a concentration of 0.03% (w/v) and 20 U/g of cellulase activated by a 2% sucrose solution were added to the solution. A simultaneous saccharification and fermentation

(SSF) process was used for the enzymatic hydrolysis and the fermentation in a citric acid-sodium citrate buffer with a pH of 4.8 at a temperature of 34 °C at 100 r/min for 96 h. During the fermentation, liquid samples were periodically taken for monitoring their glucose and ethanol concentrations (Gao *et al.* 2014).

Statistical Analysis

All experimental data were expressed as the average value. All statistical analyses were performed using SPSS 19.0 and statistically significant differences were applied for p-values less than 0.05. One-way ANOVA analyses followed by a multi-comparison of Duncan's test was used to compare the differences among the experimental treatments. All figures were plotted using SigmaPlot 10.0.

RESULTS AND DISCUSSION

Changes in the Extent of Solid Decomposition

The extent of solid decomposition means the percentage of the dry matter hydrolyzed by the raw sample after pretreatment, which exhibited an increasing trend with increasing levels of the two single factor conditions. The solid decomposition is a comprehensive index to evaluate the efficiency of a pretreatment, and can reflect the removal of cellulose, hemicellulose, lignins, and other impurities. The solid decomposition increased as the NaOH and H₂SO₄ concentration increased. However, the solid decomposition in the H₂SO₄ pretreatment was lower than the NaOH pretreatment, especially at a 3% concentration (as shown in Fig. 1A).

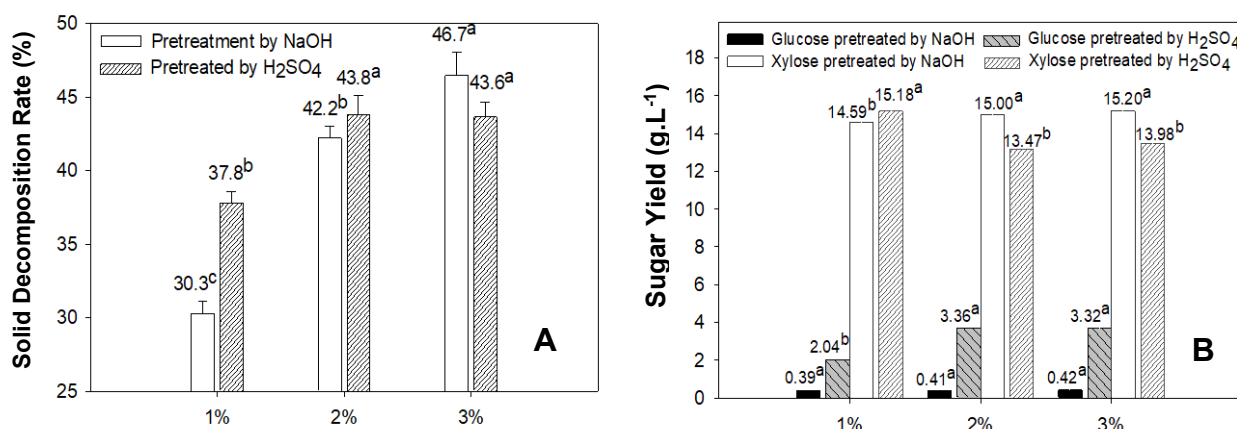


Fig. 1. Solid decomposition rate (A) and sugar yield (B) of hybrid *Pennisetum* under dilute acid and alkali pretreatments

The solid decomposition of hybrid *Pennisetum* under a H₂SO₄ pretreatment was higher than the solid decomposition of NaOH when the concentration was 1% to 2%; while at 3% concentration, the situation was reversed. The solid decomposition exhibited a large difference between the NaOH and H₂SO₄ pretreatments at 1% concentration, and a small difference at 2% concentration, and the situation was reversed at 3% concentration. The H₂SO₄ pretreatment significantly promoted the solid decomposition rate to a certain range, which was consistent with the results of Ballesteros *et al.* (2008). However, the

decomposition of lignocellulose would likely vary among different kinds of plants; so specific analyses may be needed (Ji 2016).

Chemical Composition and Degradation of Lignocellulose

The degradation ability of the H₂SO₄ pretreatment in terms of cellulose and hemicellulose was greater than the degradation ability of NaOH. However, the removal efficiency of the H₂SO₄ pretreatment in terms of lignins was much lower than the removal efficiency of NaOH at each concentration (Fig. 2B). The cellulose content could be decreased to 31.3% to 32.5% (Fig. 2A), the hemicellulose content could be degraded more than 80%, and the lignin content could be reduced 15.3% more under the H₂SO₄ pretreatment. Meanwhile, under the NaOH pretreatment, cellulose retention was greater than 35.0%, and the degradation of the hemicellulose and lignin content was 47.3% to 73.4% and 40% to 88.3%, respectively.

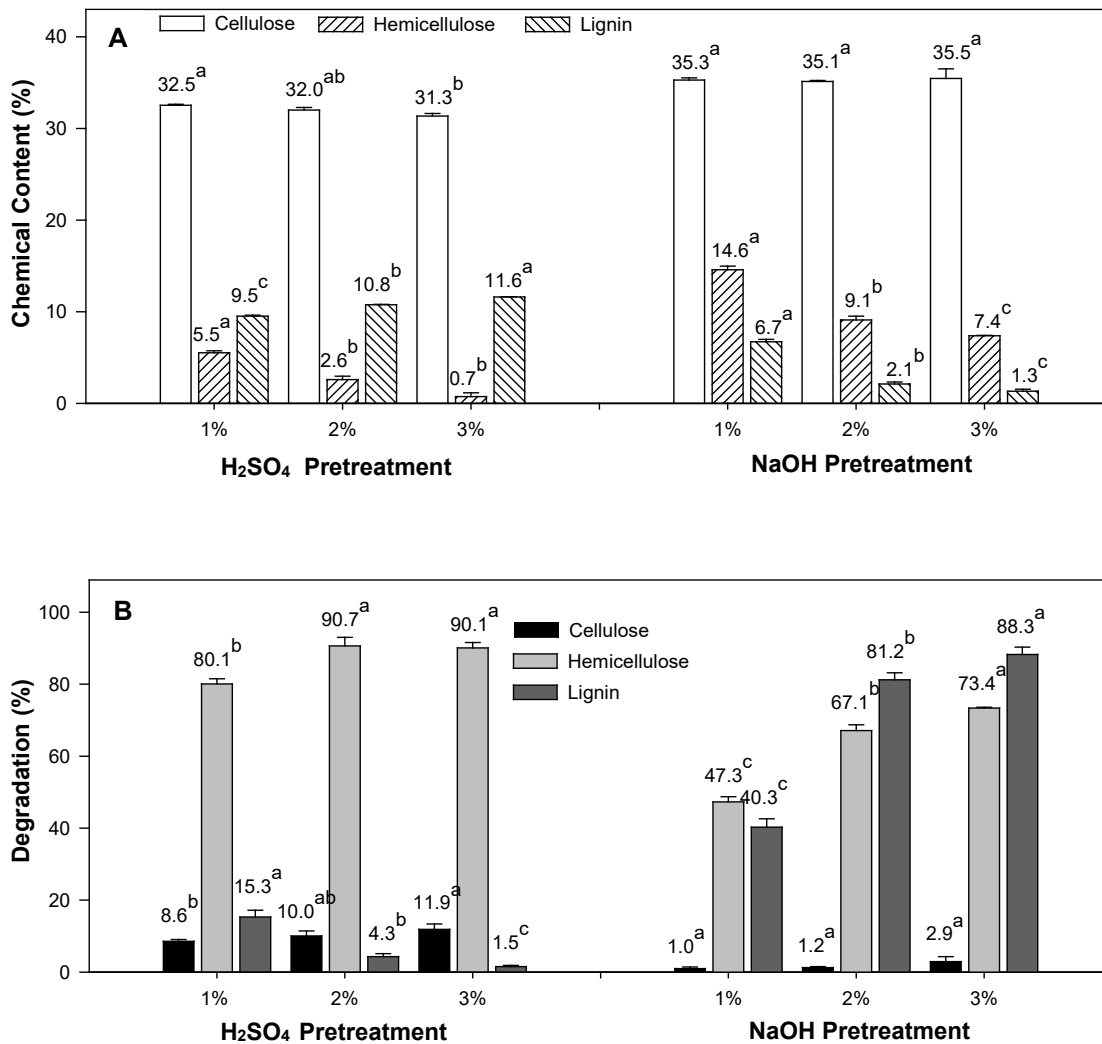


Fig. 2. The differences in the cellulose, hemicellulose, and lignin contents in hybrid *Pennisetum* under dilute acid and alkali pretreatments: (A) Chemical composition of hybrid *Pennisetum*; and (B) Degradation of the cellulose, hemicellulose, and lignins in hybrid *Pennisetum*

The NaOH pretreatment effectively removed lignins and some hemicelluloses, while the H₂SO₄ pretreatment significantly inhibited the removal of lignin. As the concentration increased, the degradation of hemicellulose increased, with the degradation effect being stronger in the H₂SO₄ pretreatment compared to the NaOH pretreatment. The lignin removal increased as the NaOH concentration increased, but it decreased as the H₂SO₄ concentration increased. This was attributed to the fact that the OH⁻ could weaken the hydrogen bond between the hemicellulose and cellulose, and saponify the ester bond between the hemicellulose and lignin, thereby promoting the dissolution of hemicelluloses and lignins (Wahab 2009; Huang 2012; Miao *et al.* 2018). By contrast, under acidic conditions, the H⁺ could form H₃O⁺ with H₂O and promote hemicellulose solubilization. Since the H₃O⁺ was selective for the hydrolysis of glycosidic bonds in hemicellulose when conditions are identical, the dissolution of hemicelluloses was higher than the dissolution of lignins (Ji 2016; Jönsson and Martín 2016). Therefore, the H₂SO₄ pretreatment caused the hemicellulose degradation efficiency to increase more rapidly than the lignin removal efficiency; therefore, the effect of the H₂SO₄ pretreatment was primarily reflected in the hydrolysis of hemicellulose. When the hemicellulose was separated from the cell wall structure, a large amount of lignins remained on the cellulose surface, hindering the access of cellulase, thereby affecting the efficiency of enzymatic hydrolysis (DeMartini *et al.* 2013; Si 2015). The NaOH pretreatment primarily dissolved lignins, destroyed the lignocellulose structure, and increased the accessibility of cellulase to cellulose, thus increasing the enzymatic hydrolysis efficiency (Himmel 2010; Kataria *et al.* 2013).

X-ray Diffraction (XRD) Analysis

The cellulose crystal polymerization degree is an important factor affecting the enzymatic hydrolysis of lignocellulose and the accessibility of cellulose (Kim and Holtzaple 2006; Park *et al.* 2010; Xu *et al.* 2019).

X-ray diffraction spectra are often used to reflect the degree of cellulose crystal polymerization. The XRD peak area can reflect the crystal content. The larger the area, the higher will be the crystal content. As shown in Figs. 3A and 3B, the XRD characteristic peaks of all the pretreated materials were higher than the characteristic peaks of the raw materials. All the curves exhibit the same trend at all angles, but the peak values are different, and the index of crystallinity is larger with the peak value. Increasing the concentration of H₂SO₄ and NaOH made the diffraction peaks more obvious, and the XRD peaks of the NaOH pretreatment was sharper than the peaks of the H₂SO₄ pretreatment at the same concentration. Therefore, increasing the pretreatment concentration increased the intensity of cellulose polymerization. This effect was stronger in the NaOH pretreatment compared to the H₂SO₄ pretreatment, which was inversely proportional to the degree of hydrolysis of cellulose. Previous research by Gao *et al.* (2014) on silvergrass with dilute alkali also reached the same conclusion, *i.e.*, the crystallinity of cellulose increases with the increase in alkali concentration. Li *et al.* (2016) considered that the hydrogen bond splitting of cellulose molecules would loosen the molecular chains, weaken the diffraction intensity, and increase the crystallinity. However, the crystallinity increase did not hinder the removal of lignins and the hydrolysis of hemicelluloses (Chundawat *et al.* 2011; Ji 2016). After the H₂SO₄ and NaOH pretreatments, most lignins and hemicelluloses were dissolved, and the cellulose leaked naked, which greatly increased the accessibility of cellulose, which decomposed into monosaccharides.

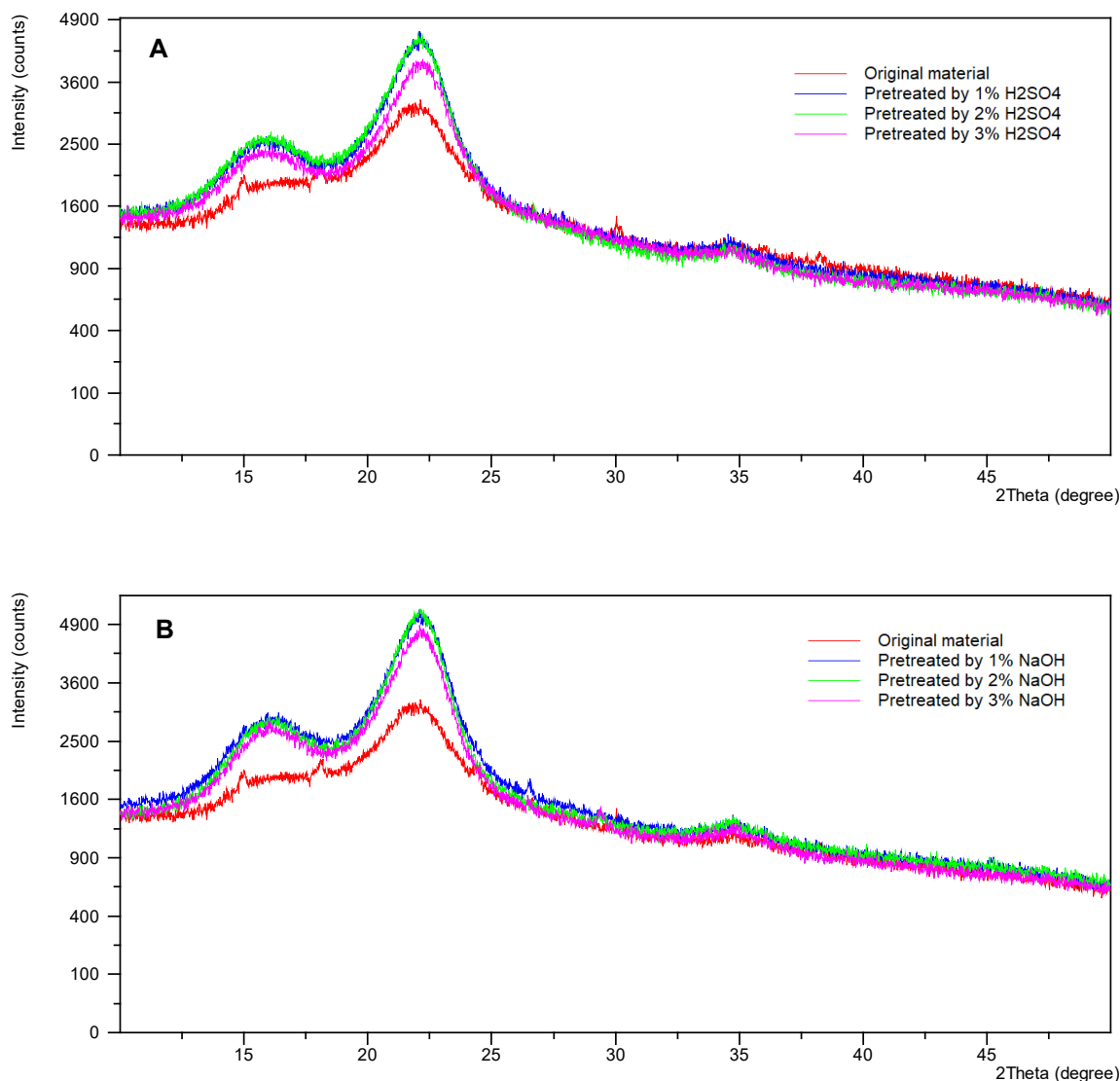


Fig. 3. XRD spectrum of hybrid *Pennisetum* under dilute acid and alkali pretreatments: (A) XRD patterns of hybrid *Pennisetum* pretreated with different concentrations of H₂SO₄; and (B) XRD patterns of hybrid *Pennisetum* pretreated with different concentrations of NaOH

Glucose and Xylose Production

The hydrolysates of cellulose and hemicellulose are primarily glucose and xylose, and their content can better reflect the effect of the pretreatment on the hydrolysis of celluloses and hemicelluloses (Chen *et al.* 2010). The concentration of xylose was significantly higher than the concentration of glucose in both the H₂SO₄ and NaOH pretreatments, which was consistent with the degree of degradation of hemicellulose and cellulose (Fig. 1B). The glucose content produced by the H₂SO₄ pretreatment at all concentrations was higher than the glucose content of the NaOH pretreatment; the difference between the 1% and 2% concentration was significant (*p*-value was less than 0.05), while the difference between the 2% and 3% concentration was insignificant (*p*-value was greater than 0.05). This meant that the H₂SO₄ pretreatment could degrade more cellulose than the NaOH pretreatment, which was also supported by the results shown in Fig. 2. In theory, if the H₂SO₄ pretreatment degrades more hemicellulose than the NaOH

pretreatment, then more xylose should be produced. However, the xylose content decreased as the H_2SO_4 concentration increased, and it increased as the NaOH concentration increased. The reason for this is that as the acid concentration increases, the xylose is further decomposed into harmful substances, *e.g.*, furfural and hydroxymethyl furfural. The alkaline pretreatment can inhibit the production of this harmful substance (Camesasca *et al.* 2015; Yong and Kim 2017). The increased NaOH concentration promoted hemicellulose degradation and inhibited xylose production, which suggests that under NaOH pretreatment, xylose readily produced furfural and other substances through further dehydration (Lee *et al.* 2015; Wang *et al.* 2015). Therefore, a high concentration NaOH pretreatment was not conducive to the preservation of xylose.

Scanning Electron Microscopy (SEM) Structure Analysis

The optimum concentrations for the H_2SO_4 and NaOH pretreatments in terms of the lignocellulose degradation of hybrid *Pennisetum* were 2% and 3%, respectively. The raw material of hybrid *Pennisetum*, and the samples pretreated with 3% NaOH and 2% H_2SO_4 were scanned *via* electron microscopy to elucidate structural differences. The inner and outer surfaces of the raw material were rough, and a large number of non-cellulose substances were interwoven before the pretreatment (Figs. 4A and 4B).



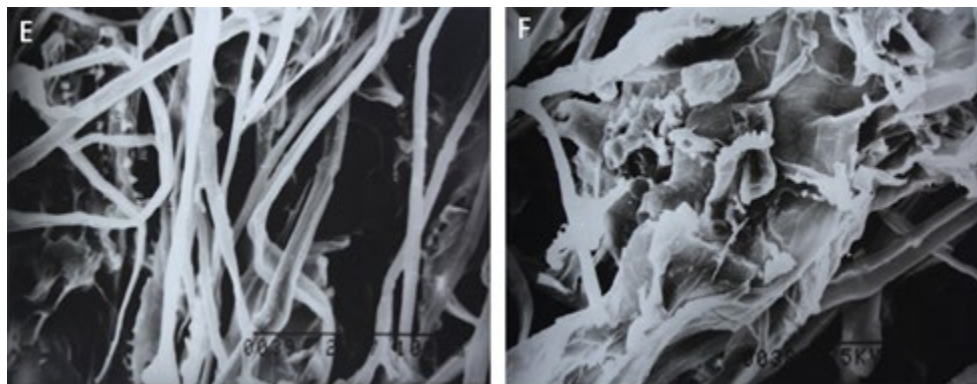


Fig. 4. SEM structure of hybrid *Pennisetum* under dilute acid and alkali pretreatments: (A) External surface of the lignocellulose before pretreatment ($\times 500$); (B) Inner surface of the lignocellulose before pretreatment ($\times 600$); (C) External surface of the lignocellulose pretreated with H_2SO_4 ($\times 500$); (D) Inner surface of lignocellulose pretreated with H_2SO_4 ($\times 600$); (E) External surface of the lignocellulose pretreated with NaOH ($\times 500$); and (F) Inner surface of the lignocellulose pretreated with NaOH ($\times 600$)

Cellulose bundles were visible and non-cellulose substances in the outer layer were clearly separated after pretreatment. The cellulose bundles were tightly arranged, and a small amount of non-cellulose substances remained after the H_2SO_4 pretreatment (Fig. 4C); these residues were lignins and celluloses, as most of the hemicelluloses were degraded.

The NaOH pretreatment could removed non-cellulose fraction more thoroughly than the H_2SO_4 pretreatment, and the cellulose bundles were loosely arranged after pretreatment (Fig. 4E and 4F). According to the regularity of plant cell structure formation, the vascular bundle structure composed of cellulose is more abundant in the outer stem of the epidermis and is scattered near the inner surface of the medulla; as such, the outer layer gradually becomes lignified as the plant grows (Himmel 2010). Therefore, the lignin and cellulose contents in the outer layer of the plant stem are higher than in the inner layer. The structure of the inner layers was compact, with a low number of cellulose bundles, which is consistent with the images shown in Fig. 4A and 4B. After H_2SO_4 and NaOH pretreatments, the structure of the non-cellulose substances changed in the inner layers, but they were not significantly removed. However, the lignocellulose structure collapsed, and a small amount of cellulose was clearly visible (as shown in Fig. 4D and 4F). The H_2SO_4 pretreatment can degrade more hemicellulose and retain more cellulose but leads to greater xylose degradation and the production of harmful substances, *e.g.*, furfural. The NaOH pretreatment can remove most of the lignins and promote the degradation of hemicellulose. Although the two methods are different in terms of destroying the lignocelluloses structure, the NaOH pretreatment can retain more cellulose, which is beneficial to subsequent ethanol production.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier transform infrared spectroscopy is often used to study changes in the composition, structure, and chemical bonds of lignocellulose during lignocellulose pretreatment (Chundawat *et al.* 2007; Li *et al.* 2009; Sun *et al.* 2013). The absorbance peaks of the three hybrid *Pennisetum* were similar, but the intensities were different (as shown in Fig. 5). The unique cellulose peaks included β -1,4-glycosidic bonds at 894 cm^{-1} (Gia *et al.* 2008), C-C bonds of the sugar ring at $400\text{ to }750\text{ cm}^{-1}$, C-O rings at 1160 cm^{-1} , and C-H stretching at 2920 cm^{-1} . Those bonds were weakened after the H_2SO_4 and NaOH

pretreatments, and the stretching of the bonds was weaker after the H₂SO₄ pretreatment compared to the NaOH pretreatment, which indicated that H₂SO₄ pretreatment could destroy cellulose structures to a greater extent than the NaOH pretreatment.

Polymers of the heteropoly saccharides of hemicelluloses are linked by covalent bonds, hydrogen bonds, ether bonds, and ester bonds. The absorption peak of the ether bond at 1252 cm⁻¹ and the acetyl ester bond at 1734 cm⁻¹, weakened after the H₂SO₄ pretreatment, and was lower than the raw material and the NaOH pretreatment (Gia *et al.* 2008). The NaOH pretreatment enhanced the absorption of the two bonds the most. This was due to the acetylation of the hemicellulose to some organic acids after the H₂SO₄ pretreatment, which reduced the number of acetyl ester bonds (Ma *et al.* 2018). However, the functional groups recombined and produced new ether/ester bonds under alkaline conditions. The absorption peaks of the lignins with aromatic rings at 1510 cm⁻¹ after the NaOH pretreatment were stronger than the peaks of the H₂SO₄ pretreatment and raw material (Sun *et al.* 2013). The 3440 cm⁻¹ band was the O-H stretching vibration of the -OH group in the lignin structure, and NaOH pretreatment made the -OH easier to replace (Zheng and Dong 2011). Both the H₂SO₄ and NaOH pretreatments weakened the absorption of the aromatic hydroxyl group (1034 to 1060 cm⁻¹) and the phenolic hydroxyl group (1375 cm⁻¹). Under the acid conditions, the concentration of OH⁻ would be very low. The absorbance peaks of the methoxy (-OCH₃) in the lignins and hemicelluloses were at 1425 and 1460 cm⁻¹, respectively, and the absorbance intensity was in the following order: H₂SO₄ was less than NaOH, which was less than the raw material. The absorption region of the carbonyl group was 1650 cm⁻¹, and its absorbance intensity increased in both the H₂SO₄ and NaOH pretreatments, which was higher than the raw material. Both the hydroxyl group and the methoxy group on the hemicelluloses and lignins were easily preserved under alkaline conditions, and easily recombined under the acid pretreatment. In agreement with Sun *et al.* (2004), the acidic and alkali pretreatments increased the amount of carbonyl groups in lignins, due to the β-O-4 linkages or another bond rearrangements, as represented by the band at 1250 cm⁻¹. Gia *et al.* (2008) pointed out that an increase in carbonyl and hydroxyl groups could make lignins more hydrophilic and degradable. The absorption peaks of the carbonyl and hydroxyl groups in the NaOH pretreatment were stronger than the peaks in the H₂SO₄ pretreatment. Therefore, NaOH could more effectively degrade lignins compared to the H₂SO₄ pretreatment.

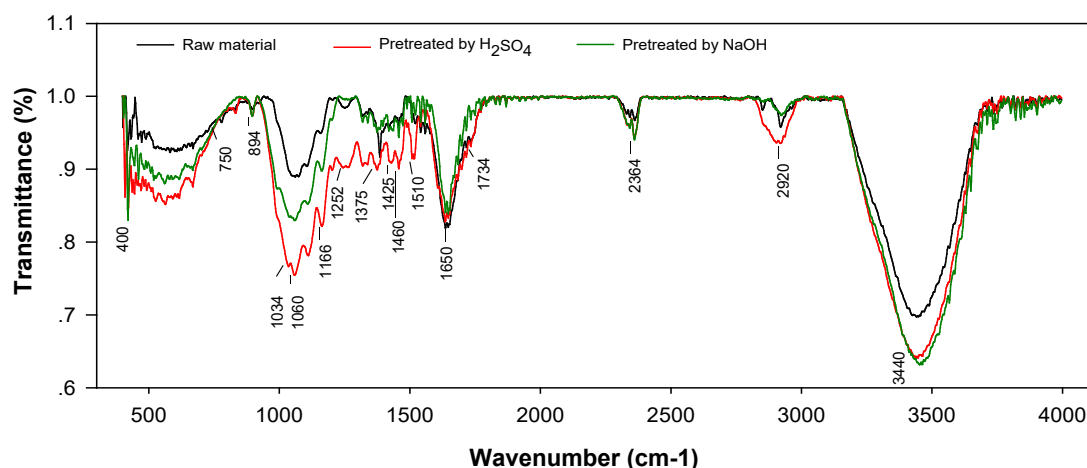


Fig. 5. FTIR analysis of hybrid *Pennisetum* under dilute acid and alkali pretreatments

Enzymatic Hydrolysis and Fermentation

The yields of the glucose and ethanol were determined. The concentration of glucose was highest after 48 h of fermentation, and then it decreased as the fermentation time further increased (Fig. 6). The results were similar to other studies following the trend of glucose concentration during fermentation (Kuo *et al.* 2014; Hu *et al.* 2016). The concentration of ethanol was highest at 72 h, and it remained unchanged as the fermentation time further increased. The concentration of the glucose and ethanol after a 3% NaOH pretreatment was higher than after a 2% H₂SO₄ pretreatment. Under the two conditions, the ethanol concentration reached its maximum at 60 h, which shortened the fermentation time and saved costs compared to other methods (Camesasca *et al.* 2015). This increased fermentability may be attributed to decreases in crystalline sizes (Fan *et al.* 2021). Otherwise, alkali pretreatment can effectively remove lignin, improve the porosity of substrates, and improve the enzymatic hydrolysis performance of cellulose (Li *et al.* 2016).

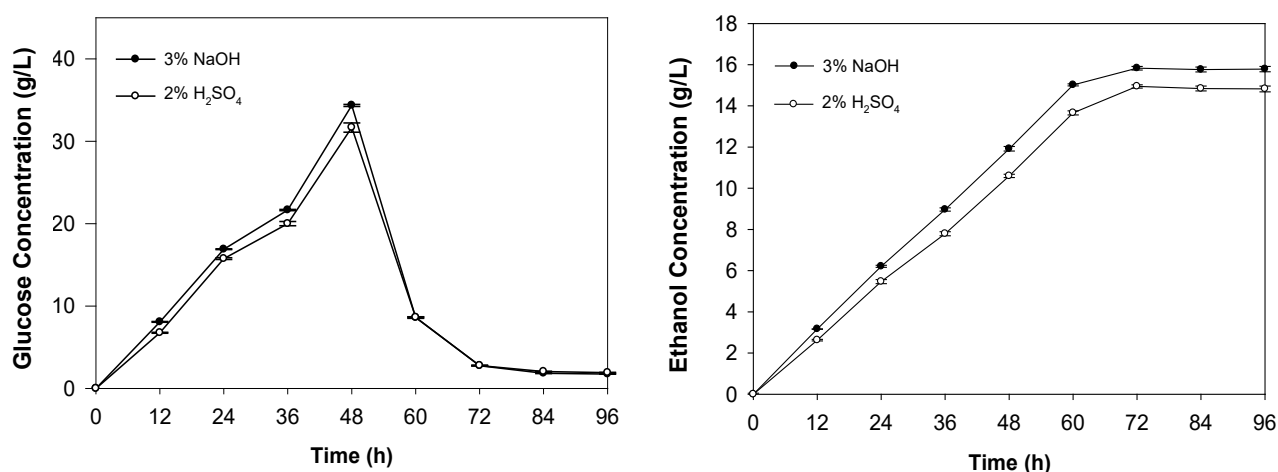


Fig. 6. Glucose and ethanol concentrations of hybrid *Pennisetum* under dilute acid and alkali pretreatments after enzymatic hydrolysis and fermentation for 96 h

CONCLUSIONS

- 1 The H₂SO₄ pretreatment primarily broke the ether-ester bonds between lignins and carbohydrates, and it effectively removed hemicelluloses, while having little effect on lignin removal. The cellulose bundles were neatly arranged, and the amount of lignin residue was low after the H₂SO₄ pretreatment.
- 2 The NaOH pretreatment increased the number of carbonyl and hydroxyl groups, making the lignins more hydrophilic and conducive to degradation, as well as effectively removing lignins and dissolving some hemicelluloses.
- 3 The H₂SO₄ pretreatment can degrade more hemicellulose, but it leads to additional xylose degradation and the production of harmful substances, *e.g.*, furfural. The NaOH pretreatment can remove most of the lignins and promote the degradation of hemicelluloses.
- 4 Although the two pretreatment methods are different in terms of destroying the lignocellulose structure, the NaOH pretreatment is more conducive to alcohol production than the H₂SO₄ pretreatment.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 31802134), the Inner Mongolia Science and Technology Project (Grant No. 201802082), and the Agricultural Science and Technology Innovation Program of CAAS (CAAS-ASTIP-IGR 2015-02).

REFERENCES CITED

- Ballesteros, I., Ballesteros, M., Manzanares, P., Negro, M. J., Oliva, J. M., and Sáez, F. (2008). "Dilute sulfuric acid pretreatment of cardoon for ethanol production," *Biochemical Engineering Journal* 42(1), 84-91. DOI: 10.1016/j.bej.2008.06.001
- Behera, S., Arora, R., Nandhagopal, N., and Kumar, S. (2014). "Importance of chemical pretreatment for bioconversion of lignocellulosic biomass," *Renewable and Sustainable Energy Reviews* 36, 91-106. DOI: 10.1016/j.rser.2014.04.047
- Camesasca, L., Ramírez, M. B., Guigou, M., Ferrari, M. D., and Lareo, C. (2015). "Evaluation of dilute acid and alkaline pretreatments, enzymatic hydrolysis and fermentation of napiergrass for fuel ethanol production," *Biomass and Bioenergy* 74, 193-201. DOI: 10.1016/j.biombioe.2015.01.017
- Chen, Y., Yang, H., Zou, H., Sun, T., and Tang, W. Z. (2020). "Effects of acid/alkali pretreatments on lignocellulosic biomass mono-digestion and its co-digestion with waste activated sludge," *Journal of Cleaner Production* 277, article no. 123998. DOI: 10.1016/j.jclepro.2020.123998
- Chundawat, S. P. S., Beckham, G. T., Himmel, M. E., and Dale, B. E. (2011). "Deconstruction of lignocellulosic biomass to fuels and chemicals," *Annual Review of Chemical and Biomolecular Engineering* 2, 121-145. DOI: 10.1146/annurev-chembioeng-061010-114205
- Chundawat, S. P. S., Venkatesh, B., and Dale, B. E. (2007). "Effect of particle size based separation of milled corn stover on AFEX pretreatment and enzymatic digestibility," *Biotechnology and Bioengineering* 96(2), 219-231. DOI: 10.1002/bit.21132
- DeMartini, J. D., Pattathil, S., Miller, J. S., Li, H. J., Hahn, M. G., and Wyman, C. E. (2013). "Investigating plant cell wall components that affect biomass recalcitrance in poplar and switchgrass," *Energy & Environmental Science* 6(3), 898-909. DOI: 10.1039/c3ee23801f
- Fan, M., Zhao, C., Huang, X., Zhang, H., and Xie, J. (2021). "Enhanced digestibility and fermentability of sugarcane bagasse in biofuel production by surfactant-assisted dilute acid pretreatment," *Industrial Crops and Products* 172, article no. 114006. DOI: 10.1016/j.indcrop.2021.114006
- Gao, F. O., Yang, F. Y., Zhou, H., Sun, Q. Z., Zhang, Y. W., and Brown, M. A. (2014). Evaluation of processing technology for *Triarrhena sacchariflora* (maxim.) nakai for ethanol production," *PLOS ONE* 9 (12), 1-16. DOI: 10.1371/journal.pone.0114399
- Himmel, M. E. (2010). *Biomass Recalcitrance: Deconstructing the Plant Cell Wall for Bioenergy*, Wiley, Hoboken, NJ.
- Hu, B. B., Zhu, S. Y., Fang, S., Huo, M. X., Li, Y. H., Yu, Y., and Zhu, M. J. (2016). "Optimization and scale-up of enzymatic hydrolysis of wood pulp for cellulosic sugar production," *BioResources* 11(3), 7242-7257. DOI: 10.15376/biores.11.3.7242-7257

- Huang, T. (2012) *Effect of Alkaline Pretreatment on the Chemical Compositions and Enzymatic Hydrolysis of Wheat Straw*, Master's Thesis, Nanjing Forestry University, Nanjing, China
- Ji, Z. (2016). *Deconstruction of Plant Cell Wall During Dilute Acid and Alkali Pretreatment*, Ph.D. Dissertation, Beijing Forestry University, Beijing, China.
- Jönsson, L. J., and Martín C. (2016). "Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects," *Bioresource Technology* 199, 103-112. DOI: 10.1016/j.biortech.2015.10.009
- Kataria, R., Ruhel, R., Babu, R., and Ghosh, S. (2013). "Saccharification of alkali treated biomass of Kans grass contributes higher sugar in contrast to acid treated biomass," *Chemical Engineering Journal* 230, 36-47. DOI: 10.1016/j.cej.2013.06.045
- Kim, S., and Holtzapfle, M. T. (2006). "Effect of structural features on enzyme digestibility of corn stover," *Bioresource Technology* 97(4), 583-591. DOI: 10.1016/j.biortech.2005.03.040
- Kuo, C. H., Lin, P. J., Wu, Y. Q., Ye, L. Y., Yang, D. J., Shieh, C. J., and Lee, C. K. (2014). "Simultaneous saccharification and fermentation of waste textiles for ethanol production," *BioResources* 9(2), 2866-2875. DOI: 10.15376/biores.9.2.2866-2875
- Lee, C. M., Zheng, Y., and VanderGheynst, J. S. (2015). "Effects of pretreatment conditions and post-pretreatment washing on ethanol production from dilute acid pretreated rice straw," *Biosystems Engineering* 137, 36-42. DOI: 10.1016/j.biosystemseng.2015.07.001
- Li, K. N., Wan, J. M., Wang, X., Wang, J. F., and Zhang, J. H. (2016). "Comparison of dilute acid and alkali pretreatment in production of fermentable sugars from bamboo: Effect of Tween 80," *Ind. Crop Prod.* 83, 414-422. DOI: 10.1016/j.indcrop.2016.01.003
- Li, L., Sun, J. S., Li, M., Wang, S. H., Pei, H. S., and Zhang, J. S. (2009). "Enhanced enzymatic hydrolysis and structural features of corn stover by FeCl₃ pretreatment," *Bioresource Technology* 100(23), 5853-5858. DOI: 10.1016/j.biortech.2009.06.040
- Li, C. L., 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 Technology* 101(13), 4900-4906. DOI: 10.1016/j.biortech.2009.10.066
- Li, Q. M., Li, X. J., Jiang, Y. L., Xiong, X. Y., Hu, Q. L., Tan, X. H., Wang, K. Q., and Su, X. J. (2016). "Analysis of degradation products and structural characterization of giant reed and Chinese silvergrass pretreated by ⁶⁰Co- γ irradiation," *Industrial Crops and Products* 83, 307-315. DOI: 10.1016/j.indcrop.2016.01.024
- Ma, T., Zhao, J., Ao, L., Liao, X. J., Ni, Y. Y., Hu, X. S., and Song, Y. (2018). "Effects of different pretreatments on pumpkin (*Cucurbita pepo*) lignocellulose degradation," *International Journal of Biological Macromolecules* 120 (Part A), 665-672. DOI: 10.1016/j.ijbiomac.2018.08.124
- Miao, L. P., Huo, L., Xu, L., Zhou, R. J., Yang, M. Y., and Fan, C. (2018). "Effects of mild alkaline hydrogen peroxide (AHP) pretreatment on enzymatic saccharification of wheat straw," *Journal of Cellulose Science and Technology* 26(4), 45-51.
- Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., and Johnson, D. K. (2010). "Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance," *Biotechnology for Biofuels* 3, 1-10. DOI: 10.1186/1754-6834-3-10

- Rabemanolontsoa, H., and Saka, S. (2016). "Various pretreatments of lignocellulosics," *Bioresource Technology* 199, 83-91. DOI: 10.1016/j.biortech.2015.08.029
- Richa, A., Sharma, N. K., Kumar, S., and Sani, R. K. (2019). "Lignocellulosic ethanol: Feedstocks and bioprocessing," in: *Bioethanol Production from Food Crops*, R. C. Ray, and S. Ramachandran (ed.), Academic Press, Cambridge, MA.
- Si, S. (2015). *Characterization of the Wall Polymer Features That Affect Biomass Saccharification and Ethanol Production in Miscanthus*, Ph.D. Dissertation, Huazhong Agricultural University, Wuhan, China.
- Soest, P. J. V., Robertson, J. B., and Lewis, B. A. (1991). "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition," *Journal of Dairy Science* 74(10), 3583-3597. DOI: 10.3168/jds.S0022-0302(91)78551-78552
- Sun, J., Xu, L., Ge, M. Y., and Zhai, M. L. (2013). "Radiation degradation of microcrystalline cellulose in solid status," *Journal of Applied Polymer Science* 127(3), 1630-1636. DOI: 10.1002/app.37544
- Sun, X. F., Xu, F., Sun, R. C., Wang, Y. X., Fowler, P., and Baird, M. S. (2004). "Characteristics of degraded lignins obtained from steam exploded wheat straw," *Polymer Degradation and Stability* 86(2), 245-256. DOI: 10.1016/j.polymdegradstab.2004.05.003
- Wahab, S. (2009). "Biotechnological approaches in the management of plant pests, diseases and weeds for sustainable agriculture," *J. Biopesticides* 2(2), 115-134.
- Wang, D., Ai, P., Yu, L., Tan, Z., and Zhang, Y. (2015). "Comparing the hydrolysis and biogas production performance of alkali and acid pretreatments of rice straw using two-stage anaerobic fermentation," *Biosystems Engineering* 132, 47-55. DOI:10.1016/j.biosystemseng.2015.02.007
- Wang, J. F., Hao, X. X., Hou, X. C., and Zhang, J. H. (2018a). "Effect of alkaline sulfite pretreatment on enzymatic hydrolysis of hybrid China wolftailgrass," *Journal of Forestry Engineering* 3(2), 95-101.
- Wang, W. Q., Zhou, H. L., and Tang, J. (2018b). "Research advances on development and utilization of forage of *Pennisetum* spp.," *Journal of Tropical Agricultural Sciences* 38(6), 49-55,78.
- Wang, J., Fishwild, S. J., Begel, M., and Zhu, J. Y. (2020). "Properties of densified poplar wood through partial delignification with alkali and acid pretreatment," *Journal of Materials Science* 55, 14664-14676. DOI: 10.1007/s10853-020-05034-2
- Ward, A. J., Hobbs, P. J., Holliman, P. J., and Jones, D. L. (2008). "Optimisation of the anaerobic digestion of agricultural resources," *Bioresource Technology* 99(17), 7928-7940. DOI: 10.1016/j.biortech.2008.02.044
- Xu, H. F., Che, X. P., Ding, Y., Kong, Y., Li, B., and Tian, W. D. (2019). "Effect of crystallinity on pretreatment and enzymatic hydrolysis of lignocellulosic biomass based on multivariate analysis," *Bioresource Technology* 279, 271-280. DOI: 10.1016/j.biortech.2018.12.096
- Yong, H. J., and Kim, K. H. (2017). "Evaluation of the main inhibitors from lignocelluloses pretreatment for enzymatic hydrolysis and yeast fermentation," *BioResources* 12(4), 9348-9356. DOI: 10.15376/biores.12.4.9348-9356
- You, Y. N., Deng, H., Meng, Y. H., Fang, J., Guo, Y. R., and Zhang, Y. (2016). "The impact of alkaline hydrogen peroxide pretreatment on the chemical composition and enzymatic hydrolyzability of apple pomace," *Scientia Agricultura Sinica* 49(8), 1559-1566. DOI: 10.3864/j.issn.0578-1752.2016.08.013

Zheng, Q. K., and Dong, Q. S. (2011). “Structural analysis of lignins from three different sources,” *Journal of Weifang University* 11(6), 58-61.

Article submitted: June 3, 2021; Peer review completed: September 11, 2021; Revised version received and accepted: May 22, 2022; Published: June 9, 2022.

DOI: 10.15376/biores.17.3.4517-4531