Comparison of Dilute Acid, Alkali, and Biological Pretreatments for Reducing Sugar Production from Eucalyptus

Haojun Lu,[a] Xinwen Zhang,[a] Aimin Wu,[a] Xiaomei Deng,[a,*] Junli Ren,[b] Fangong Kong,[c] and Huiling Li [a,b,c,*]

The effects of chemical pretreatments (dilute H_2SO_4, dilute NaOH, and NH_4OH) and biological pretreatments (*Coriolus versicolor* and *Daedalea quercina*) on the enzymatic hydrolysis of *Eucalyptus* were investigated. The results showed that *Eucalyptus* obtained from different regions possess similar chemical compositions and that the optimum particle sizes for reducing sugar production were 60- to 80-mesh. Contrary to the negative influences of a dilute H_2SO_4 pretreatment, an alkali pretreatment showed positive effects on *Eucalyptus* saccharification. This phenomenon may had been attributed to the efficient removal of lignin and the stronger structural damage during the alkali pretreatment process. In comparison with the chemical pretreatments, a higher reducing sugar yield could be achieved from the biological pretreated *Eucalyptus*. The highest reducing sugar yield of 97.14 mg/g was obtained from the Guangxi (GX) *Eucalyptus* that was pretreated with *Daedalea quercina*.

Keywords: Eucalyptus; Reducing sugar; Dilute acid pretreatment; Alkali pretreatment; Biological pretreatment

Contact information: a: Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China; b: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China; c: Key Laboratory of Pulp and Paper Science & Technology of Ministry of Education/Shandong Province, Qilu University of Technology, Jinan 250353, China;
** Corresponding authors: dxmei2006@scau.edu.cn and lihl@scau.edu.cn*

INTRODUCTION

The depletion of petroleum reserves and the increasing demand for liquid transport fuels are driving research to explore alternative renewable energy resources to relieve society's reliance on fossil raw materials and achieve a sustainable future (Kruse *et al.* 2009; Lai *et al.* 2011; Cheng *et al.* 2016). Lignocellulosic biomass, such as woody materials, agricultural and forestry residues, and energy crops, is considered as promising feedstock for the production of biofuels and high-value added chemicals under the concept of biorefinery (Ho *et al.* 2014; Cheng *et al.* 2015; Zhe *et al.* 2015). The efficient conversion of lignocellulosic biomass into value products such as fuels and chemicals could attribute to a more environmentally benign energy sector (Dedsuksophon *et al.* 2011; Soboll and Bünger 2013; Li *et al.* 2015).

Bioethanol, which presents characteristics of high octane number, low cetane number, and high heat of vaporization, is the most common liquid biofuel particularly in Brazil and the United States (Kim and Dale 2004; Balat *et al.* 2008; Alvira *et al.* 2010; Sarkar *et al.* 2012). The conversion of lignocellulosic biomass into bioethanol includes two

sequential steps: hydrolysis (saccharification) of raw materials into monosaccharides, and the additional fermentation from soluble sugars to ethanol (Zhang *et al.* 2007; Domínguez *et al.* 2017). Therefore, to produce bioenergy and chemicals from lignocelluloses, carbohydrate polymers must be first broken down into individual sugar molecules (Sarkar *et al.* 2012; Frankó *et al.* 2016). However, a severe restriction of enzymatic accessibility is caused by the complexity of the cell wall matrix, the structural heterogeneity, and the complex cross-linking of the cell-wall constituents (Li *et al.* 2014; Deng *et al.* 2015, 2016; Li *et al.* 2016). Therefore, efficient pretreatment approaches are required to destroy the recalcitrant nature structure of lignocellulosic materials, thus facilitating the vulnerability of cellulose to enzyme attack.

Intensive efforts have been devoted to the development of novel pretreatment methods to improve the accessibility of cellulose and hemicellulose (Garlock *et al.* 2011; Vallejos *et al.* 2015). These pretreatment approaches include physical pretreatment, chemical pretreatment, biological pretreatment, and their combinations. Physical pretreatment, such as ball milling, is presently the most widespread pretreatment technology. However, it has the disadvantage of high energy consumption. Compared with the physical pretreatment, the removal of hemicellulose and lignin can be addressed efficiently by chemical and biological pretreatments. Moreover, the efficiency of different pretreatment approaches differed with the reaction conditions, the physical-chemical properties of the raw materials, and so on. Although various studies have been conducted to optimize pretreatment conditions, there have been limited investigations on the comparison of pretreatment ways. Due to the various properties of different lignocellulosic biomass, it is important to select a suitable pretreatment method to alter the structure of the biomass, thus enhancing the enzyme accessibility.

Eucalyptus grows extensively and diversely and is one of the fastest-growing group of plants in the world. In the present study, chemical pretreatments (dilute H_2SO_4, dilute NaOH, and NH_4OH) and biological pretreatments by *Coriolus versicolor* and *Daedalea quercina* were performed to investigate the influences of the different pretreatment processes on the production of reducing sugar from *Eucalyptus*. The obtained comprehensive information could be useful for the future development of effective pretreatment strategies for improving reducing sugar yields.

EXPERIMENTAL

Materials

Eucalyptus robusta Smith stem chips were manually collected from the local experimental fields in the Eucalyptus Natural Germplasm Resource Center (Guangdong, China) and were defined as FJ (Fujian) and GX (Guangxi) based on their provenance, respectively. Prior to the experiments, the *Eucalyptus* stem chips were dried at 55 °C to a constant weight. The dried samples were milled by the pulverizer and stored in desiccators. Mixed cellulases (containing β-glucanase $\geq 2.98 \times 10^4$ U, cellulase ≥ 298 U, and xylanase $\geq 4.8 \times 10^4$ U) were obtained from the Imperial Jade Biotechnology Co. Ltd. (Yinchuan, China). The chemicals used in this study (H_2SO_4, NaOH, NH_4OH, KOH, HNO_3, and ethanol) were all purchased from Sigma-Aldrich (Shanghai, China) and used without further purification.

Methods

Chemical component analysis

The amounts of cellulose, hemicellulose, and lignin in the *Eucalyptus* were measured according to reported procedures (Zhao *et al.* 2014, 2017). The dried *Eucalyptus* stem chips were ground to pass through 40- to 60-mesh screens and then Soxhlet-extracted with toluene/ethanol (2:1, v/v) for 6 h. Thereafter, the dewaxed powders were delignified by sodium chlorite (pH 4.0) at 75 °C for 4 h. The solid residues were classified as holocelluloses. The cellulose content of *Eucalyptus* was determined by the Kurschner-Hoffner's method. In short, the dewaxed *Eucalyptus* was treated with nitric acid (65%) and ethanol (96%) with the volume ratio of 1 to 4 at 100 °C. It should be noted that fresh nitric acid/ethanol solution was periodically added. After the reaction, the collected solid product (cellulose) was repeatedly washed with deionized (DI) water and then dried at 60 °C overnight. The hemicellulose content in *Eucalyptus* was calculated based on the difference between the weights of holocellulose and cellulose.

The lignin content of *Eucalyptus* was measured by the standard analytical procedure of the National Renewable Energy Laboratory (NREL/TP-510-42618) (Sluiter *et al.* 2010).

Chemical pretreatments

H_2SO_4 pretreatment: First, 50 mg *Eucalyptus* samples (60- to 80-mesh) and 1.5 mL dilute H_2SO_4 (0.25%, 1%, 4%, v/v) were added into the thick-wall glass pipe and then heated at 121 °C for 20 min, respectively. After the reaction, the mixtures were maintained at 50 °C for 2 h with shaking at 150 rpm. The solid residues were collected for the further enzymatic hydrolysis reaction. Each experiment was repeated triplicate under the same conditions to ensure the reproducibility of the results.

NaOH pretreatment: The ground *Eucalyptus* samples (50 mg, 60- to 80-mesh) were dispersed into the NaOH (1.5 mL) solutions with different concentrations (0.25%, 1%, 4%, w/v), respectively, and then shaken at 150 rpm for 2 h at 50 °C. The solid residues were collected for the further enzymatic hydrolysis reaction. All of the experiments were conducted in triplicate.

NH_4OH pretreatment: Similar to the NaOH pretreatment, 50 mg of ground *Eucalyptus* powders (60- to 80-mesh) were supplemented with 1.5 mL NH_4OH with different concentrations (17%, 21%, 25%, v/v), respectively, and then heated at 60 °C for 12 h. The solid residues were collected for further enzymatic hydrolysis reaction. All of the experiments were repeated triplicate under the same conditions.

Biological pretreatment

First, 4.6 g of potato-dextrose agar (PDA) was added into 100 mL DI water and then heated at 121 °C for 20 min under high-pressure conditions. After the reaction, the medium was cooled to room temperature and made into a flat shape. *Coriolus versicolor* and *Daedalea quercina* were inoculated into the PDA medium *via* Micro-loop. The ground *Eucalyptus* powder (1g, 60- to 80-mesh) was placed in a sterile gauze (200-mesh) that was loaded on the PDA flat surface. All of the samples were placed in the incubator at 28 °C, and the washed solid products obtained after 7 days, 14 days, 21 days, and 28 days were subjected to further enzymatic hydrolysis, respectively.

Enzymatic hydrolysis of Eucalyptus

The pretreated samples were washed with distilled water for five times and dried at 60 °C for 5 h before enzymatic hydrolysis. All supernatants were collected for the soluble

sugar analysis. Pretreated *Eucalyptus* (50 mg) was mixed with 1.5 mL mixed cellulases solution (0.2 g/mL), and then shaken at 150 rpm for 48 h at 50 °C. The released monosaccharides were analyzed by high-performance anion-exchange chromatography (HPAEC, Dionex ICS-3000, Sunnyvale, USA) coupled with a pulsed amperometric detector and a Carbopac PA-20 column (4×250 mm, Dionex) as described by Li *et al.* (2015). After the reaction, the mixtures were immersed in boiling water for 10 min to inactivate the enzymes, and the hydrolysates were filtered for analysis. Neutral sugars were separated in a 5 mM NaOH (carbonate free and purged with nitrogen) solution for 20 min, followed by a 0-75 mM NaAc gradient for 15min. Then the column was washed with 200 mM NaOH for 10 min to remove carbonate, and followed a 5 min elution with 5 mM NaOH to re-equilibrate the column before the next injection. The results were expressed in milligrams of reducing sugar to the gram of the dewaxed *Eucalyptus* (mg/g). All experiments were performed in triplicate.

Morphology analysis

The morphology of impact fracture surfaces of the pretreated *Eucalyptus* were observed by a Hitachi (S-4800, Japan) scanning electron microscope (SEM) with an acceleration voltage of 2 kv. The samples were sputter-coated with gold prior to the observation.

RESULTS AND DISCUSSION

Chemical Compositions of *Eucalyptus* Obtained from Different Regions

The major compositions of *Eucalyptus* used in the current work are presented in Table 1. The results are comparable to the data shown by Romaní *et al.* (2010). The compositions of *Eucalyptus* obtained from different regions were similar, which mainly consisted of cellulose (44.40% to 45.51%), hemicellulose (21.85% to 25.12%), lignin (27.70% to 29.07%), and a minimal amount of extractives. The characteristic of high carbohydrate content made *Eucalyptus* a favorable feedstock for the reducing sugar production. In addition, the lignin content of *Eucalyptus* was relatively high, which indicated that pretreatment should be taken into account to increase the enzymatic digestibility of *Eucalyptus*.

Table 1. Major Compositions (Expressed in Relative Weight Percentage, %) of *Eucalyptus*

Samples	Extractives	Cellulose	Hemicellulose	Lignin
FJ	2.53 ± 0.11	44.80 ± 2.27	23.61 ± 1.12	29.07 ± 1.90
GX	1.45 ± 0.05	45.51 ± 2.67	25.12 ± 1.28	27.92 ± 1.88
Ref. (Romaní *et al.* 2010)	2.40 ± 0.15	44.40 ± 0.04	21.85 ± 0.98	27.70 ± 0.40

Effect of *Eucalyptus* Particle Size on Reducing Sugar Production

The saccharification of lignocellulosic biomass was greatly influenced by its particle size. In this study, experiments for the saccharification of *Eucalyptus* with different mesh sizes (40 to 60, 60 to 80, and 80 to 100) were conducted to investigate the effect of particle size on the reducing sugar production. The results are shown in Fig. 1.

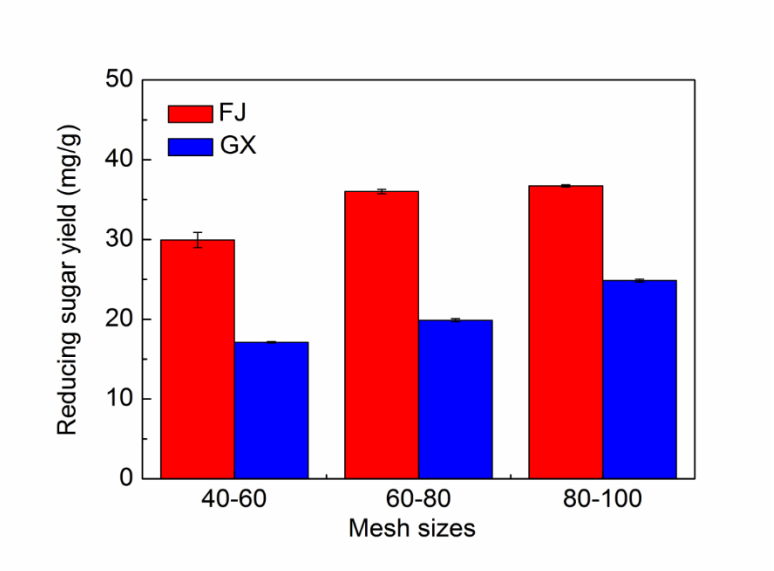

Fig. 1. Effect of mesh sizes on the *Eucalyptus* saccharification (Saccharification conditions: 50 mg Eucalyptus, 1.5 mL 0.2% (w/v) cellulase, 50 °C, 150 rpm, 48 h)

The yield of reducing sugar increased with the increment of mesh sizes, which indicated that a smaller particle size of raw material favored *Eucalyptus* saccharification. This phenomenon may be resulted from the enhancement of bulk density, porosity, and the surface area in the size reduction process, which accelerated the contact between enzymes and polysaccharides (Ruiz *et al.* 2011). In addition, although the chemical compositions of FJ and GX *Eucalyptus* were similar, the saccharification efficiency of the FJ sample was much higher than that of the GX ones, which suggested that the production of reducing sugar may be relevant to the origin of the feedstock.

Due to the size reduction process of lignocellulosic biomass being energy-intensive and expensive, it was necessary to optimize the suitable particle size of raw material to achieve higher sugar production and lower cost (Liu *et al.* 2013). In terms of the reducing sugar yield and energy consumption, mesh sizes of 60 to 80 were selected for the following experiments.

Effect of Chemical Pretreatments on the Reducing Sugar Production from *Eucalyptus*

The dilute acid pretreatment is considered to be one of the most efficient approaches to destroy the complex structure of lignocellulosic biomass. In this study, the pretreatment of *Eucalyptus* with different H_2SO_4 concentrations (0.00%, 0.25%, 1.00%, and 4.00%) was conducted to investigate the effect of a dilute acid pretreatment on the reducing sugar production (Fig. 2). Interestingly, the yield of reducing sugar decreased in the presence of dilute H_2SO_4, which was inconsistent with the results reported by Kumar *et al.* (2009), Peng *et al.* (2010), and Wang *et al.* (2013). Moreover, there was no extreme change of the reducing sugar yield when the concentration of H_2SO_4 increased from 0.25% to 4.00%. These occurrences may have been ascribed to the absorption of acids by the inner fibers during the pretreatment process, thus hindering the enzymatic hydrolysis (Jin *et al.* 2013). The chemical compositions of FJ and GX samples after 1.00% H_2SO_4 acid pretreatment are shown in Table 2. Usually, acid pretreatment would alter chemical and physical structures, thus promoting enzymatic activity; whereas size reduction mainly increases surface areas.

However, the contents of major compounds (extractives, cellulose, hemicellulose, and lignin) in the raw materials (Table 1) and the dilute acid pretreated *Eucalyptus* were similar, which suggested that dilute H_2SO_4 treatment was not an ideal pretreatment approach for *Eucalyptus*. Therefore, size reduction played a more important role for the improvement of reducing sugar yield.

Table 2. Major Compositions (Expressed in Relative Weight Percentage, %) of 1.00% H_2SO_4-pretreated *Eucalyptus*

Samples	Extractives	Cellulose	Hemicellulose	Lignin
FJ	2.65 ± 0.11	43.30 ± 1.52	24.62 ± 2.10	29.43 ± 1.02
GX	1.55 ± 0.05	43.05 ± 0.33	26.27 ± 1.09	29.13 ± 1.41

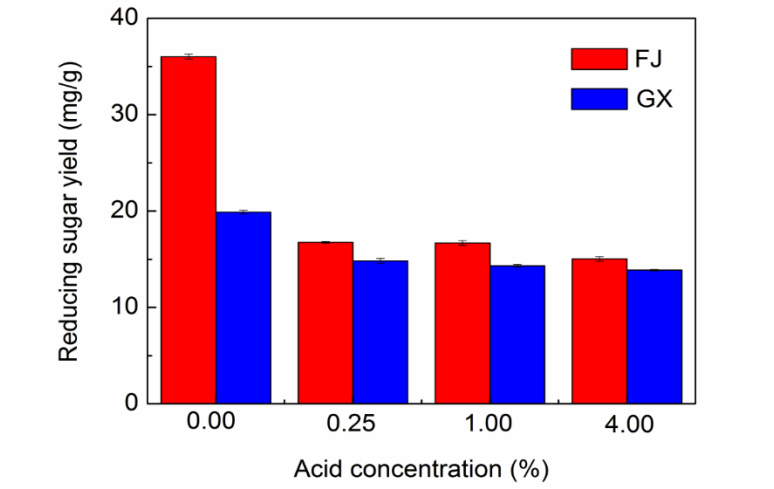

Fig. 2. Effects of dilute acid concentrations on the *Eucalyptus* saccharification (Pretreated conditions: 50 mg *Eucalyptus*, 1.5 mL H_2SO_4, 121 °C, 20 min, 50 °C, 150 rpm, 2 h; Saccharification conditions: 1.5 mL 0.2% (w/v) cellulase, 50 °C, 150 rpm, 48 h)

The NaOH pretreatment was a mild pretreatment method to swell the lignocellulosic biomass particles, which could greatly enhance the removal of lignin and hemicellulose during the pretreatment process. The effect of the dilute alkali pretreatment on *Eucalyptus* saccharification that was performed under different NaOH loadings (0.00%, 0.25%, 1.00%, and 4.00%) is shown in Fig. 3. As shown, the reducing sugar yield increased with the increment of NaOH concentration, which may have resulted from the degradation of lignin under alkaline conditions (Jin *et al.* 2013). This phenomenon was consistent with the result that the lignin content of *Eucalyptus* was reduced after the NaOH pretreatment (Table 3). The removal of lignin could have greatly accelerated the accessibility of enzymes to carbohydrates, thus enhancing the yield of reducing sugars. Moreover, in comparison with the dilute acid pretreatment, the dilute alkali pretreatment showed a positive influence on the production of reducing sugar from *Eucalyptus*, even at a lower reaction temperature. This distinct occurrence suggested that delignification had a stronger effect on the extent of enzymatic digestibility than did the removal of xylan (Cheng *et al.* 2016). In addition, when the concentration of NaOH was 4.00%, the yield of reducing sugar obtained from GX

Eucalyptus was higher than that from the FJ samples. This may have been due to the stronger interaction between lignin and cellulose in the GX *Eucalyptus*.

Table 3. Major Compositions (Expressed in Relative Weight Percentage, %) of 4% NaOH-pretreated *Eucalyptus*

Samples	Extractives	Cellulose	Hemicellulose	Lignin
FJ	2.68 ± 0.35	50.19 ± 1.21	25.26 ± 0.21	21.87 ± 0.74
GX	1.81± 0.21	51.62 ± 1.86	26.22 ± 2.05	20.35 ± 1.35

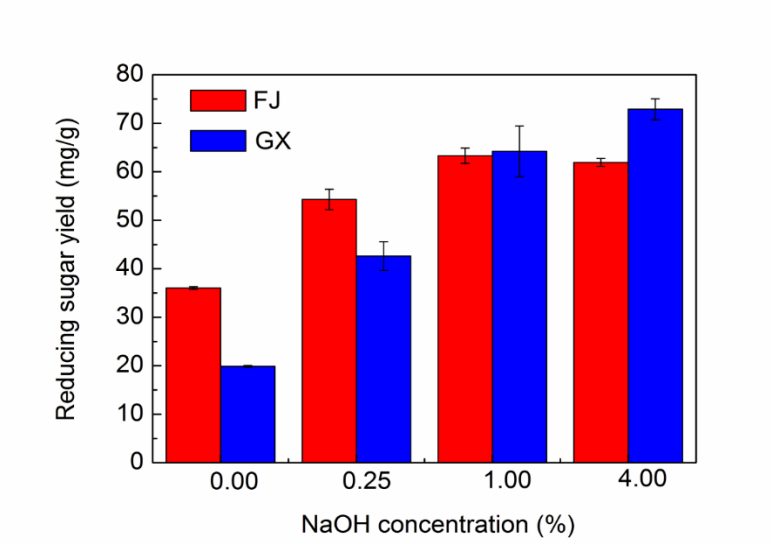

Fig. 3. Effects of NaOH concentrations on the *Eucalyptus* saccharification (Pretreated conditions: 50 mg *Eucalyptus*, 1.5 mL NaOH, 50 °C, 150 rpm, 12 h; Saccharification conditions: 1.5 mL 0.2% (w/v) cellulase, 50 °C, 150 rpm, 48 h)

The NH_4OH pretreatment was the other common alkali pretreatment method used for the conversion of lignocellulosic biomass. To investigate the effect of the NH_4OH pretreatment on *Eucalyptus* saccharification, experiments were performed at 60 °C for 12 h with different NH_4OH concentrations (0%, 17%, 21%, and 25%) (Fig. 4).

The highest reducing sugar yield of 50.46 mg/g was obtained from the FJ samples when the concentration of NH_4OH was 17%. However, it was lower than that from the NaOH-pretreated *Eucalyptus*, which suggested that the NaOH pretreatment was more efficient than the NH_4OH pretreatment. NaOH could swell the lignocellulosic biomass particles, thus accelerated the removal of lignin and enhanced the surface area of *Eucalyptus*.

Chemical composition analysis of the 4% NaOH-pretreated *Eucalyptus* and 17% NH_4OH-pretreated *Eucalyptus* (Table 3 and Table 4) showed that NaOH pretreatment possess better performance for the removal of lignin than the NH_4OH pretreatment. Moreover, compared with the acid pretreatment (Fig. 2), the alkali pretreatments (Fig. 3 and Fig. 4) were more conductive to the reducing sugar production. These phenomena may have resulted from the efficient lignin removal (Tables 3 and 4) during the alkali pretreatment (Kumar *et al.* 2009; Peng *et al.* 2010; Wang *et al.* 2013).

Table 4. Major Compositions (Expressed in Relative Weight Percentage, %) of 17% NH_4OH-pretreated *Eucalyptus*

Samples	Extractives	Cellulose	Hemicellulose	Lignin
FJ	2.38 ± 0.09	46.59 ± 1.70	24.90 ± 0.03	26.12 ± 1.24
GX	1.57 ± 0.15	46.92 ± 0.28	24.45 ± 1.13	27.06 ± 1.05

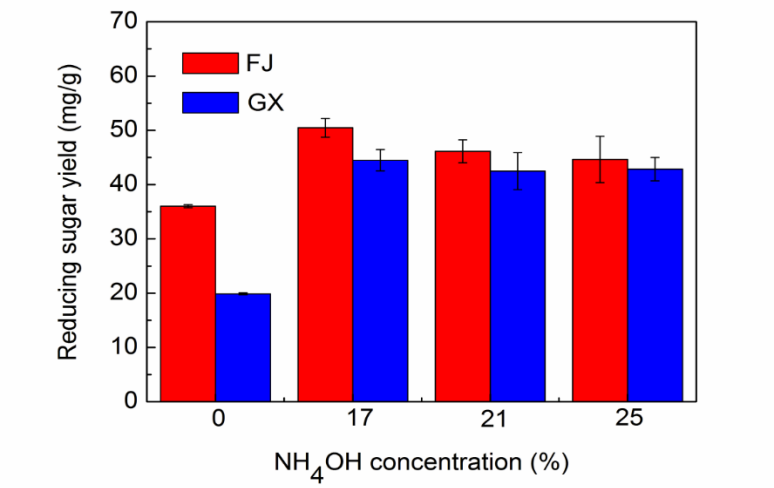

Fig. 4. Effects of NH_4OH concentrations on the *Eucalyptus* saccharification (Pretreated conditions: 50 mg *Eucalyptus*, 1.5 mL NH_4OH, 60 °C, 2 h; Saccharification conditions: 1.5 mL 0.2% (w/v) cellulase, 50 °C, 150 rpm, 48 h)

Effect of Biological Pretreatment on the Reducing Sugar Production from *Eucalyptus*

A biological pretreatment is considered a green pretreatment approach to enhance the saccharification efficiency of lignocellulosic biomass. Subsequently, *Eucalyptus* was exposed to *C. versicolor* and *D. quercina* to study the effect of fungus on the reducing sugar production (Table 5).

Table 5. Effects of Different Fungus on *Eucalyptus* Saccharification

Time (day)	FJ		GX	
	Coriolus versicolor	*Daedalea quercina*	*Coriolus versicolor*	*Daedalea quercina*
7	45.58 ± 1.32	42.46 ± 0.44	35.16 ± 1.41	54.36 ± 5.36
14	75.86 ± 7.92	44.72 ± 1.12	66.42 ± 4.03	55.92 ± 1.89
21	69.00 ± 5.66	43.64 ± 0.67	54.08 ± 2.26	80.44 ± 3.13
28	63.86 ± 0.26	43.18 ± 0.40	51.16 ± 1.14	97.14 ± 2.07

*Note: All values are in mg/g; Saccharification conditions: 1.5 mL 0.2% (w/v) cellulase, 50 °C, 150 rpm, 48 h

Coriolus versicolor, a white-hot fungus, is capable of metabolizing and depolymerizing polysaccharides and lignin (Bhandari and Bist 1989). The reducing sugar yield obtained from the *Eucalyptus* that was pretreated by *C. versicolor* first increased and then decreased with prolonging of reaction time. The highest yields of reducing sugar were 75.86 mg/g and 66.42 mg/g for FJ samples and GX samples, respectively.

Table 6. Major Compositions (Expressed in Relative Weight Percentage, %) of *Eucalyptus* after Biological Pretreatment for 28 Days

	Samples	Extractives	Cellulose	Hemicellulose	Lignin
Coriolus versicolor	FJ	2.70 ± 0.13	48.86 ± 1.09	29.12 ± 0.09	19.32 ± 0.23
	GX	1.97 ± 0.23	45.92 ± 1.37	25.51 ± 1.22	26.60 ± 1.06
Daedalea quercina	FJ	2.35 ± 0.06	46.66 ± 0.86	24.22 ± 1.95	26.77 ± 2.13
	GX	1.50 ± 0.19	53.43 ± 1.41	27.72 ± 1.01	17.34 ± 1.09

Daedalea quercina is a brown-rot fungus that grows on weak or dead stumps of deciduous trees (Rösecke and König 2000). Similar to *C. versicolor*, *D. quercina* also has the ability to degrade lignin and other wood components. In comparison with *C. versicolor*, the *D. quercina* pretreatment showed better performance on the GX Eucalyptus saccharification, which may have resulted from the higher lignin removal as shown in Table 6. The highest yield of 97.14 mg/g was obtained from the *D. quercina* pretreated samples (GX) within 28 days. In addition, the saccharification efficiency of *Eucalyptus* varied between the different fungus pretreatments, which may have been due to their different structural characteristics and the diffusibility of fungi's extracellular enzymes into the wood cell wall (Bhandari and Bist 1989).

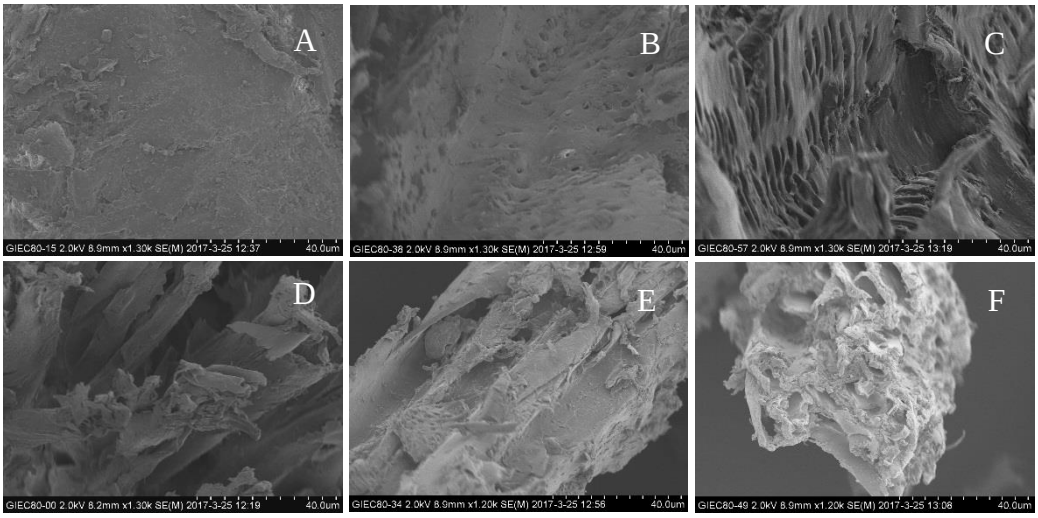

Fig. 5. SEM images of FJ *Eucalyptus* pretreated by different approaches (A: Control; B: 1% H_2SO_4 pretreatment; C:4% NaOH pretreatment; D:17% NH_4OH pretreatment; E: *Coriolus versicolor* pretreatment; F: *Daedalea quercina* pretreatment)

Figure 5 shows the SEM images of the pretreated *Eucalyptus* with different approaches. Compared with the unpretreated sample (Fig. 5A), the surface of *Eucalyptus* changed from smooth to rough after chemical pretreatments (dilute H_2SO_4, dilute NaOH and NH_4OH) and biological pretreatments. Moreover, alkali and biological pretreatments showed better performance on the deconstruction of *Eucalyptus*, which was beneficial for the solution of polysaccharides and the enzyme accessibility.

CONCLUSIONS

1. Chemical pretreatments (dilute H_2SO_4, dilute NaOH, and NH_4OH) and biological pretreatments (*Coriolus versicolor* and *Daedalea quercina*) were employed to enhance the saccharification efficiency of *Eucalyptus*.
2. Eucalyptus' high carbohydrate content made it a promising feedstock for reducing sugar production. The optimum particle sizes were 60- to 80-mesh.
3. Compared with the dilute acid pretreatment, the alkali pretreatment was more suitable for Eucalyptus saccharification *via* enzymatic hydrolysis. This could have been due to the efficient removal of lignin during the alkali pretreatment process.
4. Even though a higher reducing sugar yield could be obtained from the biologically pretreated *Eucalyptus*, a long reaction time and a narrow reaction environment impeded its utilization.
5. Future efforts will focus on the optimization of reaction conditions to improve the reducing sugar production from *Eucalyptus*.

ACKNOWLEDGMENTS

This study was supported by the Guangdong Province Science and Technology Projects (Grant Nos. 2015A050502045 and 2016A010104012) and the Foundation (No. KF201616) of Key Laboratory of Pulp and Paper Science and Technology of Ministry of Education/Shandong Province of China.

REFERENCES CITED

Alvira, P., Tomáspejó, E., Ballesteros, M., and Negro, M. J. (2010). "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review," *Bioresource Technology* 101(13), 4851-61. DOI: 10.1016/j.biortech.2009.11.093

Balat, M., Balat, H., and Öz, C. (2008). "Progress in bioethanol processing," *Progress in Energy and Combustion Science* 34(5), 551-573. DOI: 10.1016/j.pecs.2007.11.001

Bhandari, K. S., and Bist, V. (1989). "Effect of *Coriolus versicolor* on physico-chemical properties of *Eucalyptus globulus* wood," *Wood Science and Technology* 23(2), 163-169. DOI: 10.1007/BF00350938

Cheng, Y. S., Chen, K. Y., and Chou, T. H. (2015). "Concurrent calcium peroxide pretreatment and wet storage of water hyacinth for fermentable sugar production," *Bioresource Technology* 176, 267-272. DOI: 10.1016/j.biortech.2014.11.016

Cheng, Y. S., Wu, J. H., and Yeh, L. H. (2016). "Utilization of *Calophyllum inophyllum* shell and kernel oil cake for reducing sugar production," *Bioresource Technology* 212, 338-341. DOI: 10.1016/j.biortech.2016.04.069

Dedsuksophon, W., Faungnawakij, K., Champreda, V., and Laosiripojana, N. (2011). "Hydrolysis/dehydration/aldol-condensation/hydrogenation of lignocellulosic biomass and biomass-derived carbohydrates in the presence of Pd/WO3-ZrO2 in a

single reactor," *Bioresource Technology* 102(2), 2040-2046. DOI: 10.1016/j.biortech.2010.09.073

Deng, A., Ren, J., Li, H., Peng, F., and Sun, R. C. (2015). "Corncob lignocellulose for the production of furfural by hydrothermal pretreatment and heterogeneous catalytic process," *Rsc Advances* 5(74), 60264-60272. DOI: 10.1039/C5RA10472F

Deng, A., Ren, J., Wang, W., Li, H., Lin, Q., Yan, Y., Sun, R., and Liu, G. (2016). "Production of xylo-sugars from corncob by oxalic acid-assisted ball milling and microwave-induced hydrothermal treatments," *Industrial Crops & Products* 79, 137-145. DOI: 10.1016/j.indcrop.2015.11.032

Domínguez, E., Romaní, A., Domingues, L., and Garrote, G. (2017). "Evaluation of strategies for second generation bioethanol production from fast growing biomass Paulownia within a biorefinery scheme," *Applied Energy* 187, 777-789. DOI: 10.1016/j.apenergy.2016.11.114

Frankó, B., Galbe, M., Wallberg, O., and Yan, J. (2016). "Bioethanol production from forestry residues: A comparative techno-economic analysis," *Applied Energy* 184, 727-736. DOI: 10.1016/j.apenergy.2016.11.011

Garlock, R. J., Balan, V., Dale, B. E., Pallapolu, V. R., Lee, Y. Y., Kim, Y., Mosier, N. S., Ladisch, M. R., Holtzapple, M. T., and Falls, M. (2011). "Comparative material balances around pretreatment technologies for the conversion of switchgrass to soluble sugars," *Bioresource Technology* 102(24), 11063-71. DOI: 10.1016/j.biortech.2011.04.002

Ho, D. P., Ngo, H. H., and Guo, W. (2014). "A mini review on renewable sources for biofuel," *Bioresource Technology* 169(5), 742-749. DOI: 10.1016/j.biortech.2014.07.022

Jin, Y., Huang, T., Geng, W., and Yang, L. (2013). "Comparison of sodium carbonate pretreatment for enzymatic hydrolysis of wheat straw stem and leaf to produce fermentable sugars," *Bioresource Technology* 137(11), 294-301. DOI: 10.1016/j.biortech.2013.03.140

Kim, S. D., and Dale, B. E. (2004). "Global potential bioethanol production from wasted crops and crop residues," *Biomass & Bioenergy* 26(4), 361-375. DOI: 10.1016/j.biombioe.2003.08.002

Kruse, A., Bernolle, P., Dahmen, N., Dinjus, E., and Maniam, P. (2009). "Hydrothermal gasification of biomass: Consecutive reactions to long-living intermediates," *Energy & Environmental Science* 189(3), 136-143. DOI: 10.1039/B915034J

Kumar, P., Barrett, D. M., Delwiche, M. J., and Stroeve, P. (2009). "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production," *Industrial & Engineering Chemistry Research* 48(8), 3713-3729. DOI: 10.1021/ie801542g

Lai, D. M., Deng, L., Guo, Q. X., and Fu, Y. (2011). "Hydrolysis of biomass by magnetic solid acid," *Energy & Environmental Science* 4(9), 3552-3557. DOI: 10.1039/C1EE01526E

Li, H., Chen, X., Ren, J., Hao, D., Feng, P., and Sun, R. (2015). "Functional relationship of furfural yields and the hemicellulose-derived sugars in the hydrolysates from corncob by microwave-assisted hydrothermal pretreatment," *Biotechnology for Biofuels* 8(1), 1-12. DOI: 10.1186/s13068-015-0314-z

Li, H., Deng, A., Ren, J., Liu, C., Lu, Q., Zhong, L., Peng, F., and Sun, R. (2014). "Catalytic hydrothermal pretreatment of corncob into xylose and furfural *via* solid

acid catalyst," *Bioresource Technology* 158(4), 313-320. DOI: 10.1016/j.biortech.2014.02.059

Li, H., Wang, X., Liu, C., Ren, J., Zhao, X., Sun, R., and Wu, A. (2016). "An efficient pretreatment for the selectively hydrothermal conversion of corncob into furfural: The combined mixed ball milling and ultrasonic pretreatments," *Industrial Crops & Products* 94, 721-728. DOI: 10.1016/j.indcrop.2016.09.052

Liu, Z. H., Qin, L., Pang, F., Jin, M. J., Li, B. Z., Kang, Y., Dale, B. E., and Yuan, Y. J. (2013). "Effects of biomass particle size on steam explosion pretreatment performance for improving the enzyme digestibility of corn stover," *Industrial Crops & Products* 44(2), 176-184. DOI: 10.1016/j.indcrop.2012.11.009

Peng, F., Bian, J., Ren, J. L., Peng, P., Xu, F., and Sun, R. C. (2010). "Fractionation and characterization of alkali-extracted hemicelluloses from peashrub," *Biomass & Bioenergy* 39, 20-30. DOI: 10.1016/j.biombioe.2010.08.034

Rösecke, J., and König, W. A. (2000). "Constituents of the fungi *Daedalea quercina* and *Daedaleopsis confragosa* var. Tricolor," *Phytochemistry* 54(8), 757-762. DOI: 10.1016/S0031-9422(00)00130-8

Romaní, A., Garrote, G., Alonso, J. L., and Parajó, J. C. (2010). "Bioethanol production from hydrothermally pretreated *Eucalyptus globulus* wood," *Bioresource Technology* 101(22), 8706-12. DOI: 10.1016/j.biortech.2010.06.093

Ruiz, H. A., Ruzene, D. S., Silva, D. P., Quintas, M. A. C., Vicente, A. A., and Teixeira, J. A. (2011). "Evaluation of a hydrothermal process for pretreatment of wheat straw-effect of particle size and process conditions," *Journal of Chemical Technology and Biotechnology* 86(1), 88-94. DOI: 10.1002/jctb.2518

Sarkar, N., Ghosh, S. K., Bannerjee, S., and Aikat, K. (2012). "Bioethanol production from agricultural wastes: An overview," *Renewable Energy* 37(1), 19-27. DOI: 10.1016/j.renene.2011.06.045

Sluiter, J. B., Ruiz, R. O., Scarlata, C. J., Sluiter, A. D., and Templeton, D. W. (2010). "Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods," *Journal of Agricultural and Food Chemistry* 58(16), 9043-9053. DOI: 10.1021/jf1008023

Soboll, S., and Bünger, R. (2013). "Simultaneous pretreatment and sacchariffication of rice husk by *Phanerochete chrysosporium* for improved production of reducing sugars," *Bioresource Technology* 128C(1), 113-117. DOI: 10.1016/j.biortech.2012.10.030

Vallejos, M. E., Felissia, F. E., Kruyeniski, J., and Area, M. C. (2015). "Kinetic study of the extraction of hemicellulosic carbohydrates from sugarcane bagasse by hot water treatment," *Industrial Crops & Products* 67, 1-6. DOI: 10.1016/j.indcrop.2014.12.058

Wang, K., Yang, H., Qian, C., and Sun, R. C. (2013). "Influence of delignification efficiency with alkaline peroxide on the digestibility of furfural residues for bioethanol production," *Bioresource Technology* 146C(10), 208-214. DOI: 10.1016/j.biortech.2013.07.008

Zhang, X., Xu, C., and Wang, H. (2007). "Pretreatment of bamboo residues with *Coriolus versicolor* for enzymatic hydrolysis," *Journal of Bioscience and Bioengineering* 104(2), 149-51. DOI: 10.1263/jbb.104.149

Zhao, X., Ouyang, K., Gan, S., Zeng, W., Song, L., Zhao, S., Li, J., Doblin, M. S., Bacic, A., Chen, X. Y., *et al*. (2014). "Biochemical and molecular changes associated with heteroxylan biosynthesis in *Neolamarckia cadamba* (Rubiaceae) during xylogenesis," *Frontiers in Plant Science* 5(602), 602-602. DOI: 10.3389/fpls.2014.00602

Zhao, X., Tong, T., Li, H., Lu, H., Ren, J., Zhang, A., Deng, X., Chen, X., and Wu, A. M. (2017). "Characterization of hemicelluloses from *Neolamarckia cadamba* (Rubiaceae) during xylogenesis," *Carbohydrate Polymers* 156, 333-339. DOI: 10.1016/j.carbpol.2016.09.041

Zhe, J., Xun, Z., Zhe, L., Xia, Z., Ramaswamy, S., and Feng, X. (2015). "Visualization of *Miscanthus* × *giganteus* cell wall deconstruction subjected to dilute acid pretreatment for enhanced enzymatic digestibility," *Biotechnology for Biofuels* 8(1), 1-14. DOI: 10.1186/s13068-015-0282-3

Article submitted: January 10, 2017; Peer review completed: March 26, 2017; Revised version received and accepted: July 6, 2017; Published: July 17, 2017.
DOI: 10.15376/biores.12.3.6353-6365