Effects of Different Pretreatment Methods on the Enzymatic Hydrolysis of Cassava Residue

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The enzymatic hydrolysis of cassava residue treated by a hot water (HW) pretreatment, an extreme-low acid (ELA) pretreatment, and an alkaline hydrogen peroxide (AHP) pretreatment was investigated. The results showed that the ELA pretreatment dissolved greater xylan and glucose quantities than the HW pretreatment under the same conditions, and the xylan and glucan contents of the pretreated substrate affected the subsequent cellulase hydrolysis. The conversion to glucose by cellulase hydrolysis reached 81.4% after the HW pretreatment, while the glucose yields under the ELA and AHP pretreatment conditions were 78.3% and 71.0%, respectively. In addition, supplementation with xylanase improved cellulase efficiency. At an equal xylanase dosage, a higher glucose yield (*i.e.*, 91.3%) was achieved for the ELA-pretreated substrates that contained a lower xylan content. Xylanase supplementation in the AHP pretreatment had little effect on the glucose conversion. Finally, X-ray diffraction studies showed that the HW and ELA pretreatments increased the cassava residue crystallinity, while the AHP pretreatment had little effect.

Keywords: Cassava residue; Alkaline hydrogen peroxide pretreatment; Hot water pretreatment; Extreme-low acid pretreatment; Enzymatic hydrolysis; Glucose yield

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INTRODUCTION

Among the various lignocellulosic biomasses, agricultural and agro-industrial residues are abundant sources of renewable energy (Ragauska *et al.* 2006). For example, cassava residue (CR) is the solid residue obtained after starch separation and is a major lignocellulosic plant residue in many tropical countries such as Thailand, China, and India (Jansson *et al*. 2009; Okudoh *et al*. 2014). China produced 4.60 MMT of cassava in 2013 (Ozoegwu *et al*. 2017), with the majority of this being produced for cassava starch. The processing of 250 to 300 tons of cassava tubers for isolating starch results in approximately 1.6 tons of solid peels and 280 tons of wet residues (moisture content, 85%) (Pandey *et al*. 2000). A considerable amount of cassava residue is generated as a by-product. Therefore, the effective disposal of this residue is a substantial issue. Cassava residue contains 30 to 50% starch, 10 to 20% cellulose, 10 to 20% hemicellulose, and a small amount of lignin (Canilha *et al.* 2011; Wu *et al.* 2011; Zhao *et al.* 2012). Previous laboratory research has shown that cellulo-starch waste from the cassava starch industry is a potential low-cost substrate for bioethanol production (Lin *et al*. 2016; Jia *et al*. 2017). Due to its high starch

content and low cellulose and hemicellulose contents, high-value products could be easily obtained from cassava residue through relatively inexpensive conversion techniques. As cassava residue is a lignocellulosic substrate with a complex and rigid structure, pretreatment is generally required to separate its recalcitrant structure and render the cellulose and non-cellulosic fractions more accessible to the hydrolytic enzymes that can generate fermentable sugars.

Pretreatments can be classified as either mechanical, thermal, chemical, or biological (Girolamo *et al*. 2013; Ge *et al*. 2016). For example, the alkaline hydrogen peroxide (AHP) pretreatment of lignocellulosic materials increases the cellulose accessibility and hydrolysis degree due to lignin removal and leads to high glucose yields. In addition, AHP results in cellulose swelling, which leads to an increase in the internal surface area available for an enzymatic treatment (Rabelo *et al*. 2011).

A dilute acid pretreatment is also an effective method for dissolving hemicellulose while retaining the majority of the cellulose component (Llyod and Wyman 2005). During the acid pretreatment, partial hemicelluloses may be hydrolyzed, which can improve the accessibility of enzymes to the cellulose. Typically, dilute sulfuric acid with a concentration of 0.4 to 2.5% (w/w) is employed at temperatures ranging from 100 to 200 °C. Recently, extremely low acid (ELA) (less than 0.1%) and high temperature techniques have been employed for the hydrolysis of cellulosic materials, which has further improved glucose yields. For example, Kim *et al.* (2001) reported that yields of approximately 61% are attainable with pure cellulose hydrolysis, while Lee *et al.* (2013a) reported that in the pretreatment of *Spiraea japonica* by ELA and subsequent enzymatic hydrolysis, the glucan yields were 4.2 and 2.4 times higher than that of the untreated substrate. In addition, Lee *et al. (*2013b) also found that an ELA pretreatment could considerably enhance the glucan fraction and enzymatic digestibility of pretreated *Lonicera japonica*. Because ELA conditions are similar to a neutral aqueous system, the weak acidity simplifies downstream processes, such as neutralization and waste treatment, in addition to noticeably reducing equipment costs (Thomsen *et al*. 2010).

Hot water (HW) pretreatments use water as a reaction medium without the requirement of additional chemicals. They are reported to cleave the hemiacetal linkages and liberate organic acids (Laser *et al*. 2002; Zhang *et al*. 2011) and have a low recycling and environmental cost. However, the HW pretreatment is commonly performed at high temperatures (140 to 220 °C). Generally, cellulose, hemicellulose, and lignin show different stabilities to acid, with the decomposition of hemicellulose being more facile than that of cellulose or lignin. In addition, the majority of hemicellulose and substantial proportions of lignin can be removed prior to cellulose degradation under dilute acid or HW pretreatment conditions, which reduces the biomass recalcitrance (Xiao *et al*. 2013). However, an increase in the harshness of the dilute acid and HW pretreatment conditions may cause hemicellulose degradation to furfural.

An effective pretreatment can reduce downstream pressure by rendering cellulose more accessible to enzymes and minimize degradation product formation that inhibit the growth of fermentative microorganisms. Studies have reported that the addition of xylanase during the biomass saccharification process can improve cellulose saccharification efficiency (Murashima *et al*. 2003; Selig *et al*. 2008; Hu *et al*. 2011) because the removal

of the hemicellulose by xylanase improves the accessibility of the cellulase (Yu *et al.* 2003).

Thus, three pretreatment methods (*i.e.*, ELA, HW, and AHP) were compared based on their efficacy in enhancing fermentable sugars released from remaining starch-free cassava residue (RSFCR) *via* cellulolytic enzymes and xylanase. The study also aimed to gain an understanding of the structural changes that took place during pretreatment and after enzymatic treatment.

EXPERIMENTAL

Materials

The cassava residue was obtained from the Guangxi Mingyang Starch Factory (Nanning, China) and was dried under sunlight, filtered using an 18-mesh sieve (6 mm), and then stored in a polyethylene container at 20 to 25 °C. The RSFCR was obtained from the cassava residue (Guangxi Mingyang Starch Factory, Nanning, China) using amylase to eliminate starch interference during the lignocellulosic pretreatment process. The main components of the cassava residue and RSFCR were analyzed according to the methods provided by the National Renewable Energy Laboratory (NREL) (Sluiter *et al.* 2012). The chemical composition of the RSFCR was as follows: arabinose (1.74 ± 0.01%), galactose (2.20 ± 0.02%), glucose (52.43 ± 1.23%), mannose (1.80 ± 0.01%), xylose (13.51 ± 0.03%), lignin (17.59 ± 0.16%, of which acid-insoluble lignin was 15.50 ± 0.16% and acid soluble lignin 2.09 ± 0.01%), and others (10.73%).

The cellulase (Initial activity: 69.38 $FPU \cdot mL^{-1}$) and xylanase (Initial activity: 28.32 $FPU \cdot mL^{-1}$) were provided by Novozymes Biotechnology Co. Ltd. (Beijing, China). The amylase was purchased from Aladdin Chemical Co. Ltd. (Shanghai, China).

Methods

Extreme-low acid and hot water pretreatment methods

The RSFCR pretreatment was performed in a 1-L reactor with an electric heater and magnetic agitation. The solid-to-liquid ratio employed during pretreatment was 1/15 (w/v). The pretreatment temperatures were 160 °C and 170 °C with a retention time ranging from 40 to 80 min. The dosage of H_2SO_4 (for ELA) was 0.05% (w/w). After pretreatment completion, the reactor was rapidly moved to an ice-water bath for cooling. The hydrolysate was then separated by filtration, and the solid fraction was washed 3 to 4 times with warm water until the filtrate was neutral. The solid fraction was air-dried after washed and stored for the enzymatic hydrolysis and mass balance analysis. As shown in Fig. 1, the residues obtained following the HW pretreatment and enzymatic hydrolysis were labeled A1 through A5 (160 °C; 40 to 80 min), A6 through A10 (170 °C; 40 to 80 min), B1 through B5 (enzymatic hydrolysis residue of A1 to A5), and B6 through B10 (enzymatic hydrolysis residue of A1 through A10). As shown in Fig. 2, the residues obtained after the ELA pretreatment and enzymatic hydrolysis were labeled C1 through C5 (160 °C; 40 to 80 min; 0.05% H_2SO_4), C6 through C10 (170 °C; 40 to 80 min; 0.05% H_2SO_4), D1 through D5 (enzymatic hydrolysis residue of C1 through C5), and D6 through D10 (enzymatic hydrolysis residue of C6 through C10).

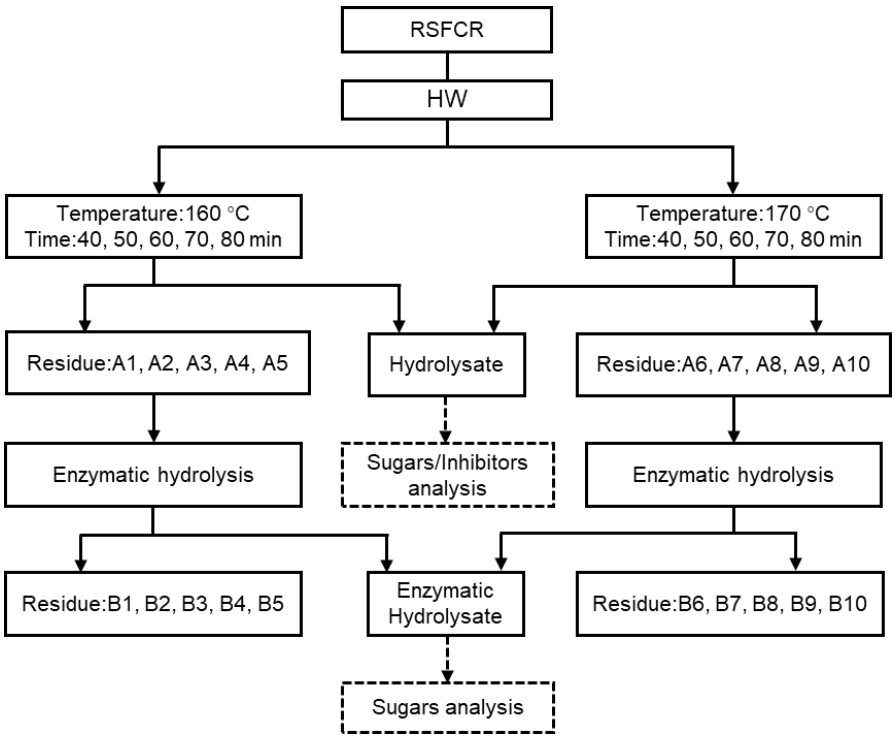

Fig. 1. Flow chart of HW pretreatment collaborative enzyme digestion

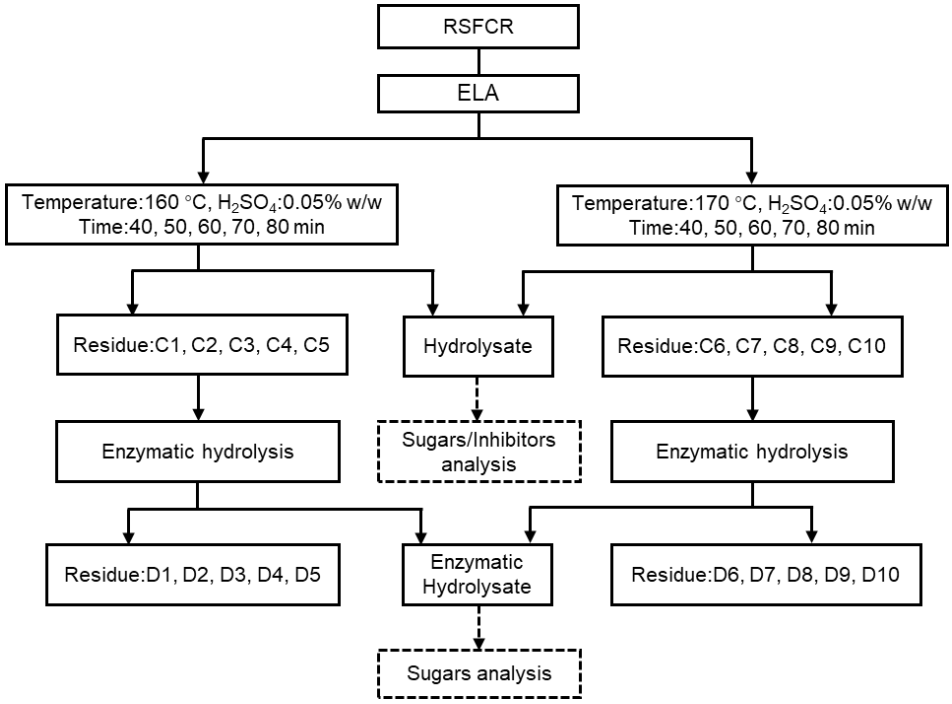

Fig. 2. Flow chart of ELA pretreatment collaborative enzyme digestion

Alkaline hydrogen peroxide pretreatment

The pH of the H_2O_2 solution was adjusted to 11.5 using 5 M NaOH, after which the AHP pretreatment was conducted in a shaker at 150 rpm for 240 min. Figure 3 shows the flow chart of AHP pretreatment collaborative enzyme digestion. The pretreatment temperature ranged from 30 to 50 °C with a retention time ranging from 2 to 6 h. The dosage of alkaline H_2O_2 ranged from 3 to 6% (w/w), and the solid-to-liquid ratio was 1/20 (w/v). After the pretreatment, the reactor was cooled to below 70 °C and the samples were collected as described earlier. The residues after the AHP pretreatment and enzymatic hydrolysis were labeled E1 through E3 (30 °C, 40 °C, or 50 °C, 6% H_2O_2, 4 h), E4 through E7 (3%, 4%, 5%, or 6% H_2O_2, 50 °C, 4 h), E8 through E12 (2 h, 3 h, 4 h, 5 h, or 6 h, 6% H_2O_2, 50 °C), F1 through F3, F4 through F7, and F8 through F12.

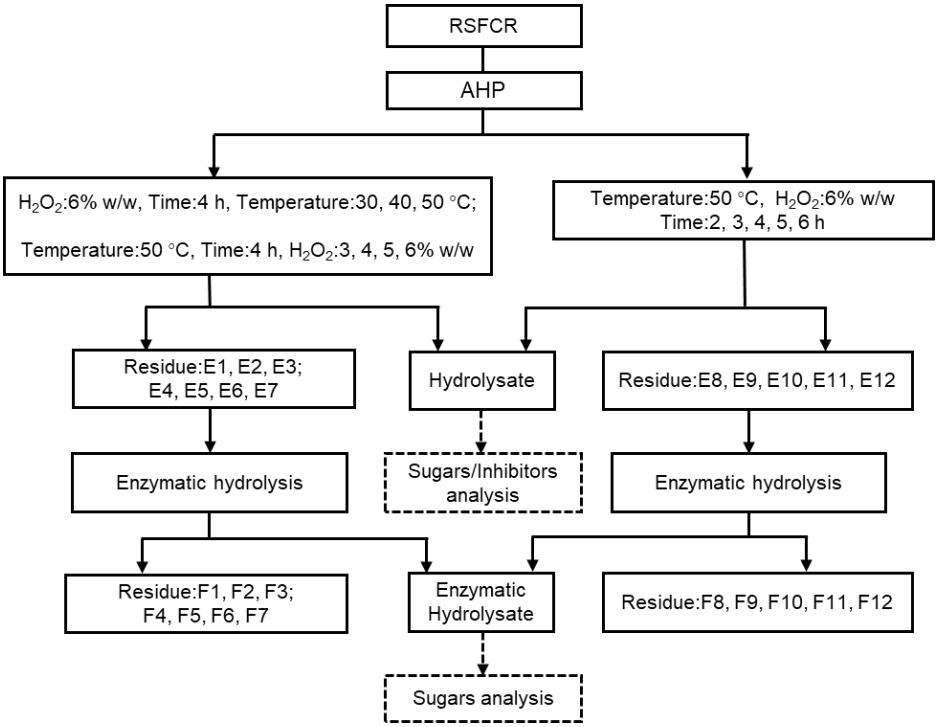

Fig. 3. Flow chart of AHP pretreatment collaborative enzyme digestion

Cellulase hydrolysis

The enzymatic hydrolysis was performed in 250-mL flasks using 0.05 mol/L citric acid-sodium citrate buffer (pH 4.8) and 2% dry matter (w/w) at 50 °C in a shaker at 200 rpm for 48 h. The air-dried pretreated solid was used for the enzymatic hydrolysis without any additional treatment. In all hydrolysis experiments, the enzyme dosage was 30 FPU/g residue (cellulase). After reaction completion, the enzyme was inactivated using a boiling water bath, and the solid-liquid separation was completed prior to subsequent analysis.

Analytical Methods

The chemical compositions of the original and pretreated biomass samples were measured using the methods of National Renewable Energy Laboratory (Sluiter *et al.* 2012). The sugars were quantitatively analyzed using ion chromatography (IC) (Dionex ICS–5000; Thermo Fisher Scientific, Waltham, MA, USA) with a CarboPac PA10 column, EC detector, column temperature of 30 °C. The eluent was 200 mmol • L^{-1} sodium hydroxide solution and ultrapure water with a flow rate of 0.8 mL/min, with proportional flows of 13% and 87%, respectively. The formic acid, acetic acid, hydroxymethyl furfural (HMF), and furfural (F) concentrations were analyzed at 30 °C using a Diamonsil C18(2) column attached to a ultraviolet (UV) detector (Agilent1260, Agilent Technologies, Santa Clara, CA, USA) with a flow rate of 1.0 mL/min. The mobile phases for the determination of F and HMF were methanol and 1% acetic acid, with the ratios of 10% and 90%, respectively. The eluents for formic acid and acetic acid determination consist of 65% methanol and 35% ultrapure water.

Fourier transform infrared spectroscopy (FTIR) was used to analyze the changes in the RSFCR functional groups after the pretreatment and enzymatic hydrolysis (TENSOR II; Bruker, Karlsruhe, Germany). The spectra were recorded between 4000 and 400 cm^{-1}.

An X-ray diffractometer (MiniFlex600; Rigaku, Tokyo, Japan) was used to analyze the degree of RSFCR crystallinity after the pretreatment and enzymatic hydrolysis.

The crystallinity (CrI) was determined using Eq. 1,

$$CrI\ (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

where I_{002} is the maximum intensity of the (2θ = 22.5°) diffraction peak and I_{am} is the scattering intensity of the amorphous phase at a diffraction angle of 18°.

Data analysis

Data analysis, statistical analysis, and linear relationship fitting were performed using Origin software (Origin Lab, v. 9.1, Northampton, MA, USA).

RESULTS AND DISCUSSION

Under the described pretreatment conditions, the hemicellulose, cellulose, and lignin were degraded and dissolved, which altered the structure of the lignocellulose raw materials, increased the enzyme accessibility, and improved the enzymatic hydrolysis efficiency.

Pretreatment Hydrolysis

Effect of the pretreatment method on the sugar dissolution

Tables 1, 2, and 3 show the total xylose and glucose yields (*i.e.*, monomer and oligosaccharide), the inhibitor concentrations during the three pretreatment methods, and the residue yields after the pretreatments. Under HW and ELA conditions, after increasing either the treatment temperature or time, initial increases were observed for the total xylose and glucose yields in the hydrolysate prior to a subsequent decrease.

The obtained results suggest that the sugars present in the hydrolysate mainly existed in the form of polysaccharides, with only a small number of monosaccharides present.

Table 1. Analysis of the HW Pretreatment Hydrolysate and Residue Yields

Conditions		Pre-hydrolysate						
Temp. (°C)	Time (min)	Yield (g/100 g RSFCR)		HMF (g/L)	Formic Acid (g/L)	Acetic Acid (g/L)	Furfural (g/L)	Residue (%)
		Xylose	Glucose					
160	40	2.71 ± 0.15	2.02 ± 0.14	0.01 ± 0.00	0.34 ± 0.02	0.32 ± 0.03	0.04 ± 0.00	79.65 ± 0.85
	50	2.98 ± 0.04	2.18 ± 0.13	0.02 ± 0.00	0.38 ± 0.05	0.36 ± 0.03	0.05 ± 0.00	77.21 ± 1.31
	60	3.52 ± 0.13	2.35 ± 0.09	0.02 ± 0.00	0.37 ± 0.01	0.30 ± 0.04	0.07 ± 0.00	75.80 ± 1.26
	70	3.46 ± 0.10	2.50 ± 0.02	0.03 ± 0.01	0.38 ± 0.01	0.33 ± 0.02	0.10 ± 0.00	75.10 ± 1.82
	80	3.39 ± 0.12	2.59 ± 0.03	0.03 ± 0.00	0.45 ± 0.03	0.38 ± 0.03	0.12 ± 0.01	74.63 ± 0.87
170	40	3.67 ± 0.13	2.62 ± 0.03	0.01 ± 0.00	0.39 ± 0.02	0.31 ± 0.01	0.06 ± 0.00	72.04 ± 0.93
	50	3.79 ± 0.04	2.65 ± 0.12	0.03 ± 0.01	0.44 ± 0.03	0.34 ± 0.01	0.10 ± 0.00	70.23 ± 1.87
	60	3.75 ± 0.09	2.68 ± 0.08	0.03 ± 0.00	0.47 ± 0.02	0.40 ± 0.01	0.13 ± 0.01	69.16 ± 1.10
	70	3.59 ± 0.05	2.71 ± 0.06	0.05 ± 0.00	0.45 ± 0.04	0.35 ± 0.01	0.19 ± 0.00	68.32 ± 1.36
	80	3.51 ± 0.15	2.72 ± 0.03	0.05 ± 0.01	0.45 ± 0.03	0.39 ± 0.00	0.22 ± 0.01	67.98 ± 1.57

At a reaction temperature of 160 °C, the xylose yield following the HW pretreatment reached 3.52 g/100 g material (60 min), while the highest yield following the ELA pretreatment was 3.67 g/100 g material (70 min). Under HW conditions, a glucose yield of 2.59 g/100 g material was reached at 80 min, while the highest yield was 3.08 g/100 g material under ELA conditions at 40 min. Under the same conditions, the addition of H_2SO_4 resulted in greater bond breakage between the hemicellulose and cellulose, which produced a higher sugar yield during the ELA pretreatment.

After the temperature was increased to 170 °C in the HW pretreatment, the xylose yield increased to a maximum of 3.79 g at 50 min prior to a subsequent decrease, while the glucose yield reached 2.72 g at 80 min. Both the xylose and glucose yields decreased under ELA conditions, with the highest yields being 4.04 and 3.19 g (40 min), respectively. This was attributed to the pentose degradation to furfural and hexose to HMF under acidic conditions. In addition, the inhibitor concentration increased after sugar degradation, and the sugar yield following the AHP pretreatment was lower than that during the HW and ELA pretreatments, with the highest yields of xylose and glucose being only 0.23 and 0.27 g, respectively (50 °C, 6% H_2O_2, 6 h).

Table 2. Analysis of the ELA Pretreatment Hydrolysate and Residue Yields

Conditions		Pre-hydrolysate						
Temp. (°C)	Time (min)	Yield (g/100 g RSFCR)		HMF (g/L)	Formic Acid (g/L)	Acetic Acid (g/L)	Furfural (g/L)	Residue (%)
		Xylose	Glucose					
160	40	3.15 ± 0.02	3.08 ± 0.18	0.02 ± 0.00	0.27 ± 0.02	0.21 ± 0.01	0.05 ± 0.00	76.19 ± 1.51
	50	3.37 ± 0.05	2.99 ± 0.14	0.0 2± 0.01	0.26 ± 0.03	0.20 ± 0.01	0.08 ± 0.00	73.71 ± 1.91
	60	3.44 ± 0.03	3.03 ± 0.10	0.02 ± 0.00	0.27 ± 0.02	0.21 ± 0.01	0.08 ± 0.01	72.96 ± 0.36
	70	3.67 ± 0.04	2.86 ± 0.12	0.03 ± 0.00	0.33 ± 0.04	0.29 ± 0.01	0.13 ± 0.00	72.41 ± 2.13
	80	3.63 ± 0.04	2.69 ± 0.15	0.04 ± 0.01	0.35 ± 0.03	0.29 ± 0.00	0.16 ± 0.01	72.24 ± 0.82
170	40	4.04 ± 0.02	3.19 ± 0.19	0.04 ± 0.00	0.39 ± 0.03	0.26 ± 0.01	0.15 ± 0.00	73.31 ± 1.36
	50	3.80 ± 0.05	2.82 ± 0.13	0.05 ± 0.00	0.40 ± 0.03	0.31 ± 0.01	0.21 ± 0.00	71.05 ± 1.74
	60	3.75 ± 0.07	2.62 ± 0.13	0.06 ± 0.00	0.40 ± 0.02	0.34 ± 0.01	0.26 ± 0.00	69.28 ± 2.21
	70	3.45 ± 0.08	2.40 ± 0.10	0.07 ± 0.00	0.41 ± 0.03	0.36 ± 0.01	0.32 ± 0.00	68.03 ± 1.93
	80	3.05 ± 0.13	2.30 ± 0.11	0.09 ± 0.00	0.42 ± 0.02	0.37 ± 0.01	0.38 ± 0.01	66.54 ± 1.99

Effect of the pretreatment method on inhibitor formation

Under the harsh conditions of the HW and ELA pretreatment methods, the dissolution and degradation of sugars proceeded simultaneously. The sugar yield in the hydrolysate considerably decreased while the saccharide formation rate was lower than the degradation rate. The inhibitors were not conducive to the subsequent utilization of hydrolysates (Palmqvist and Hahn-Hägerdal 2000), and no inhibitors were produced in the AHP hydrolysate due to the mild conditions employed.

As shown in Tables 1 and 2, the F and HMF concentrations increased when the pretreatment temperature and time were increased. At 160 °C over 80 min, the F concentrations in the HW and ELA hydrolysates were 0.12 g/L and 0.16 g/L, while the xylose yields were 3.39 and 3.63 g, respectively. A greater quantity of F was produced at 170 °C under HW and ELA conditions. With a reaction time of 80 min, the F concentration in the HW hydrolysate was 0.22 g/L, while the xylose yield was 3.51 g. Similarly, the F concentration in the ELA hydrolysate was 0.38 g/L, and the xylose yield was only 3.05 g, which was considerably lower than the value obtained at 40 min (4.05 g).

At a lower temperature (160 °C), the HMF concentration in the ELA hydrolysate was slightly higher than that obtained following the HW pretreatment, *i.e.*, 0.03 g/L and 0.04 g/L (80 min), respectively. At 170 °C, the HMF concentration was considerably increased, particularly in the ELA hydrolysate, which reached 0.09 g/L at 80 min, while it was only 0.05 g/L in the HW hydrolysate. The HMF formation was accompanied by glucose degradation. In this case, the glucose yield was only 2.3 g (80 min), which was less

than that obtained under HW conditions (2.72 g). The F and HMF concentrations in RSFCR ELA (200 °C, 20 min, 0.01%(w/w) H_2SO_4) hydrolysate were 0.57g/L and 0.07g/L, respectively (Yu *et al*. 2018). Their concentration in the hydrolysate obtained by Paulownia HW method (160 °C, 150 min) was 0.51g/L and 0.50g/L, respectively (Yan *et al*. 2016), which were more than in this study. F and HMF can be metabolized into furoic acid and 2,5-bis-hydroxymethylfuran, resulting in decreased growth rates of strains and ethanol productivity, and inhibition is related to strain species (Pienkos and Zhang 2009). When F concentration was lower than 5.8g/L, it did not inhibit the production of ethanol (Sarvari *et al*. 2003). Qureshi fermented butanol from hydrothermal pretreatment hydrolysate of sweet sorghum residue. The results showed that the fermentation process was not inhibited until the pre-hydrolysis temperature reached 200 °C (Qureshi *et al*. 2016). Therefore, the inhibitors content in this study were within the tolerance limit of the strains.

Furthermore, the formic acid and acetic acid concentrations considerably increased at higher temperatures, although no obvious increase was observed when the hold time was increased. In this context, the 5-hydroxymethylfurfural (5-HMF) produced from the monosaccharide can be degraded further into formic acid and acetic acid under harsh conditions (Shen and Gu 2009; Nilsson *et al*. 2016). However, the acetic acid formation was mainly attributable to abscission of the acetyl groups from the hemicellulose, with stronger acidic conditions increasing such abscission.

Effect of pretreatment on the residual solid yields

As shown in Tables 1 through 3, the residual solid yield after the HW pretreatment ranged from 68.0 to 79.6%, which was slightly higher than that following the ELA pretreatment (66.5 to 76.2%). However, the highest residual yield was obtained after the AHP pretreatment (*i.e.*, 86.5 to 95.3%). The yield of B1 (HW) was 79.6 g/100 g material, which contained 45.7 g glucan, 7.89 g xylan, and 2.8 g of other sugars. The yield of D5 (ELA) was 72.2 g/100 g material, which consisted of 48.0 g glucan, 7.08 g xylan, and 2.32 g of other sugars.

Table 3. Analysis of the AHP Pretreatment Hydrolysate and Residue Yields

Conditions			Pre-hydrolysate		
Temp. (°C)	H_2O_2 (%)	Time (h)	Yield (g/100 g RSFCR)		Residue (%)
			Xylose	Glucose	
30	6	4	0.07 ± 0.00	0.06 ± 0.00	95.32 ± 1.21
40			0.08 ± 0.00	0.08 ± 0.00	95.21 ± 1.21
50			0.16 ± 0.01	0.19 ± 0.02	90.82 ± 1.10
50	3	4	0.11 ± 0.00	0.15 ± 0.00	93.20 ± 1.11
	4		0.13 ± 0.00	0.16 ± 0.00	92.59 ± 1.17
	5		0.15 ± 0.00	0.17 ± 0.01	92.31 ± 1.22
	6		0.16 ± 0.01	0.19 ± 0.02	90.82 ± 1.10
50	6	2	0.09 ± 0.00	0.14 ± 0.00	92.36 ± 1.14
		3	0.09 ± 0.00	0.18 ± 0.01	91.65 ± 1.26
		4	0.16 ± 0.01	0.19 ± 0.02	90.82 ± 1.10
		5	0.20 ± 0.01	0.25 ± 0.02	87.83 ± 1.08
		6	0.23 ± 0.01	0.27 ± 0.02	86.54 ± 1.24

In addition, F3 (AHP) contained 48.0 g glucan, 12.2 g xylan, and 4.9 g of other sugars, and had a solid yield of 90.8 g/100 g material. The xylan contents of the HW and ELA pretreatment residues were lower than that obtained after the AHP pretreatment. Furthermore, the hemicellulose, which consists of xylose and other sugars, hindered cellulase accessibility and reduced the enzymatic hydrolysis efficiency.

Crystallinity analysis

Figures 4(a) through 4(c) show the X-ray diffraction images representing the RSFCR crystallinity during HW (A1), HLA (C1), and AHP (E3) pretreatments with synergistic enzymatic hydrolysis (B1, D1, and F3), respectively. As indicated, the RSFCR (002) diffraction peaks of cellulose remained at 22.5°, which indicated that the pretreatment and enzymatic hydrolysis processes did not alter the crystal structure of cellulose.

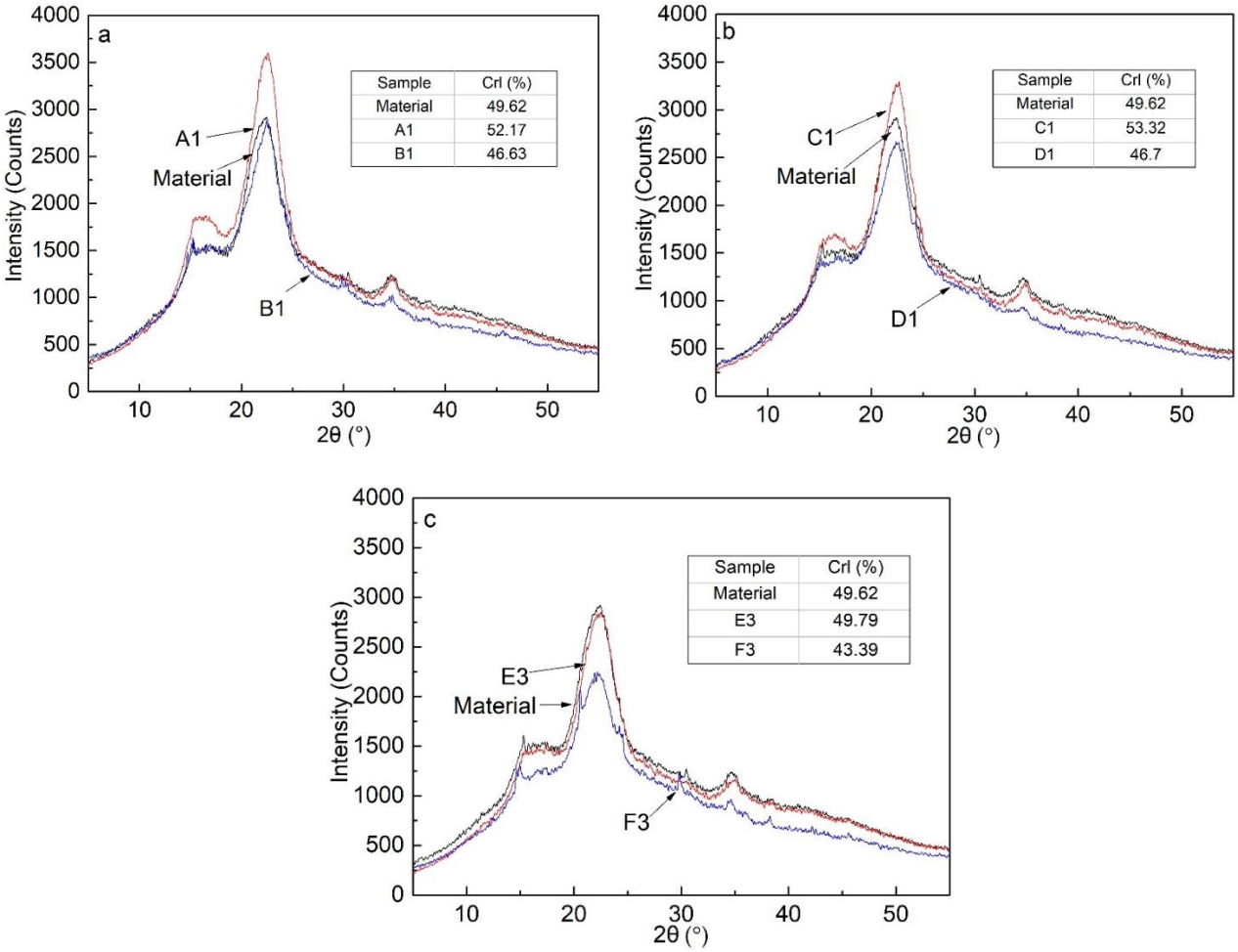

Fig. 4. X-ray diffraction patterns of the residues obtained under different treatment conditions: (a) A1 (HW pretreatment 160 °C, 40 min), (b) C1 (ELA pretreatment 160 °C, 40 min, 0.05% H_2SO_4), and (c) E3 (AHP pretreatment 50 °C, 240 min, 6% H_2O_2). The B1, D1, and F3 are the solids obtained following the three different pretreatment processes.

For the HW pretreatment and enzymatic hydrolysis process (160 °C, 40 min), an initial increase in the RSFCR crystallinity was observed from 49.6 to 52.2%, followed by a subsequent decrease to 46.6%. During the ELA pretreatment (160 °C, 40 min, 0.05% H_2SO_4) and enzymatic hydrolysis, the RSFCR crystallinity increased to 53.5% prior to decreasing to 46.7%. Under AHP conditions (50 °C, 240 min, 6% H_2O_2), the RSFCR crystallinity slightly increased to 49.8%, and decreased again after the enzymatic hydrolysis. A similar crystallinity was observed after the HW and ELA treatments and enzymatic hydrolysis, which was likely due to degradation and dissolution of the amorphous areas of the cellulose and hemicellulose under acidic conditions. A degree of lignin dissolution also took place during the pretreatments that led to an increased proportion of crystalline cellulose areas (Qiu *et al*. 2012). The ELA pretreatment had a slightly more pronounced effect than the HW pretreatment due to the acid addition. However, during the AHP pretreatment process, the lignin was partially removed and a proportion of the crystalline region increased, which increased the RSFCR crystallinity (Ramadoss and Muthukumar 2015). The alkaline conditions promoted swelling of the cellulose crystalline zone. However, this swelling effect was weaker than that of the lignin and hemicellulose removal on the overall crystallinity.

Furthermore, the enzymatic cellulose degradation decreased the crystallinity. Despite cellulose dissolution during the enzymatic hydrolysis, the crystallinity of B1, E1, and F3 remained at 46.6%, 46.7%, and 43.4%, respectively, thereby indicating that a small amount of cellulose was still present. Moreover, the crystallinity of F3 (43.4%) indicated that the AHP pretreatment imparted a strong swelling effect on the cellulose.

FTIR analysis

Figure 5 shows the infrared spectra of the raw RSFCR materials, the pretreated samples (A1, C1, and E3), and the enzymatic residues (B1, D1, and F3). The characteristic absorption peak intensity of the carbonyl or acetyl group of the hemicellulose at a wavelength of 1740 cm^{-1} was reduced after pretreatment with HW (A1: 160 °C, 40 min) and ELA (C1: 160 °C, 40 min, 0.05% H_2SO_4), which indicated that the hemicellulose dissolved during the pretreatment. After the AHP pretreatment, this peak was also slightly reduced, which confirmed that trace hemicellulose was degraded and dissolved during the process. In addition, after the HW and ELA pretreatments, the peak corresponding to the aromatic lignin moieties at 1510 cm^{-1} remained relatively unchanged, while this peak notably reduced in intensity after the AHP pretreatment (E3: 50 °C, 240 min, 6% H_2O_2). This was mainly due to the increased lignin degradation and dissolution under AHP pretreatment conditions. Compared with the raw material, the characteristic β-D glucoside absorption peak (898 cm^{-1}) of the cellulose did not noticeably change after any of the three pretreatment processes, likely due to the limited cellulose degradation under these conditions. In addition, the absorption peak intensities (898 cm^{-1}) of the three pretreated enzymatic residues (B1, D1, and F3) were considerably reduced, which indicated a large amount of cellulose was degraded during the enzymatic hydrolysis process. The peak at 1740 cm^{-1} disappeared in the ELA and HW pretreated enzymatic residues, while this signal was reduced in intensity for the AHP pretreated enzymatic residue. This was due to a lower hemicellulose removal rate under AHP pretreatment conditions, which hindered cellulase enzymatic hydrolysis.

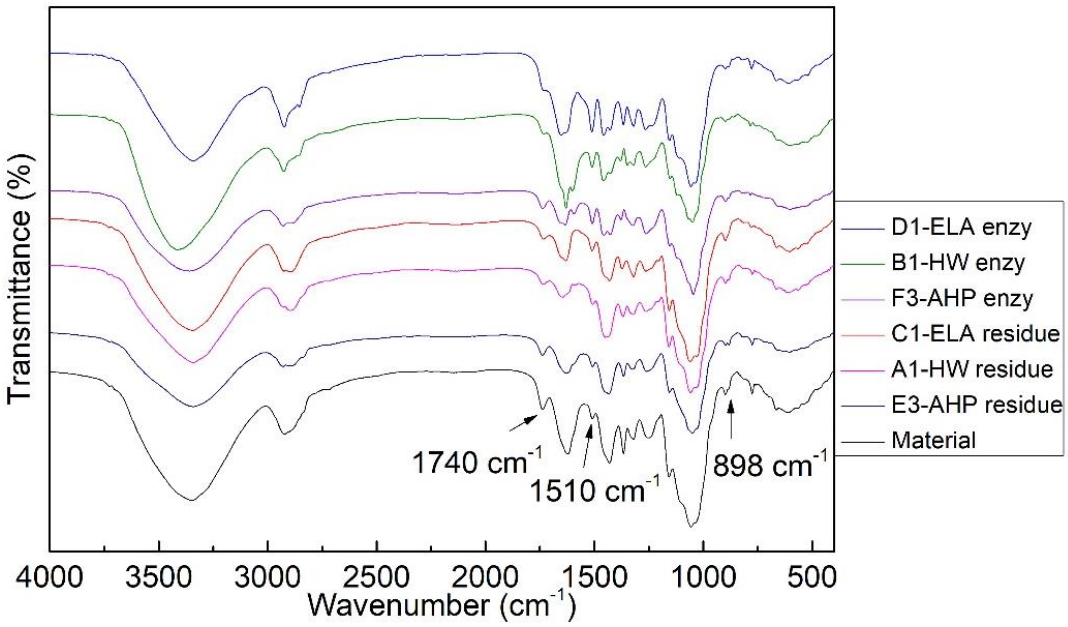

Fig. 5. Infrared analysis of the various fibers following pretreatment

Effect of pretreatment on enzymatic hydrolysis

Following the pretreatments, the obtained A1, C5, and E3 residues were employed as substrates. Figure 6 shows the total glucose yields in the cellulase hydrolysates when these three samples were employed.

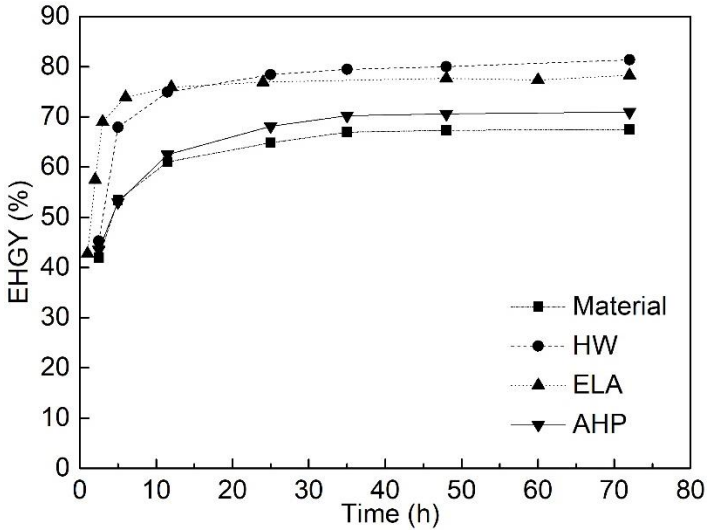

Fig. 6. Effect of different pretreatment methods on the enzymatic hydrolysis of cellulase (A1: HW pretreatment 160 °C, 40 min; C5: ELA pretreatment 160 °C, 80 min, 0.05% H_2SO_4; and E3: AHP pretreatment 50 °C, 240 min, 6% H_2O_2)

As shown in Fig. 6, the glucose yield rapidly increased with increased reaction times and stabilized after 36 h. However, the highest glucose yields were reached at 72 h

(HW, 81.4%; ELA, 78.3%; and AHP, 71.0%). After the pretreatment, the glucose conversion of the RSFCR was higher than that of the raw material (67.5%). The results were consistent with previous reports on the increasing enzymatic hydrolysis rate of corn stover by the HW pretreatment. The highest reported conversion of glucose was 82% (Zhou *et al*. 2014). The A1 residue (HW pretreatment 160 °C, 40 min) contained 58.4% (7.89 g) xylan and 48.8% (2.8 g) other sugars, including arabinose, galactose, and mannose that are hemicellulose components. Similarly, the C5 residue (ELA pretreatment 160 °C, 80 min, 0.05% H_2SO_4) retained 52.4% xylan (7.08 g) and 40.4% (2.32 g) other sugars, while the retentions of xylan and other sugars in the E3 residue were 90.6% (12.24 g) and 85.4% (4.9 g), respectively. Under HW and ELA conditions, the barrier between the hemicellulose, lignin, and cellulose was broken, allowing nearly 50% of the hemicellulose to dissolve in the hydrolysate. The presence of hemicellulose in the residue had an important influence on the glucose conversion of the cellulase hydrolysis (Zhang *et al*. 2013). In addition, a greater amount of carbohydrates was dissolved and degraded under ELA conditions, and the residual yields were slightly lower than under HW pretreatment conditions.

The AHP pretreatment enhanced enzyme accessibility by removing lignin, reducing the ineffective adsorption of lignin to cellulase, and swelling the cellulose crystalline zone (Aita and Kim 2010). However, due to the trace dissolution of hemicellulose and the high yields of the pretreatment residue (90.82%, Table 3), the AHP pretreatment had no obvious effect on improving the enzymatic hydrolysis efficiency.

The promoting effect of xylanase on cellulase

Studies have shown that free xylan can easily form hydrogen bonds with the hydroxyl groups on cellulose in solutions and adhere to the cellulose surface to prevent any contact between the cellulose and the enzymes (Kabel *et al*. 2007). As shown in Fig. 7, the xylanase considerably improved the degree of glucose conversion in the HW and ELA pretreated residues, where the glucose hydrolysis conversion reached 89.0% and 91.3% after 72 h, respectively. These yields were 9.4% and 16.6% higher, respectively, than those obtained in the absence of xylanase, which is likely due to the addition of xylanase reducing the inhibition of cellulase absorption by xylan (Zhang *et al*. 2013). Similar results were reported by Li *et al.* (2015), in which compared with using cellulase alone, the glucose yield of bamboo timber treated by xylanase and cellulase increased from 39.3% to 65.9%.

In addition, the glucose conversion in the enzymatic hydrolysates of A1, C5, and E3 were 93.5%, 94.5%, and 71.8%, respectively (based on substrates mass). Due to the relatively severe ELA pretreatment conditions, a low hemicellulose content was detected. As described earlier, the hemicellulose content in the C5 residue (9.4 g) was slightly lower than that in the A1 residue (10.7 g), which resulted in a slightly higher enzymatic hydrolysis efficiency. Furthermore, xylan was converted into oligosaccharides or monosaccharides, which reduced the cellulose adsorption and promoted cellulase hydrolysis. Therefore, a small hindrance to the enzymes resulted in slightly higher enzymatic hydrolysis efficiency. Moreover, the exposure of greater cellulose amounts improved the hydrolysis efficiency by increasing the enzyme accessibility. However, the presence of hemicellulose in the AHP residue reduced enzyme accessibility, and therefore the AHP pretreatment had no substantial effect on the glucose dissolution enzymatic hydrolysis, giving a glucose yield of only 68.9% at 72 h.

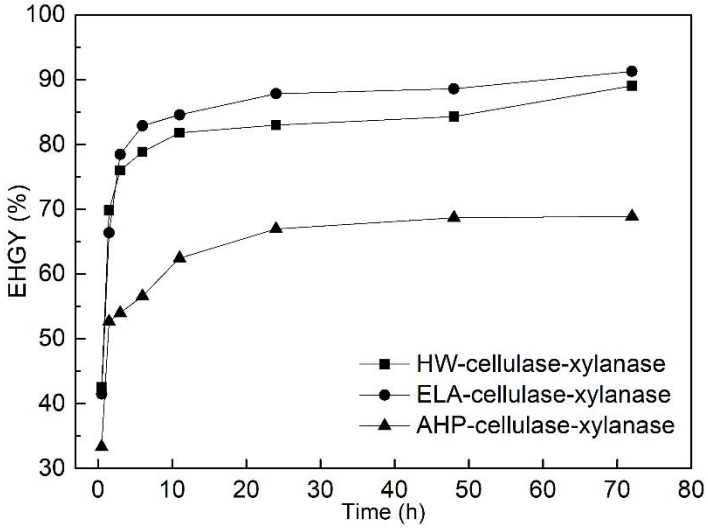

Fig. 7. The promoting effect of xylanase on cellulase; cellulase dosage: 30 FPU/g; xylanase dosage: 10 U/g; substrates: A1 (HW pretreatment 160 °C, 40 min), C5 (ELA pretreatment 160 °C, 80 min, 0.05% H_2SO_4), and E3 (AHP pretreatment 50 °C, 240 min, 6% H_2O_2)

Effect of xylan content in the enzymatic residue on the cellulase hydrolysis efficiency

Figure 8 shows the variation in the total glucose conversion with the total xylose removal rate in different enzymatic substrates. Substrates A1 (HW pretreatment, 160 °C, 40 min) and C5 (ELA pretreatment, 160 °C, 80 min, 0.05% H_2SO_4) were treated with xylanase (0.125, 0.25, 0.375, or 0.5 U/g) for 24 h, and then with cellulase (5 FPU/g, substrate) for 72 h. Under HW pretreatment conditions, the total glucose conversion increased from 46.4 to 51.1% with an increase in the total xylose removal rate.

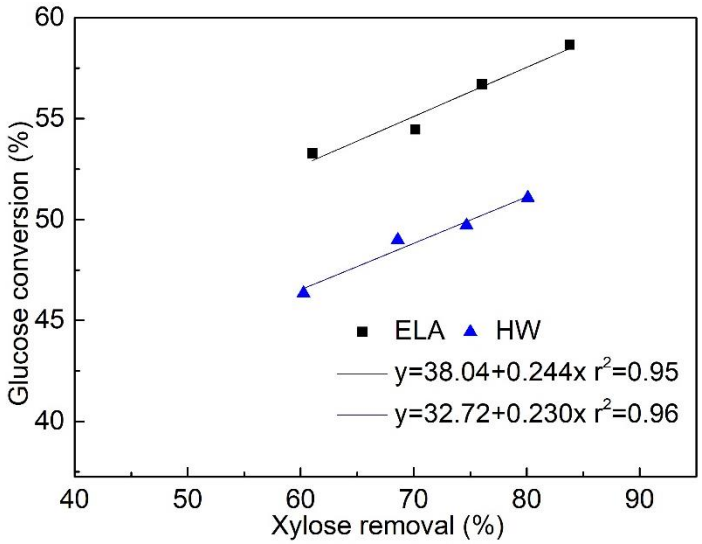

Fig. 8. Relationship between the xylan removal and the glucose conversion. A1 (LHW pretreatment 160 °C, 40 min) and C5 (ELA pretreatment 160 °C, 80 min, 0.05% H_2SO_4)

The equation y = 32.72 + 0.230x (R^2 = 0.96) was obtained by fitting the one-dimensional equation. When the ELA pretreatment residue was used as the substrate, the total glucose conversion increased when the total xylose removal increased. More specifically, the total glucose conversion increased from 53.3% to 58.7%, and in this case, the equation y = 38.04 + 0.244x (R^2 = 0.95) was obtained by fitting the one-dimensional equation. In addition, the linear relationship obtained between the xylose removal and the glucose conversion under different pretreatment methods differed, likely due to the severity of the pretreatment method on the raw materials.

CONCLUSIONS

1. Enzymatic hydrolysis of the ELA-pretreated residue gave higher glucose and xylose yields, with the low xylan content of the hydrolysis substrate improving the efficiency of cellulase hydrolysis.
2. The addition of xylanase promoted the conversion of glucose in the pretreated substrates. A total of 50.5 g glucose/100 g material was obtained from ELA pretreatment and subsequent enzymatic hydrolysis (cellulase and xylanase), with 47.8 g of this glucose content originating from the enzymatic hydrolysate. These results are of importance as they could lead to the production of high-value products from the cassava residue by-product obtained following the treatment of cassava starch.

ACKNOWLEDGEMENTS

This project was supported by the National Natural Science Foundation of China (No. 2136605) and the Guangxi Natural Fund (2013GXNSFFA019005).

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Article submitted: March 21, 2019; Peer review completed: May 25, 2019; Revised version received and accepted: June 9, 2019; Published: June 12, 2019.
DOI: 10.15376/biores.14.3.6060-6078