Effect of Kraft Pulping Pretreatment on the Chemical Composition, Enzymatic Digestibility, and Sugar Release of Moso Bamboo Residues

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In this work, kraft pulping was carried out on moso bamboo residues as a pretreatment and its impact on the chemical compositions and the digestibility of the sample was investigated. Meanwhile, steam explosion and sulfuric acid pretreatments were also carried out on the sample to determine their impacts on enzymatic saccharification. Results showed that kraft pulping pretreatment removed a significant amount of lignin from the sample, and its enzymatic saccharification was enhanced. Approximately 95% of the lignin was removed with the optimized kraft pulping pretreatment (26% effective alkali charge, 24% sulfidity, 160 °C, and 70 min cooking time). Consequently, about 79% and 77% yields of glucan and xylan, respectively, were achieved with enzymatic saccharification from the pretreated sample. As a result, 352 g, 128 g, and 88 g sugars were generated from 1000 g of samples pretreated by kraft pulping, steam explosion, and sulfuric acid, respectively. The results suggested that kraft pulping can be a remarkably effective pretreatment applied on moso bamboo residues (*i.e*., lignin-rich biomass) for sugars released, compared to steam explosion and sulfuric acid pretreatment.

Keywords: Moso bamboo residues; Kraft pulping; Delignification; Enzymatic hydrolysis; Fermentable sugars

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INTRODUCTION

Lignocellulosic materials such as agriculture and forestry residues, which contain high carbohydrate content, have been extensively investigated as renewable sources of hexoses and pentoses for the production of bio-ethanol or bio-based chemicals (Gusakov *et al.* 2007; Zhu *et al.* 2010; Jin *et al.* 2010). Bamboo as a lignocellulosic biomass, is a great potential resource and has been widely used for papermaking, food sources, and construction materials (Zhang *et al.* 2007). The utilization of bamboo for the production of ethanol, methane, and lactic acid has been studied for years (Kobayashi *et al.* 2004; Asada *et al.* 2005; Yamashita *et al.* 2010). Moso bamboo is an important ecological and economic product, which covers an area of 3.19 million hectares in China. In the course of utilization of perennial moso bamboo stem, the bamboo industry usually produces significant amounts of waste residuals, which are not used practically. Only 30% of the bamboo residues are burned to recover energy or made into activated carbon (Choy *et al.* 2005; Liu *et al.* 2010); the remainder is discarded, which results in environment

pollution. Therefore, efficient utilization of bamboo residues is an ideal choice for bio-based chemical production and reducing the risk of pollution.

The holocellulose components of lignocellulosic materials are generally embedded with amorphous lignin, and the cellulose comprises a highly resistant crystalline structure. These complex structures of lignocellulosic materials are the major obstacles that obstruct the degradation of carbohydrates by hydrolytic enzymes to release fermentable sugars (Rahikainen *et al.* 2011). Therefore, an effective pretreatment is required to treat the recalcitrant structure of lignocellulosic materials and to increase the accessibility of cellulolytic enzymes to the cellulose. Pretreatments such as dilute sulfuric acid, steam explosion, and sodium hydroxide (NaOH) have been investigated for decades. Mosier *et al.* (2005) reported that higher concentrations of dilute sulfuric acid solutions and higher hydrolyzing temperatures could effectively remove hemicellulose and improve the yield of enzymatic hydrolysis. Steam explosion can break down the lignocellulosic structure and remove a portion of the lignin and hemicelluloses, leading to high enzymatic hydrolysis yields (Liu *et al.* 2013; Chen *et al.* 2014). Sodium hydroxide pretreatment can successfully break up the ester bonds between lignin and carbohydrates (*i.e.*, lignin-carbohydrate complexes (LCCs)), resulting in the exposure of carbohydrates to enzymes (Li *et al.* 2010; Sambusiti *et al.* 2013). In addition, sodium hydroxide can disrupt the crystalline of cellulose to facilitate enzymatic saccharification.

Using pretreatments such as dilute acid and steam explosion, notable gains in delignification and enzymatic saccharification have been observed in several species, especially in annual plant wastes such as corn stover (Liu *et al.* 2013; Chen *et al.* 2014), sorghum bagasse (Sambusiti *et al.* 2013), and sugarcane bagasse (Chen *et al.* 2011). However, when dilute sulfuric acid and steam explosion pretreatments were applied to bamboo, it was found that delignification was not as high as that observed for other herbaceous plants, and the enzymatic hydrolysis yield of pretreated bamboo was low. (Yamashita *et al.* 2010; Li *et al.* 2014). Although sodium hydroxide could remove most of the lignin from lignocellulosic materials and improve the efficiencies of enzymatic saccharification, the pretreatment was applied at a high concentration of sodium hydroxide, and the carbohydrates were severely degraded (Li *et al.* 2014). To selectively extend delignification while retaining the more carbohydrates, Hartler (1978) proposed four modified kraft cooking principles, one of which was to maintain a level alkali concentration during pulping by splitting and injecting the white liquor (Na_2S+NaOH) at different points in the digester. The proposed rules laid the foundation of modern kraft cooking. From that point on, modified kraft cooking has been industrially applied in both batch and continuous pulping systems on a large scale (Wallberg and Jönsson 2006; Biswas *et al.* 2011).

Lou *et al.* (2010) found that the major phenylpropane units of moso bamboo lignin were syringyl and guaiacyl, which were bonded by *β*-aryl ethers (Sun *et al.* 2014). HS^- is a strong nucleophile. It can promote the cleavage of phenolic *β*-aryl ether bonds of the lignin such that most of lignin can be removed (Hartler 1978). Hence, it is speculated kraft pulping pretreatment could be an effective pretreatment for moso bamboo to enhance the enzymatic saccharification by the effective removal of lignin. Although kraft pulping as a pretreatment has been studied for decades, only a few researchers have reported the effect of kraft pulping on the chemical composition, enzymatic digestibility, and saccharification of carbohydrates on bamboo residues.

The purpose of this paper was to investigate systematically the effect of kraft pulping pretreatment on moso bamboo residues used to obtain fermentable sugars

(glucose and xylose) after enzymatic hydrolysis. Steam explosion and sulfuric acid pretreatments were also carried out on the samples to determine the impact of pretreatment on the enzymatic saccharification and the sugars yield. As a result, the effectiveness of different pretreatments was evaluated by the amount of released sugars from substrates treated by different pretreatments.

EXPERIMENTAL

Materials

The residues used in this study were from the stems of 3-year-old or older moso bamboo (*Phyllostachys heterocycla*) provided by the Shaowu Bamboo Processing Factory in Fujian, China. Air-dried moso bamboo residues without classification were collected and stored in sealed plastic bags at room temperature. Cellulase (No. C2730, with an activity of 102 FPU/g) and *β*-glucosidase (No. NZ188, with an activity of 504 IU/g) were purchased from Sigma-Aldrich Inc. (USA).

Methods

Kraft pulping pretreatment

Kraft pulping liquors were prepared with Na_2S and NaOH. The effective alkali (EA) charge (as Na_2O on dry material) was varied from 14 to 30%. Sulfidity, $Na_2S/(Na_2S$ and NaOH) (on Na_2O basis), was varied from 14 to 28%. The pretreatment was carried out in 1-L autoclave bombs that were heated with an oil bath. The liquid: solids ratio was 6:1. Dry bamboo residues (100 g) were first impregnated with the liquor at 60 °C for 30 min. After impregnation, the temperature was raised at a rate of 2 °C/min to the target cooking temperature (*i.e.*, 120, 140, or 160 °C) and maintained for 20 to 80 min. The pretreated bamboo residues were washed with distilled water at a solids: liquid ratio of 1:10 to remove the spent chemicals. The resulting pulp was stored at 4 °C for subsequent experiments.

Steam explosion pretreatment

Steam explosion of bamboo residues was carried out in 1-L autoclave bombs that were heated with an oil bath. The reactor was charged with 100 g of dried bamboo residues that were treated at various temperatures (190, 200, or 210 °C) for 10 min. When the pretreatment was completed, the bottom valve of the bomb was opened to depressurize the contents to atmospheric pressure. The steam-exploded bamboo residue solids were washed with distilled water at a solids: liquid ratio of 1:10. The resulting pulp was stored at 4 °C for subsequent experiments.

Dilute sulfuric acid pretreatment

The dilute sulfuric acid pretreatment was carried out in 1-L autoclave bombs that were heated with an oil bath at 120 to 160 °C for 60 min. Dry bamboo residues (100 g) were packed into the bomb, and a prescribed amount of sulfuric acid solution was added. The concentration of sulfuric acid was varied in the range of 1 to 4% (w/v) in the liquor, and the ratio of solid-to-liquid was 1:10. After pretreatment, the solids were washed with distilled water at a solids: liquid ratio of 1:10 to remove the spent chemicals. The resulting pulp was stored at 4 °C for subsequent experiments.

Enzymatic saccharification

Enzymatic saccharification (30 mL) was conducted at a substrate loading of 5% (w/v) with a cellulase loading of 20 FPU/g glucan supplemented with 3 IU *β*-glucosidase/g glucan (Chu *et al.* 2013). The experiments were performed in a 150-mL Erlenmeyer flask at 50 °C using 50 mM citrate buffer (pH 4.8), which was shaken at 150 rpm for 48 h. Aliquots were withdrawn and centrifuged for 10 min at 4000 rpm; the supernatants were subsequently filtered through a 0.22-μm syringe filter and analyzed to determine the sugar content.

Analysis methods

The primary constituents of bamboo residues and the pretreated samples were determined based on the procedure developed by the National Renewable Energy Laboratory for analyzing biomass materials (Sluiter *et al.* 2011). The activity of cellulase and *β*-glucosidase were determined according to the International Union of Pure and Applied Chemistry (IUPAC) standard (Ghose 1987).

The concentration of sugars was measured using a high-performance liquid chromatography (HPLC) system equipped with an Aminex HPX-87H column (300 × 7.8 mm) and a refractive index (RI) detector, and 5 mM H_2SO_4 solution was used as the eluent at a flow rate of 0.6 mL/min (Tengborg *et al.* 2001). The yields of glucose or xylose after enzymatic hydrolysis, which were based on the dried bamboo residues, were calculated as follows:

$$\text{Yield (\%)} = \frac{\text{glucose or xylose in enzymatic hydrolysate (g)}}{\text{inital glucose or xylose in substrate (g)}} \times 100\% \quad (1)$$

$$\text{Recovered Yield (\%)} = \frac{\text{glucan or xylan in pretreated bamboo residue (g)}}{\text{glucan or xylan in unpretreated bamboo residue (g)}} \times 100\% \quad (2)$$

The amount of delignification was calculated as follows:

$$\text{Delignification (\%)} = 1 - \frac{\text{lignin in pretreated bamboo residue (g)}}{\text{lignin in the raw bamboo residue (g)}} \times 100\% \quad (3)$$

The amount of fermentable sugars (*M*) from 1000 g of dry bamboo residuals was determined as follows:

$$M \text{ (g)} = \text{The amount of glucose produced (g)} + \text{The amount of xylose produced (g)} \quad (4)$$

RESULTS AND DISCUSSION

Characterization of Bamboo Residues

The primary chemical components of moso bamboo residues used in this work were as follows (%, dry weight basis): 39.2% glucan, 17.3% xylan, and 32.8% lignin (acid-insoluble (Klason) + acid-soluble). The lignin content in the bamboo residues was higher than the lignin content in corn stover and other annual plants (Wallberg and

Jönsson 2006). A possible explanation is that the conditions used for lignification during the pulping process leave behind higher lignin content in mature the 3-year-old or older moso bamboo in comparison to younger bamboo (Lin *et al.* 2002). Recently, Li *et al.* (2014) also found the lignin content was over 30% in the perennial moso bamboo.

Delignification and Composition of Pulped Bamboo Residues under Different Kraft Pulping Conditions

Technically, several parameters such as EA (effective alkali), sulfidity, cooking temperature, and cooking time should be investigated during the kraft pulping process (Reina *et al.* 2014). The effect of these parameters on delignification and pulp composition of moso bamboo residues are illustrated in Tables 1, 2, and 3. Data in these tables indicate that the solid, glucan, and xylan recovery yields decreased with increasing EA charge, sulfidity, cooking time, and cooking temperature.

It should be pointed out that there are some experimental errors between the cross data, which were at the condition of EA charge 26%, sulfidity 20%, and cooking time 60min, or at the condition of EA charge 26%, sulfidity 24%, and cooking time 60 min. The explanation was that the starting material used for investigation was received as a mixture of different age bamboo and the compositions could be different, which induced some experimental errors inevitably.

Table 1. The Effect of EA Charge on Chemical Composition and Delignification*

Temp. (°C)	EA charge (%)	Recovered yield (%)			Delignification (%)
		Solid	Glucan	Xylan	
120	14	66.4	94.1	62.2	68.4
	18	59.0	90.4	50.0	71.5
	22	56.6	90.3	42.8	73.9
	26	58.0	91.9	40.2	74.7
	30	51.9	85.1	34.9	78.6
140	14	59.6	90.9	53.4	77.9
	18	55.6	89.9	49.9	80.4
	22	52.4	88.2	42.5	85.1
	26	48.4	88.4	34.0	88.1
	30	45.5	83.3	28.9	88.4
160	14	51.9	88.4	50.9	87.7
	18	47.6	86.0	46.9	92.0
	22	45.4	85.2	38.4	95.0
	26	45.6	84.6	33.4	95.7
	30	39.8	80.2	27.9	96.6

* Kraft pulping was performed at the condition of sulfidity 20%, solids:liquid ratio 1:6, and cooking time 60 min (according to Gu *et al.* 2013)

As shown in Table 1, the cooking temperature increased as the solid yield decreased in the range of 51.9 to 66.4% for 120 °C, 45.5 to 59.6% for 140 °C, and 39.75 to 51.9% for 160 °C. These low solid yields of solid were mostly caused by the degradation of hemicelluloses and the removal of lignin, as well as some alkali degradation of the cellulose. It was also observed that the recovered yields of the carbohydrates decreased as the amount of delignification increased. For example, at 140 °C, the amount of delignification increased from 77.9% (at 14% EA charge) to 88.4% (at 30% EA charge), while the recovered yield of glucan and xylan dropped from 90.9% and 53.4%, respectively, to 83.3% and 28.9%, respectively.

Table 1 also suggests that kraft pulping had the capacity to improve degree of delignification with increasing EA loading. For instance, at 160 °C, the degree of delignification was 87.7% at a 14% EA charge, while over 90% of the lignin was removed when the EA charge was increased to 18%. Furthermore, when more severe pretreatment conditions were used, the glucan degradation losses were lower than that of the xylan. When bamboo residues were pulped at 22% EA, 20% sulfidity, and 140 °C, the recovered yield of glucan was 88.2% while that of the xylan was only 42.5%. This observation was in agreement with the report by Gu *et al.* (2013), which indicated that cellulose in the lignocellulosic biomass was more stable than xylan to alkali exposure due to the crystalline nature and high degree of polymerization of the natural cellulose.

At 160 °C, it was observed that a further increase of EA charge failed to promote delignification markedly after 26% EA charge. Meanwhile, the glucan and xylan were reduced sharply with more alkali addition at 26% EA charge, which consequently decreased the sugars released during enzymatic saccharification. A similar result was also observed at 120 °C and 140 °C. Hence, EA charge was set up at 26% to investigate the impacts of sulfidity and cooking time on chemical composition and enzymatic hydrolysis.

Table 2. Effects of Sulfidity on the Chemical Composition and Delignification*

Temp. (°C)	Sulfidity (%)	Recovered yield (%)			Delignification (%)
		Solid	Glucan	Xylan	
120	14	62.2	93.9	48.8	69.4
	16	61.5	92.8	47.3	69.5
	20	58.2	91.8	40.3	71.9
	24	57.1	90.9	38.3	74.8
	28	55.2	87.8	38.0	75.9
140	14	62.2	93.9	38.8	78.6
	16	49.9	89.6	35.8	81.3
	20	49.2	89.5	35.4	86.7
	24	49.1	89.3	35.4	87.4
	28	45.7	83.5	34.1	89.2
160	14	44.6	84.6	36.7	91.4
	16	44.0	84.2	35.9	93.1
	20	43.3	84.5	34.9	93.9
	24	42.7	84.7	34.4	94.2
	28	40.2	78.3	33.6	95.0

*Kraft pulping was performed at the condition of EA charge 26%, solids:liquid ratio 1:6, and cooking time 60 min

Table 2 shows the effect of sulfidity on the chemical composition of the pulped bamboo residuals. An increased degree of delignification was obtained with higher sulfidity charges. For example, the degree of delignification increased from 69.4% (at 14% sulfidity) to 77.0% (at 28% sulfidity) at 120 °C because a higher sulfidity provided more available HS^-, which promoted more phenolic β-aryl ether cleavage of the lignin (Gu *et al.* 2012). Table 2 also indicates that higher cooking temperatures promoted lignin removal during kraft pulping. At 14% sulfidity and 120 °C, the amount of delignification was 69.4%, which is significantly less than when the cook was conducted at 140 °C (85.2%) and 160 °C (91.4%). It was also observed that pretreatment with milder sulfidity conditions (*i.e.*, 14 to 24%) retained more glucan in the pulped solids, whereas most of the xylan was removed during cooking. Over 80% of glucan was retained in the pulped bamboo residues, while the retained xylan was only 33 to 49%. That could be explained

as the hemicelluloses have a lower degree of polymerization and more branched structure than cellulose, which make them more susceptible to being degraded during kraft pulping.

As can be seen in Table 2, the glucan, xylan recovery yields and degree of delignification were maintained at a point with the increase sulfidity from 16% to 24% at 120 °C, 140 °C, and 160 °C. Meanwhile, the compositions recovery yields were decreased sharply at 28%, and little enhancement of delignification was obtained. Hence, 24% sulfidity was used for further investigation of cooking time.

The impact of the kraft cooking time on delignification and pulp composition is illustrated in Table 3. It was shown that cooking time played an important role in the extent of delignification. At 120 and 140 °C, when the cooking time was increased from 20 min to 80 min, the amount of additional lignin removed increased by 10.2% and 8.6%, respectively. That was because moso bamboo is a highly lignified species, and it has some unique characteristics of high density and hardness (Chand *et al.* 2006). Thus, a longer cooking time could ensure chemical liquor transfer to the inner structure of bamboo and accelerate the degradation of lignin. Meanwhile, it was observed that the recovery yields of glucan and xylan were decreased with increasing cooking time. The reason for the degradation of carbohydrate was the result of the random alkali degradation and peel reaction that occurred at a long reaction time. These results provide insights into the effects of cooking time on the levels of carbohydrates and lignin in the pulped bamboo residuals.

Table 3. Effects of Cooking Time on the Chemical Composition and Delignification*

Temp. (°C)	Cooking time (min)	Recovered yield (%)			Delignification (%)
		Solid	Glucan	Xylan	
120	20	61.0	93.8	41.9	66.5
	40	57.3	91.4	39.3	69.8
	60	56.6	90.5	39.7	71.7
	70	56.1	90.3	39.4	74.9
	80	53.7	88.9	37.1	76.7
140	20	55.0	91.8	38.4	80.5
	40	51.0	90.8	35.8	85.3
	60	49.0	88.8	37.0	88.4
	70	47.2	87.3	36.5	88.9
	80	46.7	86.8	37.8	89.1
160	20	45.2	88.2	35.8	92.3
	40	44.6	87.4	35.3	93.7
	60	43.0	85.7	38.0	94.8
	70	42.9	85.8	38.8	95.5
	80	40.6	81.2	37.2	96.0

*Kraft pulping was performed at the condition of EA charge 26%, sulfidity 24%, solids:liquid ratio 1:6.

The degradation of lignin was enhanced by increasing the EA charge, sulfidity, cooking temperature, and cooking time of the kraft pulping process. More than three-fourths of the lignin in the bamboo residues could be removed using 14 to 30% EA and 14 to 28% sulfidity charge when pulped at a temperature of 120 to 160 °C for 20 to 80 min.

In addition, more than 80% of the original glucan and only 35% of the original xylan were retained in the pulped bamboo residues. When compared with other alkali pretreatment (Zhu *et al.* 2005; Kim and Han 2012), kraft pulping is an excellent method for removing lignin from the residuals, which might be attributable to the addition of Na_2S.

Yield of Enzymatic Hydrolysis and Mass Balance for the Amount of Fermentable Sugars

An ideal pretreatment is able not only to produce a readily digestible substrate with low content of lignin, but also to recover the maximum amount of available sugars in feedstocks (Li *et al.* 2014). Hence, fermentable sugars released from pretreated substrates should be calculated, and the amount of sugars should be considered as a standard to evaluate the pretreatment. The yields of enzymatic hydrolysis and the amount of fermentable sugars released with the different kraft conditions are shown in Figs. 1, 2, and 3. These figures revealed that the four pulping parameters were correlated with the enzymatic digestibility and the amount of fermentable sugars released.

From Fig. 1a, increasing the EA charge from 14% to 30% caused the yields of glucose and xylose to be enhanced significantly, from 21.9 to 28.1% for glucose and from 9.39 to 27.8% for xylose. This observation was attributed to the removal of lignin and the partial removal of hemicelluloses at different EA charge, sulfidity, and cooking time.

In addition, more LCC bonds were broken up by the extended NaOH addition, which may be another factor of the better enzymatic digestibility (Min *et al.* 2014). Yu *et al.* (2011) indicated that the efficiency of enzymatic hydrolysis of lignocelluloses was enhanced as the level of lignin removal was increased. Meanwhile, the removal of lignin could reduce the strong surface interaction between the lignin and the enzyme (Lou *et al.* 2013). These figures also demonstrate that higher sulfidity and cooking time helped obtain higher glucose and xylose yield (Figs. 2a and 3a).

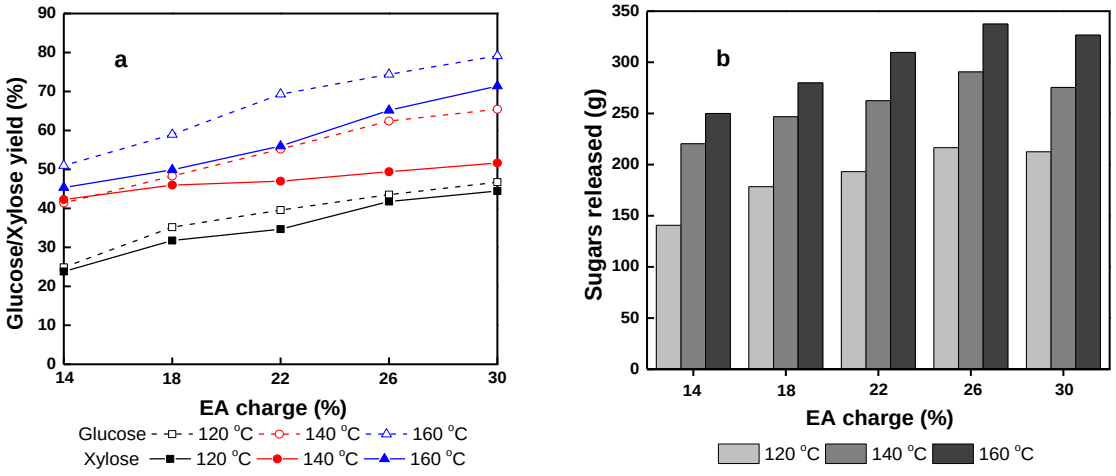

Fig. 1. (a) Effect of EA charge on glucose and xylose yields and (b) sugars released after enzymatic hydrolysis**.** Sugar production (glucose and xylose) was calculated by mass balance based on 1000 g of dry bamboo residues factoring kraft cooking and enzymatic hydrolysis processes

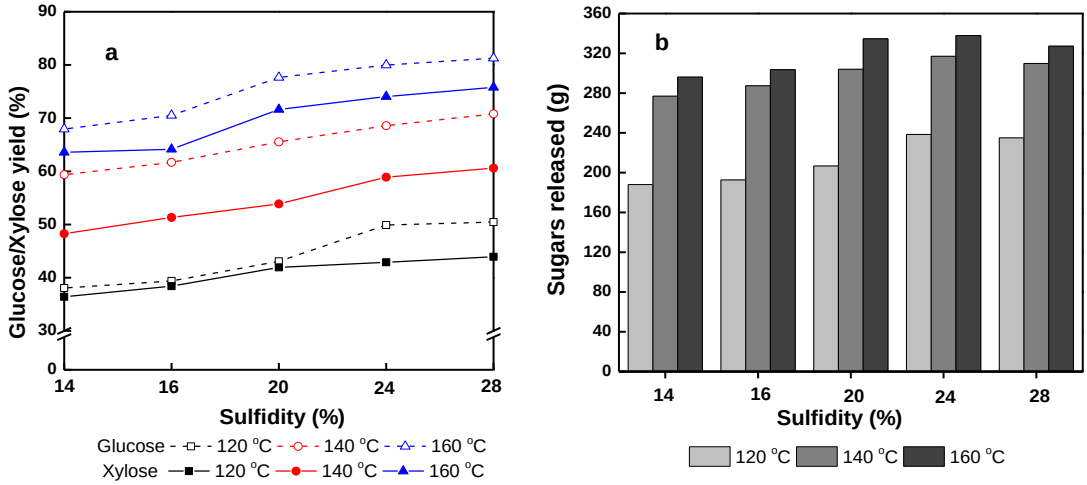

Fig. 2. (a) Effect of sulfidity on glucose/xylose yields and (b) sugars released after enzymatic hydrolysis**.** Sugars production (glucose and xylose) was calculated by mass balance based on 1000 g of dry bamboo residues factoring kraft cooking and enzymatic hydrolysis processes.

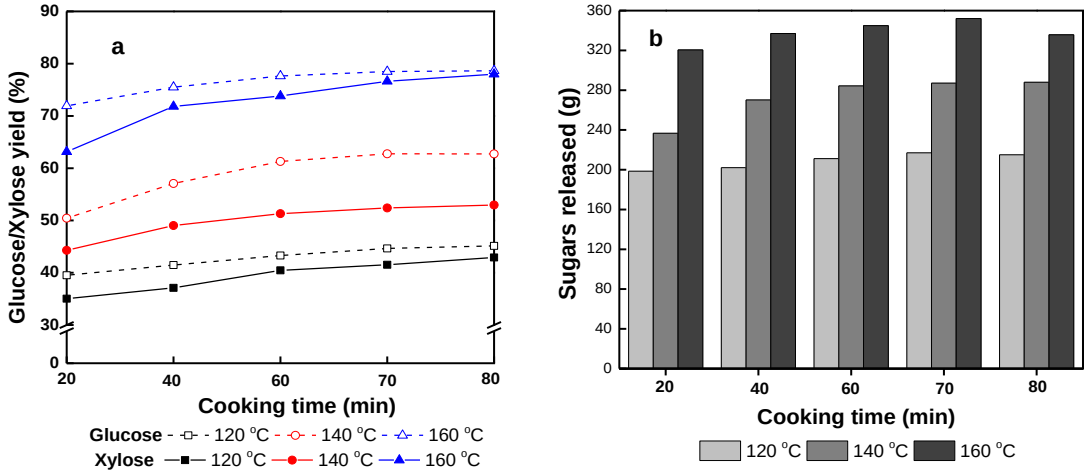

Fig. 3. (a) Effect of cooking time on glucose/ xylose yields and (b) sugars released after enzymatic hydrolysis**.** Sugars released (glucose and xylose) was calculated by mass balance based on 1000 g of dry bamboo residues factoring kraft cooking and enzymatic hydrolysis processes.

Figures 1b, 2b, and 3b show the amount of fermentable sugars derived from 1000 g of dry bamboo residues under different pretreatment conditions. The fermentable sugars yields were enhanced with the increasing pretreatment severity. The increased sugars yield may be due to the higher enzymatic digestibility with less lignin content in the pretreated residues. Many researchers have found that the enzymatic saccharification and sugars released are correlated with the lignin content in the substrates. Jin *et al.* (2010) reported the fermentable sugars released from alkali-pretreated lignocellulosic materials during enzymatic hydrolysis are correlated with lignin content. Additionally, there is a strong negative correlation between the amount of sugars liberated and the amount of lignin contained in the pretreated substrate (Gu *et al.* 2012; 2013).

These figures all showed increasing pretreatment severity can effectively enhance sugars yield. However, lower sugars yield was found when pretreatment conditions exceeded a certain point. For instance (Fig. 1b), at 160 °C, the amount of sugars

decreased from 337 g (at 26% EA charge) to 326 g (at 30% EA charge). As observed in Fig. 2b, increasing sulfidity from 14% to 24% increased the amount of sugars released, whereas the production changed less with further increase of sulfidity from 24% to 28% (at 120 °C, 140 °C, and 160 °C). A similar tendency was also observed in cooking time (Fig. 3b). At 120 °C, 140 °C, and 160 °C, the amount of sugars was enhanced with cooking time from 20 to 70 min. However, increasing cooking time from 70 min to 80 min failed to promote the release of sugar. These phenomena might be attributed to more holocellulose losses at serve pretreatment conditions, which is not beneficial for sugars recovery (Monavari *et al.* 2009). As expected, 26% EA charge and 24% sulfidity were the optimal parameters for sugars released from moso bamboo.

Integrating all parameters, kraft pulping pretreatment at 160 °C, 26% EA charge, 24% sulfidity, and a cooking time of 70 min were the optimized conditions for sugars released, yielded 352.0 g of fermentable sugars from 1000 g dry moso bamboo residues.

Dilute Sulfuric Acid Pretreatment

Dilute sulfuric acid, an alternative pretreatment method, was applied to the bamboo residues. As shown in Table 4, increasing the sulfuric acid charge and the pretreatment temperature caused lower recovered yields of solids, glucan, and xylan. It was observed that the dilute sulfuric acid pretreatment apparently hydrolyzed most of the xylans in the bamboo residues when high temperature and high acid charge were employed. At these conditions, only glucan and lignin were left in the pretreated substrates. This was true when all of the xylans were removed at 160 °C and acid loading of 3% to 4%. The low recovered yields of glucan and xylan in the pretreated bamboo residues were due to the acidic hydrolysis of the carbohydrates during pretreatment (Saha *et al.* 2005). Unlike kraft pulping pretreatment, the dilute sulfuric acid treatment had no significant influence on the delignification of bamboo residues, as only 10.1% to 17.4% of the lignin was removed at various conditions. These observations are consistent with the results reported by Li *et al.* (2014), who indicated that the lignin levels in the substrates were little enriched after the sulfuric acid pretreatment.

Table 4. Effects of Dilute Sulfuric Acid Pretreatment on the Residual's Composition, Delignification, Glucose and Xylose Yield, and Sugars Released*

Temp. (°C)	Acid charge (%)	Recovered yield (%)			Delignification (%)	Yield of enzymatic hydrolysis (%)		Sugars Released (g)
		Solid	Glucan	Xylan		Glucose	Xylose	
120	1	80.0	97.2	49.1	10.1	4.6	7.1	26.3
	2	71.2	91.9	26.2	13.5	8.1	10.7	37.8
	3	68.1	89.4	23.6	15.3	8.5	14.5	39.8
	4	67.4	89.9	20.1	16.9	8.9	16.5	41.3
140	1	68.2	92.4	17.3	12.1	15.3	12.6	65.8
	2	66.0	90.5	9.8	14.9	17.9	15.0	73.5
	3	65.5	87.3	4.7	16.0	19.6	16.9	76.0
	4	64.0	84.6	3.0	16.3	21.7	24.5	81.4
160	1	66.7	89.2	11.0	14.3	17.9	12.3	72.2
	2	64.2	83.3	7.3	15.3	19.8	0.0	71.6
	3	63.6	83.5	0.0	16.2	22.3	0.0	80.9
	4	62.9	82.2	0.0	17.4	24.7	0.0	88.1

*The sugars (glucose and xylose) released were calculated by mass balance based on 1000 g of dry bamboo residues, combining pretreatment and enzymatic hydrolysis processes

Previous studies have shown that dilute sulfuric acid pretreatment could remove hemicellulose and lignin, generating pores of pretreated lignocellulosic materials and improving the enzymatic hydrolysis efficiency (Yu *et al.* 2011; Li *et al.* 2014). Indeed, the efficiency of enzymatic hydrolysis of bamboo residue was enhanced to a certain extent when the pretreatment temperature and sulfuric acid concentration was increased. The maximal glucose yield was only 24.6%, even when all of the xylan content was removed under the harshest pretreatment conditions. One possible explanation for the low glucose yield was that most lignin was retained in pretreated solid after dilute sulfuric acid pretreatment, which could not expose enough cellulose to enzymes (Kumar *et al.* 2012). Additionally, lignin may adsorb enzymes ineffectively, reducing enzymatic digestibility.

The highest sugar released, 88.1 g of fermentable sugars, was obtained from 1000 g of dry bamboo residues pretreated at 160 °C and an acid charge of 4%. When compared with kraft pulping, fewer fermentable sugars were released from the acid pretreated bamboo residues due to the higher weight loss of xylan and the lower enzymatic hydrolysis yield. Therefore, dilute sulfuric acid pretreatment may not be an ideal process for converting bamboo residues into fermentable sugars.

Steam Explosion Pretreatment

Table 5 illustrates the impact of steam explosion pretreatment conditions on the composition of the treated bamboo residues. The recovered yields of glucan and xylan were reduced as the pretreatment temperature was increased; for the glucan and xylan, the yields were 97.0% and 35.5%, respectively, at 190 °C, whereas the yields were 89.5% and 19.7%, respectively, at 210 °C. Meanwhile, 21.8 to 45.8% of lignin was removed with increased temperature from 190 to 210 °C. Technically, steam explosion pretreatment could cause the structure of the bamboo residues to break-down, which led to a large reduction in the amount of holocellulose (Yamashita *et al.* 2010) and to a small amount of lignin degradation (Cara *et al.* 2008).

Table 5. Effects of Steam Explosion Pretreatment on the Residual's Composition, Delignification, Glucose and Xylose Yield, and Sugars Released*

Temp. (°C)	Cooking time (min)	Recovered yield (%)			Delignification (%)	Yield of enzymatic hydrolysis (%)		Sugars released (g)
		Solid	Glucan	Xylan		Glucose	xylose	
190	10	85.4	97.0	35.5	21.8	17.7	15.7	85.6
200	10	79.1	92.9	31.1	32.8	28.0	16.9	123.3
210	10	75.2	89.5	19.7	45.8	31.0	18.3	127.9

* The sugars (glucose and xylose) released were calculated by mass balance based on 1000 g of dry bamboo residues, combining pretreatment and enzymatic hydrolysis processes

Generally, it is believed that lignin removal of 20 to 65% is sufficient to increase the accessibility of cellulose to enzymes (Gu *et al.* 2013). In this work, approximately 21.8% to 45.8% delignification occurred during the steam explosion pretreatment; however, the saccharification efficiency did not improve as expected. The glucose and xylose yields were 17.7% to 31.0% and 15.7% to 18.3%, respectively, over the 190 to 210 °C pretreatment range. Additionally, because of low enzymatic hydrolysis yield, only 127.9 g of fermentable sugars (based on 1000 g of dry bamboo residues) was obtained after the consecutive steam explosion pretreatment (210 °C, 10 min) and enzymatic

hydrolysis process. As discussed above, although steam explosion pretreatment could remove 45.8% of the original lignin content, the enzymatic hydrolysis efficiency was still low. In the dilute sulfuric acid pretreatment, even if the hemicelluloses were almost completely dissolved, the enzymatic hydrolysis yield was still not as great. As expected, kraft pulping pretreatment had the capacity to remove more lignin; over 90% of the original lignin content could be removed under the severe kraft pulping conditions. Meanwhile, the highest enzymatic hydrolysis yield could reach 79% under the optimized kraft pulping pretreatment. Therefore, when the three pretreatments are compared for their enzymatic hydrolysis efficiencies, it is observed that the extent of delignification was the key factor affecting enzymatic digestibility. Similarly, Li *et al.* (2014) and Yamashita *et al.* (2010) also found that the lignin content played a significant role on the saccharification of lignocellulosic materials.

Among these three pretreatment methods, the kraft pretreatment yielded the highest amount of fermentable sugars from the bamboo residuals. Table 6 shows that after pretreatment and enzymatic hydrolysis, kraft pulping yielded 352.0 g of sugars; however, only 127.9 g and 88.1 g of sugars were obtained from the steam explosion and sulfuric acid pretreatment (based on 1000 g of dry bamboo residues), respectively. These results suggest that kraft pulping pretreatment may be an effective method for fermentable sugars production from moso bamboo residues (*i.e.*, lignin-rich biomass).

When compared with green liquor (Gu *et al.* 2013) and SPORL (Li *et al.* 2014) pretreatments, kraft pulping yielded higher levels of delignification, but negatively affected the recovery of the carbohydrates, which would reduce the total sugar recovery after pretreatment (Biswas *et al.* 2011). To achieve the goals of high holocellulose recovery yields, enzymatic hydrolysis yields, and fermentable sugars yields, pulping additives (Biswas *et al.* 2011) and optimized enzyme mixtures can be applied in the pretreatment and enzymatic saccharification process. Technically, kraft pulping additives such as anthraquinone (AQ) can inhibit alkali peeling reactions by oxidizing the end groups of polysaccharides while accelerating the delignification kinetics (Sjöström 1993). Zhang *et al.* (2010), Li *et al.* (2014), and Alvira *et al.* (2011) reported that the addition of multiple enzymes (*β*-glucosidase, xylanase, *a*-L-arabinofuranosidase, and pectinase) can enhance the conversion of the cellulose and hemicellulose into fermentable sugars. Hence, these methods should be further investigated for increasing the amount fermentable sugars released from moso bamboo residues in a future study.

Table 6. Effects of Three Pretreatment on the Residual's Composition, Delignification, Glucose and Xylose Yield, and Sugars Released*

Pretreatment	Recovered yield (%)			Delignification (%)	Yield of enzymatic hydrolysis (%)		Sugars released (g)
	Solid	Glucan	Xylan		Glucose	xylose	
Kraft pulping*	42.9	85.8	38.8	95.5	78.5	76.6	352.0
Steam explosion*	75.2	89.5	19.7	45.8	31.0	18.3	127.9
Sulfuric acid*	62.9	82.2	0.0	17.4	24.6	0.0	88.1

*The sugars (glucose and xylose) released were calculated by mass balance based on 1000 g of dry bamboo residues, combining pretreatment and enzymatic hydrolysis processes.
* Kraft pulping pretreatment was at the condition of EA charge of 26%, sulfidity of 24%, 160 °C, and cooking time of 70 min.
* Steam explosion pretreatment was at the condition of 210 °C and cooking time of 10 min.
* Sulfuric acid pretreatment was at the condition of 160 °C and cooking time of 60 min.

CONCLUSIONS

1. Kraft pulping pretreatment can remove most of the lignin, and the amount of delignification was a key factor for enhancing the enzymatic hydrolysis of moso bamboo residues.
2. The three pretreatment methods improved the release of fermentable sugars from bamboo residues; the best results were obtained by the kraft pulping pretreatment. Based on 1000 g of dry moso bamboo residues, 352.0 g, 88.1 g, and 121.5 g of sugars could obtained after kraft pulping, dilute sulfuric acid, and steam explosion pretreatments, respectively, when combined with enzymatic hydrolysis.
3. These results suggest that kraft pulping pretreatment may be an effective method for the saccharification of moso bamboo residues (*i.e.*, lignin-rich biomass). The optimized kraft pulping pretreatment conditions were EA charge of 26%, sulfidity of 24%, 160 °C, and cooking time of 70 min.

ACKNOWLEDGMENTS

This research was supported by the International Advanced Forestry Technology Introduction Project Funding (No. 2012-4-18) and the Major Program of the Natural Science Foundation of Jiangsu Higher Education (No. 11KJA220004). The authors thank the Priority Academic Program Development of Jiangsu Higher Education Institution (PAPD) and Natural Science Foundation of Jiangsu Provincial University (No. 14KJB220006) and the Natural Science Foundation of Jiangsu Province (No. BK20131426) for supporting the work presented in this paper.

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Article submitted: August 25, 2014; Peer review completed: October 15, 2014; Revised version received and accepted: November 4, 2014; Published: November 17, 2014.