Comparison of Enzymatic Hydrolysis of Bamboo Using Steam Explosion and Acid Sulfite, Alkali, and Alkaline Sulfite Pretreatments

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A combination of steam explosion (SE) and chemical pretreatments, such as acid sulfite (AS), alkali (AL), and alkaline sulfite (ALS), were evaluated using bamboo. Low pressure steam explosion at 1.25 MPa for 4 min was first applied to the bamboo. Then, the pretreated bamboo was delignified using three chemical pretreatments. Enzymatic hydrolysis was also compared among the pretreated bamboo samples. It was found that SE-ALS could be a potential method for bamboo pretreatment, which led to the reduction of lignin from 25.15% to 1.74% at 165 °C for 2 h in 5% (w/v) Na_2SO_3 and 0.7% (w/v) NaOH; however, little cellulose was solubilized during ALS pretreatment. A maximum glucose yield of 99.35% was achieved during the enzymatic hydrolysis process when combined with the SE-ALS pretreatment. The SE-ALS method resulted in a lower degree of lignin condensation and increased delignification compared to the SE-AS method. In addition, the SE-ALS pretreatment protected carbohydrates from degradation better than the SE-AL methods.

Keywords: Combined pretreatment; Bamboo; Glucose; Steam explosion; Sulfonation; Alkalization

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INTRODUCTION

With increasing concerns about global warming and air pollution, indispensable studies have been done to enhance the digestibility of lignocellulosic biomass, mainly for the transformation of carbohydrates into ethanol (Scurlocka *et al*. 2000). Among the potential alternative bioenergy resources, bamboo is a widely available raw material, notably in Asia and South America. Bamboo, which belongs to the Gramineae family, has been widely used as a feedstock for paper, textiles, food, construction, and reinforcing fibers (Zhang *et al.* 2007), and it has been considered as a potential feedstock for biofuel and biochemical production because of its fast growth, short period of regrowth, and easy propagation (Scurlocka *et al*. 2000).

The most important advantage of bamboo is the short duration (3 to 5 years) to maturity compared with other plants (Krzesinska 2009). However, the pretreatment of bamboo was found to be more difficult than that of agricultural residues and other species of wood. Such samples required loading with relatively high enzyme levels to achieve complete hydrolysis (Boussaid *et al*. 2000). For example, Chen and Liu (2007) found during their experiments that low-pressure steam explosion without the addition of

chemicals could result in an easy conversion from wheat straw to monosaccharides, and the cellulose-to-glucose conversion yield in enzymatic hydrolysis was above 90%. Comparatively, a lower cellulose-to-glucose conversion yield (42.6%) was obtained with a relatively high-pressure steam explosion at 243 °C during a bamboo experiment (Yamashita *et al*. 2010).

Most fractionation methods employ either acidic or alkaline conditions, which result in lignin and hemicellulose depolymerisation *via* solvolysis reactions. Steam explosion (SE), which has been extensively investigated and tested in several pilot and demo plants worldwide (Galbe and Zacchi 2012), is considered useful for the production of second-generation ethanol and other value-added products (Oliveira *et al*. 2012). For feedstocks like bamboo, a possible way to improve acidic or alkaline pretreatment is to combine it with an external, low-pressure SE pretreatment. In addition, the effects of the pretreatment are very dependent on the material species, biomass composition, and operating conditions. Among the chemical methods, fragmentation through alkalization or sulfuration, and hydrophilisation through sulfonation are considered to be pretreatments with good potential. The alkaline sulfite pretreatment causes both alkalization and sulfonation.

The goal of this paper was to evaluate the impact of pretreatment methods of acid sulfite (AS), alkali (AL), and alkaline sulfite (ALS) on bamboo that was first pretreated through low-pressure SE solubilisation. This paper reports the effect of the pretreatments on enzymatic hydrolysis using a mixture of commercial enzymes (*i.e.*, cellulase and cellobiase). The chemical compositions of bamboo were compared before and after each pretreatment at both the solid and liquid phases, respectively. Total glucose and xylose recovery from each pretreatment was also evaluated.

EXPERIMENTAL

Materials

The bamboo (*Neosinocalamus affinis*) samples were obtained from ChiShui, Guizhou Province, China. Air-dried stems of bamboo were chopped into relatively homogenously sized pieces of 50 mm × 5 mm for SE pretreatment. Celluclast 1.5 L, a cellulase preparation from *Trichoderma reesei*, and Novozyme 188, a β-glucosidase preparation from *Aspergillus niger*, were purchased from Novozymes Investment Co., Ltd. (China). The activity of Celluclast 1.5 L and Novozyme 188 were detected to be 108 FPU/mL and 175 BGU/mL, respectively.

Methods

Pretreatments

The SE pretreatment was carried out in a batch pilot unit (STG19-40, Andritz Austria) equipped with a 7.5 L reaction vessel and exposed to saturated steam at 190 °C (1.25 MPa) for 4 min. After exposure to the saturated steam, a ball valve at the bottom of the reactor was suddenly opened to bring the reactor rapidly to atmospheric pressure.

Chemical pretreatments of bamboo were carried out after SE pretreatment. The chemical pretreatments were carried out using a reactor with a volume of 200 mL and manufactured with polytetrafluoroethylene (PTFE). For each experiment, bamboo samples were mixed with distilled water containing the desired amounts of chemicals. For the AS pretreatment, the sodium bisulfite loading was 5% (w/v), and 0.5% (w/v)

H_2SO_4 was added to adjust the pH to 2.3. For the AL pretreatment, the sodium hydroxide loading was 0.7% (w/v), the sodium sulfide loading was 0.2% (w/v), and 0.0125% (w/v) of anthraquinone (AQ) was added to prevent excess carbohydrates from degrading, with an initial pH of 14.

For the ALS pretreatment, the sulfite loading was 5% (w/v) and the sodium hydroxide loading was 0.7% (w/v), with an initial pH of 12. Each pretreatment was heated at a rate of 5 °C/min to reach the desired temperatures of 120 °C, 140 °C, and 165 °C, respectively. All pretreatments were conducted at the same pretreatment time (10 min to the temperature and 2 h at the temperature), and the ratio of pretreatment liquor to bamboo was 20:1 (v/w).

After the pretreatment process, the system was rapidly cooled with tap water, and the solid fraction was washed with distilled water until a neutral pH was achieved for enzymatic hydrolysis and composition analysis.

Enzymatic hydrolysis

Enzymatic hydrolysis was performed in a 100 mL flask using 50 mL of 0.05 M sodium acetate buffer (pH 4.8) at 48 °C. The flasks were agitated at 150 rpm in a rotary shaker (Certomat-R, B-Braun, Germany) for 72 h. Wet materials were added at a concentration of 5.0% (w/v) on a dry basis. The enzyme loading process for the substrate consisted of 18FPU/g-cellulose for cellulase and 27 CBU/g-cellulose for β-glucosidase. Hydrolysis of the raw bamboo samples and SE of the bamboo were performed as controls.

Samples were taken at 1, 12, 24, 48, and 72 h, respectively. The glucose yield was calculated by assuming that 1 g of cellulose present in solution equaled approximately 1.11 g of glucose. The total glucose recovery was calculated by glucose mass from enzymatic hydrolysis of chemical processed sample divided by glucose mass from SE bamboo sample.

Analytical methods

The carbohydrate content of bamboo was analyzed using a two-step acid hydrolysis method, according to the procedure published by the National Renewable Energy Laboratory (NREL) (Sluiter *et al*. 2004). A 0.3 g dry sample was weighed in a pressure bottle, into which 3 mL of 72% sulfuric acid and 84 mL of distilled water were added. Then, the pressure bottle was transferred to an autoclave where the temperature was set at 121 °C for 1 h.

After hydrolysis, the sample was cooled, neutralized, and filtered through a medium-coarseness sintered glass crucible. The filtrate was analyzed using a high-performance liquid chromatography system (Waters 2695e, USA) and an Aminex HPX-87P (Bio-Rad, USA) at 85 °C with a refractive index detector at 35 °C (Xing *et al*. 2015). The injection volume of the sample was 10 μL, and water (0.6 mL/min) was used as the eluent, with the total analysis time of 50 min. The Klason lignin content was measured as the ash free residue remaining after acid hydrolysis.

Each pretreatment was carried out in duplicates, and reported as the average of the two runs. Figure 1 shows the schematic for the treatment of bamboo samples with the combination of low-pressure SE and the chemical pretreatments.

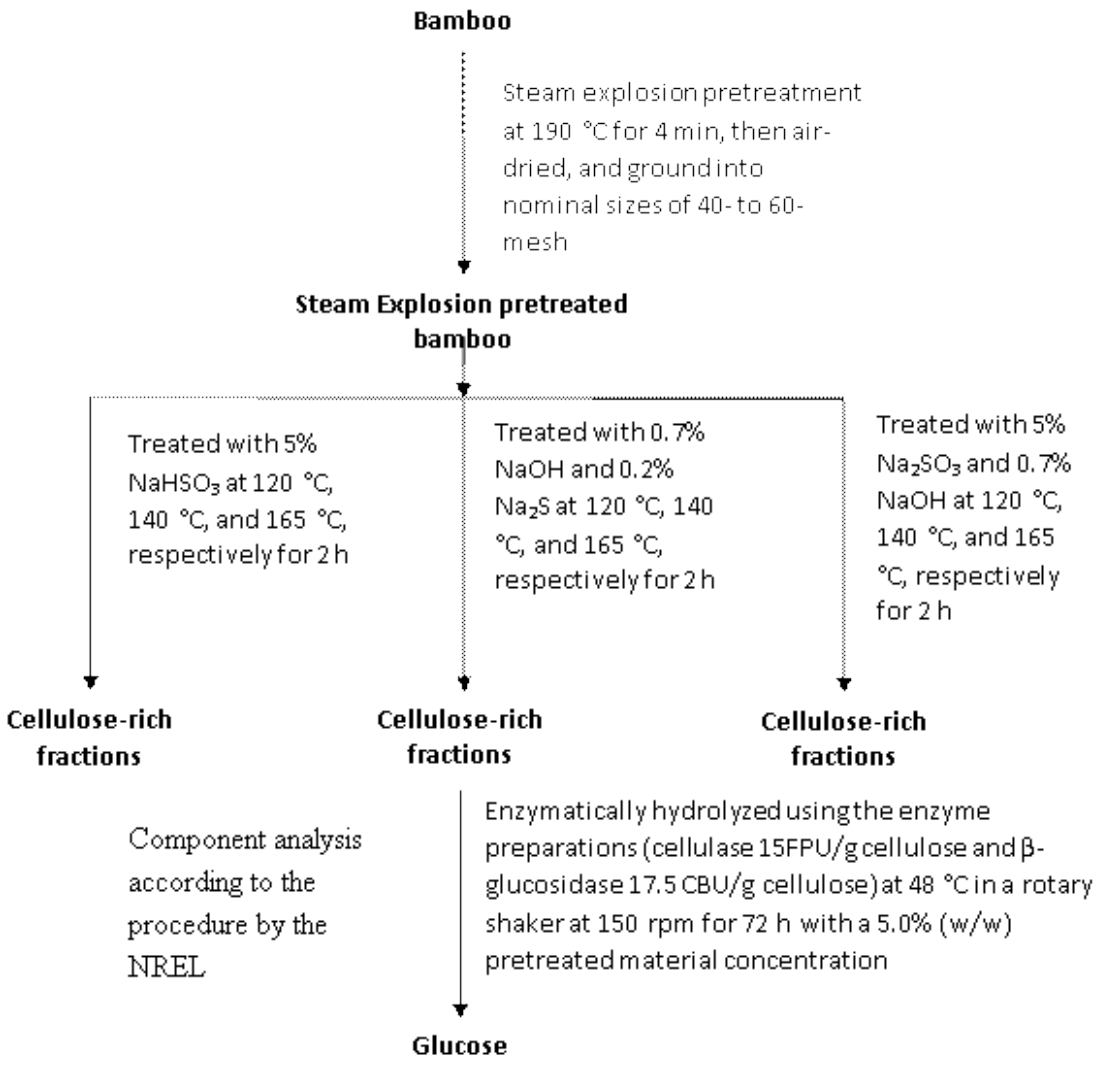

Fig. 1. Schematic showing the treatment of bamboo samples with the combination of steam explosion pretreatment and chemical pretreatments

RESULTS AND DISCUSSION

Composition of Original Bamboo and Low-Pressure SE Materials

Table 1 shows the compositional analysis of bamboo before and after the low-pressure SE pretreatment. For the raw material, glucan was the dominant component, followed by lignin and xylan. These results were similar to those previously reported (Scurlock *et al*. 2000; Xing *et al*. 2013). The ash content of the original bamboo was low. After the SE pretreatment, the content of xylan in the substrate decreased from 17.88% to 11.18%, leading to an addition of glucan. However, the hemicellulose removal was minimal because of the low intensity of the SE pretreatment. The SE pretreatment had a positive effect because a part of the hemicellulose was removed. The structure became

loose and the surface area of the fibers increased in terms of delignification and enzymatic hydrolysis.

Table 1. Chemical Composition of Original Bamboo and SE-Pretreated Bamboo

	Glucan (%)	Xylan (%)	Acid-insoluble lignin (%)	Acid-soluble lignin (%)	Arabinan (%)	Galactan (%)	Mannan (%)	Ash(%)
RM	43.59 ±1.42	17.88 ±0.74	25.87 ±0.82	3.91 ±0.28	1.16 ±0.09	0.24 ±0.05	0.74 ±0.02	1.32 ±0.10
SE	54.89 ±1.72	11.18 ±0.53	25.15 ±0.84	4.29 ±0.26	0.97 ±0.10	0.20 ±0.03	ND	0.88 ±0.05

RM: raw material (original bamboo); SE: low-pressure steam exploded material; ND: not detected

Comparison of Chemical Pretreatment on Bamboo Composition

Many factors, such as lignin content, crystallinity of cellulose, and particle size, limit the digestibility of the hemicellulose and cellulose present in the lignocellulosic biomass (Hendriks and Zeeman 2009). The mechanism by which the three pretreatments improved delignification was quite different. The rate and extent of enzymatic hydrolysis is inversely related to the lignin content (Limayem and Ricke 2012); thus delignification is an important process. Therefore, results yielded significant differences among the pretreated types for the bamboo samples.

For the AS pretreatment, the amount of lignin removed was slightly increased with increasing temperature; however, the variation of the quantity of cellulose and hemicellulose was not pronounced (Fig. 2a). It should be noted that the AS pretreatment is normally used for softwood. From the result, it can be concluded that the AS pretreatment may not be suitable for bamboo samples in delignification. However, the final properties and effect of the products should be evaluated through enzymatic hydrolysis. Li *et al*. (2014) mentioned that during the AS pretreatment, the addition of sodium sulfite, as a buffer, elevated the pH value of the pretreatment liquor, thus achieving a higher amount of hemicellulose that was retained in the AS substrates. Consequently, the digestibility of the AS substrates was worse than that of the other substrates. In this work, the pH value was strictly limited below 2.3, and some of the hemicelluloses had been removed during the SE pretreatment. However, the amount of lignin was still slightly decreased. Bamboo has some unique characteristics in terms of its chemical composition and anatomical structure (Li *et al*. 2014). As a result of the extremely low pH, some condensation reactions took place between different lignin units, which formed non-cleavable, carbon-carbon bonds, which significantly impacted delignification. (Xing *et al*. 2015).

It is expected that the delignification reaction was pronounced in the AL fractionation because of the fragmentation through alkalization and sulfuration, compared to the AS cooking. Hsu (1997) reported that the alkaline pretreatment was generally more effective for agricultural residues and herbaceous crops than woody materials (Hsu 1997). Chen *et al*. (2007) reported that a large amount of lignin removal was observed with sodium hydroxide concentrations between 0.5 and 2.0 wt%, when using raw herbaceous feedstock (Chen 2007). In this work, the content of lignin changed from an original 25.15%, to 9.18%, 6.70%, and 4.31% when operating at temperatures at 120 °C, 140 °C, and 165 °C, respectively (Fig. 2b). On the other hand, extensive carbohydrate

degradation decreased the yield of polysaccharides before enzymatic hydrolysis when the temperature was above 140 °C.

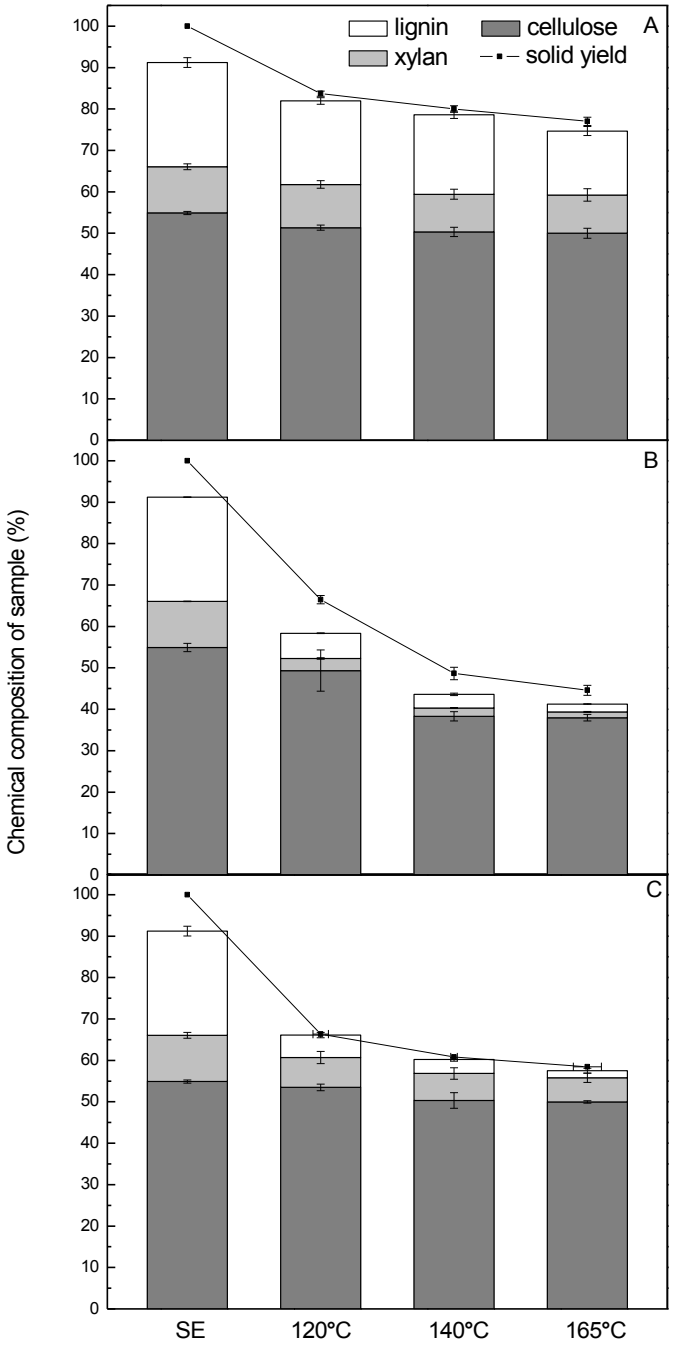

Fig. 2. A comparison of the chemical composition after the AS (A), AL (B), and ALS (C) pretreated bamboo samples

Almost all of the carbohydrates were retained in the solid phase for the AS pretreatment even though the delignification rate was relatively low (Fig. 2A). On the contrary, the lignin removal only reached 38.55% when using AS treatment at 165 °C.

Similar results in lignin removal were found in the ALS and AL pretreatments (93.1% and 92.4%, respectively), thus fragmentation was better than hydrophilisation for bamboo in delignification, while peeling reactions may have led to the consumption of cellulose and hemicellulose. The ALS pretreatment removed lignin with both sulfonation and alkalization. The composition of the feedstocks after the ALS pretreatment is shown in Fig. 2c. The content of lignin notably decreased with increasing temperature; the content of lignin was 5.39%, 3.38%, and 1.74% at 120 °C, 140 °C, and 165 °C, respectively. This change may have been attributed to both sulfonation and alkalization. On the other hand, pronounced carbohydrates degradation was absent from the ALS pretreatment at the detected temperatures, with the absence of AQ. The peeling reaction of polysaccharide removal, caused by NaOH, was much lower under mild alkaline conditions compared to the AL pretreatment. Sulfite can compensate lignin removal to achieve high delignification through sulfonation.

Table 2 summarizes the composition of the liquid phase after each chemical pretreatment. These results provided insights into the effects of pretreatment on the changes of carbohydrates. The AS and ALS liquors contained less sugars than AL liquors, which corresponded to the results of solid phase. The higher concentration of glucose and xylose in the AL spent liquors verified that extensive carbohydrate degradation started during AL pretreatment when the temperature was above 140 °C.

Table 2. Concentration of Monomeric Sugars in Pretreated Spent Liquors

Pretreatment	Temperature(°C)	Glucose(g/L)	Xylose(g/L)	Arabinose(g/L)	Galactose(g/L)
AS	120	1.80±0.14	0.41±0.06	0.35±0.09	0.04±0.00
	140	2.31±0.42	1.05±0.11	0.36±0.04	0.03±0.00
	165	2.50±0.24	1.00±0.10	0.40±0.08	0.04±0.00
AL	120	2.81±0.35	4.12±0.32	0.30±0.01	ND
	140	8.33±0.25	4.63±0.35	0.32±0.05	ND
	165	8.48±0.82	4.97±0.48	0.33±0.09	ND
ALS	120	0.80±0.13	1.93±0.14	0.34±0.10	ND
	140	2.36±0.42	2.37±0.32	0.35±0.04	ND
	165	2.57±0.44	2.73±0.21	0.34±0.01	ND

The results showed that the ALS pretreatment was a better way to avoid degradation of carbohydrates and achieve bulk delignification, compared to the AS and AL pretreatments. The following reasons are suggested as possible explanations: 1) The ALS pretreatment provided a mild reaction stage, which reduced the alkaline degradation of carbohydrates compared to the AL pretreatment; 2) During the AS method, sulfonation was completed by forming a benzyl carbonium ion. The condensation reaction may compete with sulfonation at a lower pH value. On the other hand, during the ALS pretreatment, sulfonation was done by nucleophilic substitution reaction. Alkalization and sulfonation reactions caused by nucleophilic ions (OH^- and SO_3^{2-}) made it easier for both phenolic lignin and non-phenolic lignin to fragment and dissociate; 3) Condensation reactions hardly occurred under mild alkali conditions. Shuai *et al*. (2010) reported that the increasing of pH value can prevent lignin from extensive condensation. The newly formed carbon-sulfur bond was relatively strong; therefore, the formation of the sulfonic acid group protects lignin from condensation reactions; 4) The sulfonation reaction originated in the cell layer. Bamboo exhibits a compact structure; thus it was impregnated slowly and was not homogeneous during AS pretreatment. However, during the AL and

ALS pretreatments, the presence of NaOH achieved fiber swelling, and the bamboo was impregnated quickly and uniformly. Therefore, the ALS pretreatment provide a potential way for lignin removal and a high content of carbohydrates to be reserved from the bamboo.

Enzymatic Hydrolysis of Pretreated Bamboo

Further research on enzymatic hydrolysis was done to compare the differences in the three pretreatments. After the low-pressure SE pretreatment, only 27.4% of glucan in the bamboo samples was converted to glucose during enzymatic hydrolysis.

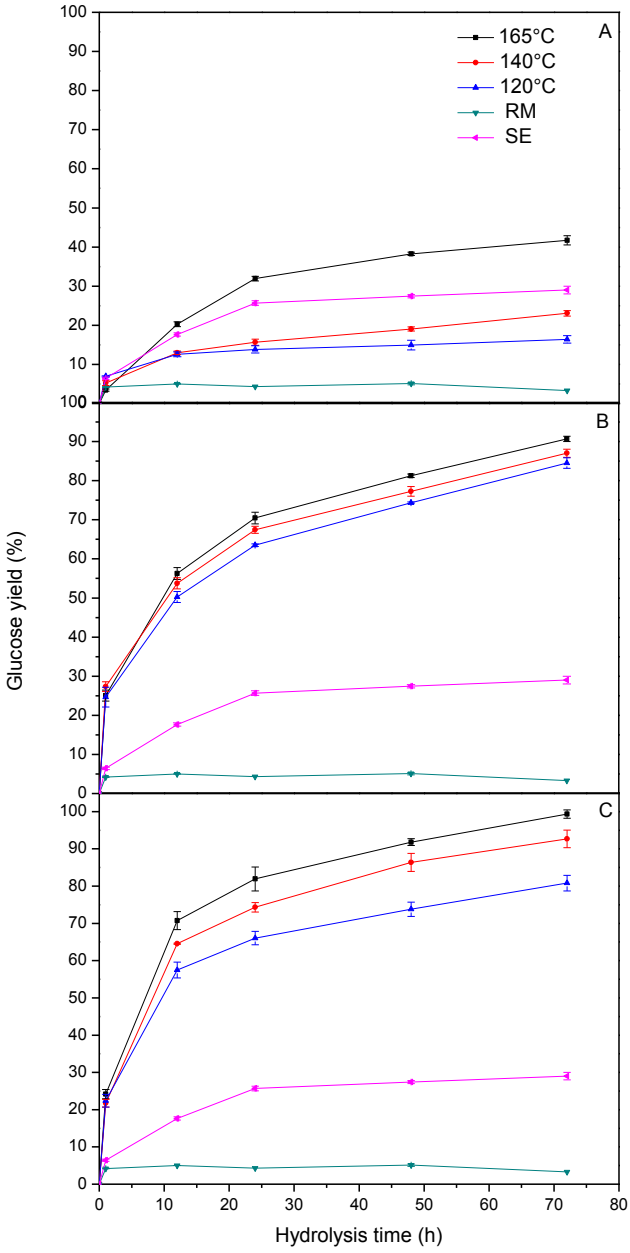

Fig. 3. Comparison of enzymatic hydrolysis profiles of the AS (A), AL (B), and ALS (C) pretreated bamboo

Figure 3 summarizes the enzymatic hydrolysis of bamboo using the three pretreatments. The glucose yield only reached 4.82% for the original bamboo after 72 h. The removal of lignin during the AS pretreatment was more effective at 165 °C for most grasses; the elevated temperature of 165 °C was found to enhance the enzymatic saccharification process. However, the AS pretreatment was ineffective for the delignification of bamboo, which was attributed to the structure of bamboo. Strangely enough, no obvious improvements were found in the enzymatic hydrolysis of SE-AS-treated bamboo compared to only SE-treated bamboo. Possible reasons for this could be that the lignin formed by condensation acted as a barrier, which led to the non-productive binding of cellulase to the substrate. Lower pH value of the acid sulfite liquors promoted condensation reaction (Li *et al*. 2014). The total glucose recovery of the samples from each pretreatment and enzymatic hydrolysis after 72 h is presented in Table 3.

Table 3. Total Glucose Recovery of the Bamboo after Enzymatic Hydrolysis

Pretreatment	120 °C (%)	140 °C (%)	165 °C (%)
AS	15.34±0.48	21.13±0.43	38.00±0.41
AL	75.96±0.69	60.71±0.53	62.74±0.47
ALS	78.74±0.64	84.93±0.76	90.42±0.54

In this work lignin removal was clearly found to be a positive factor for improving the enzymatic digestibility of bamboo, when treated with AL. Enzymes can saccharify cellulose to produce glucose (90.7%), when pretreated at a temperature of 165 °C (Fig. 3b). The total sugar recovery was significantly elevated, compared to the AS pretreatment. However, this value of recovery was unsatisfactory because the sugars were degraded by AL pretreatment. Among the temperatures tested, it was found that 120 °C was the most beneficial because a large quantity of sugars remained in the pretreated solids, compared to 140 °C and 165 °C.

Figure 3c shows the effect of 5.0% alkaline sulfite at different temperatures on the production of glucose. The addition of alkaline sulfite increased the sugars production, and the maximum cellulose-to-glucose conversion yield was 99.35% after 72 h at 165 °C. Due to the high amount of cellulose and hemicellulose that remained in the pretreated solids and the high cellulose-to-glucose conversion yield, the total glucose recovery reached 90.42%. Therefore, the ALS pretreatment was the most effective pretreatment method when combined with steam explosion for enzyme saccharification of bamboo.

In addition, the content of xylan changed from 17.88% to 11.18% after the SE pretreatment. However, during the chemical pretreatments, it was apparent that the ALS pretreatment retained a high yield of xylan. The total xylose recovery from the ALS pretreated samples was the highest, compared with the other two pretreatments (data not shown).

CONCLUSIONS

1. The maximum lignin removal from bamboo reached 93.1% when using the steam explosion - alkaline sulfite (SE-ALS) treatment; however, the lignin removal for the corresponding acid sulfite treatment (SE-AS-treated bamboo) was only 38.55%. Similar results in lignin removal were found in the SE-ALS and SE-AL pretreatments

(93.1 and 92.4%, respectively), however using the SE-AL pretreatment method resulted in 40.48% of the carbohydrates degraded, even with the addition of anthraquinone (AQ).

2. The maximum glucose yield reached 99.35% from enzymatic hydrolysis of bamboo when the SE-ALS pretreatment method was used. Meanwhile, the total glucose recovery obtained was 90.42%, with the culminating effects of high lignin removal and high reserved carbohydrates.

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REFERENCES CITED

Boussaid, A., Esteghlalian, A. R., Gregg, D. J., Lee, H. L., and Saddler, J. N. (2000). "Steam pretreatment of Douglas-fir wood chips," in: *Proceedings from the Twenty-First Symposium on Biotechnology for Fuels and Chemicals*, Fort Collins, CO, USA, pp. 693-705. DOI: 10.1007/978-1-4612-1392-5_53

Chen, H. Z., and Liu, L. Y. (2007). "Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction," *Bioresour. Technol.* 98(13), 666-676. DOI: 10.1016/j.biortech.2006.02.029

Chen, Y., Sharma-Shivappa, R. R., Keshwani, D., and Chen, C. (2007). "Potential of agricultural residues and hay for bioethanol production," *Appl. Biochem. & Biotechnol.* 142(3), 276-290. DOI: 10.1007/s12010-007-0026-3

Galbe, M., and Zacchi, G. (2012). "Pretreatment: the key to efficient utilization of lignocellulosic materials," *Biomass & Bioenergy* 46, 70-78. DOI: 10.1016/j.biombioe.2012.03.026

Hendriks, A. T. W. M., and Zeeman, G. (2009). "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresour. Technol.* 100(1), 10-18. DOI: 10.1016/j.biortech.2008.05.027

Hsu, T. A. (1997). "Pretreatment of biomass," *Fuel and Energy Abstracts* 38(2), 103-103. DOI: 10.1016/S0734-9750(97)00065-7

Krzesińska, M., Zachariasz, J., and Lachowski, A. I. (2009). "Development of monolithic eco-composites from carbonized blocks of solid iron bamboo (*Dendrocalamus strictus*) by impregnation with furfuryl alcohol," *Bioresour. Technol.* 100(3), 1274-1278. DOI: 10.1016/j.biortech.2008.08.039

Li, Z. Q., Jiang, Z. H., Fei, B. H., Cai, Z. Y., and Pan, X. J. (2014). "Comparison of bamboo green, timber and yellow in sulfite, sulfuric acid and sodium hydroxide pretreatments for enzymatic saccharification," *Bioresour. Technol.* 151 (2014), 91-99. DOI: 10.1016/j.biortech.2013.10.060

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

Oliveira, F., Pinheiro, I. O., Souto-Maior, A. M., Martin, C., Goncalves, A. R., and Rocha, G. J. (2012). "Industrial-scale steam explosion pretreatment of sugarcane straw for enzymatic hydrolysis of cellulose for production of second generation ethanol and value-added products," *Bioresour. Technol.* 130, 168-173. DOI: 10.10016/j.biortech.2012.12.030

Scurlocka, J. M. O., Daytonb, D. C., Hamesb, B. (2000). "Bamboo: An overlooked biomass resource," *Biomass & Bioenergy* 19(4), 229-244. DOI: 10.1016/S0961-9534(00)00038-6

Shuai, L., Yang, Q., Zhu, J. Y., Lu, F. C., Weimer, P. J., Ralph, J., and Pan, X. J. (2010). "Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production," *Bioresour. Technol.* 101(9), 3106-3114. DOI: 10.1016/j.biortech.2009.12.044

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2004). "Determination of structural carbohydrates and lignin in biomass," LAP-002 NREL Analytical Procedure, National Renewable Energy Laboratory.

Yamashita, Y., Shono, M., Sasaki, C., and Nakamura, Y. (2010). "Alkaline peroxide pretreatment for efficient enzymatic saccharification of bamboo," *Carbohydr. Polym.* 79(4), 914-920. DOI: 10.1016/j.carbpol.2009.10.017

Xing, Y., Ji, L., Liu, Z. P., Zhang, W. M., Jiang, J. X. (2015a). "Effects of *Gleditsia saponin* on high-solids enzymatic hydrolysis of furfural residues," *Industr. Crops & Prod.* 64, 209-214. DOI: 10.1016/j.indcrop.2014.09.055

Xing, Y., Bu, L., Sun, D., Liu, Z., Liu, S., and Jiang, J. X. (2015b). "High glucose recovery from direct enzymatic hydrolysis of bisulfite-pretreatment on non-detoxified furfural residues," *Bioresour. Technol.* 193, 401-407. DOI: 10.1016/j.biortech.2015.06.118

Xing, Y., Yu, H. L., Zhu, L. W., and Jiang, J. X. (2013). "Efficient enzymatic hydrolysis of bamboo by pretreatment with steam explosion and alkaline peroxide," *BioResources* 8(4), 5392-5408. DOI: 10.15376/biores.8.4.5392-5408

Zhang, X., Yu, H., Huang, H., and Liu, Y. (2007). "Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms," *Internat. Biodeter. & Biodegrad. J.* 60(3), 159-164. DOI: 10.1016/j.ibiod. 2007.02.003

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