Dilute Acid Pretreatment of Bamboo for Fermentable Sugar Production

W. Leenakul and N. Tippayawong*

Department of Mechanical Engineering, Faculty of Engineering, Chiang Mai University, Chiang Mai, 50200, Thailand
*Corresponding author. Tel.: +66 5394 4146, Fax: +66 5394 4145, E-mail: n.tippayawong@yahoo.com

Abstract: Biochemical conversion of lignocellulosic biomass to ethanol provides a sustainable energy production system. Bamboo is a fast growing woody grass that has great potential to be used as a domestic feedstock for fuel ethanol production. It consists of about 40% cellulose, and 27% hemicelluloses. In this investigation, bamboo (*Dendrocalamus asper*) was pretreated with dilute sulfuric acid prior to enzymatic hydrolysis process to produce fermentable sugars. The amount of dry feedstock solid/liquid loading at 10% w/w was pretreated in an autoclave at different temperatures (120, 140°C) with different residence times (30, 60, 90 min) and different sulfuric acid concentrations (0.6, 0.9, 1.2% w/w). Maximum glucose and xylose yields were achieved at 140°C, 1.2% sulfuric acid concentration and 90 min. After enzymatic saccharification with cellulase and β-glucosidase at the same pretreatment conditions, the yields of total reducing sugars were found to be low (56 mg/g). On the other hand, the maximum yields of total reducing sugar (85 mg/g) were obtained for the pretreatment conditions at 120°C, 1.2% sulfuric acid concentration and 60 min. Within these conditions, increasing temperature, residence time and acid concentration led to higher total sugar yields and cellulose conversion rates.

Keywords: Bamboo; Hydrolysis; Lignocellulosic materials; Pretreatment; Reducing sugars

1. Introduction

Future contributions of renewable energy are vital as non-renewable energy becomes more scarce and expensive. The use of diverse biomass resources is projected to contribute to a major fraction of future energy demands. Biomass is one of the most important raw materials in bio-ethanol production [1]. Normally, plants containing sugar or starch can be easily converted into sugars and fermented into ethanol. Examples of the two main materials include sugarcane, rice, cassava, and corn. Nonetheless, competition between biomass supplies for fuel and for food applications has intensified in recent years. This concern has led to growing interests in alternative, non-edible biomass resources. Lignocellulosic biomass, such as wood, straw and grasses, are viewed as important sources [2].

The cellulose and hemicellulose content of biomass material can be hydrolyzed chemically or enzymatically. Processing lignocellulosic into bioethanol consists of four steps: pretreatment, hydrolysis, fermentation and distillation [2]. Pretreatment process is the most important step in cellulose-to-ethanol technology, because it can remove hemicelluloses, reduce cellulose crystallinity and increase the porosity of materials. Pretreatments improves the digestibility of the lignocellulosic materials [3]. By opening up the biomass to enzymes that breakdown the hemicellulose and cellulose of the material into sugars, which are then fermented into ethanol for recovery. It is crucial to improve the release of sugars from both hemicelluloses and cellulose fractions while avoiding both the carbohydrate degradation and product that may inhibit hydrolysis and fermentation. Among all the pretreatment methods, dilute acid pretreatment is one of the most widely adopted methods [4]. Significant efforts have been made to treat various agricultural wastes [5-8].

Thailand is abundant in lignocellulosic materials which are largely untapped. One noticeable biomass is bamboo. Bamboo is the common term applied to a broad group of woody grasses (family Poaceae, subfamily Bambusoideae) ranging from 100 mm to 40 m in height. It encompasses 1250 species within 75 genera. It is distributed mostly in the tropics, comprising natural stands of native species. Bamboo has been used for handicrafts, paper-making and construction materials as well as cultivated for edible shoots. Although bamboo is recognized as a useful resource, its utilisation and further development may be required. It may have potential as a bioenergy and bioproduct crop [9] because it has high cellulose content and is available in great amounts. There have been reports on improved processes that convert bamboo for bioethanol [10-13]. This study focuses on *Dendrocalamus asper*, known as pai tong kheaw in Thai, which is one of the most widely available bamboos in Thailand. It is a species well adapted for local conditions. In 1994, the cultivation of this species was extended to 67 provinces with a total planting area of more than 67,000 ha. Yields of *D. asper* were reported to be over 1.2 and 0.3 million ton for stem and shoot productions, respectively. Exports of bamboo shoot products, mainly of *D. asper*, amounted to more than one thousand million baths annually [14]. The objective of this study was to characterize the effects of dilute acid pretreatment on *D. asper* as a biofuel source. The effects of hydrolysis parameters on the production of monomeric sugars by dilute acid hydrolysis of bamboo was investigated. The variables considered were temperature, residence time and acid concentration. The enzymatic digestibility of the pretreated bamboo after dilute acid pretreatment will be discussed.

2. Experimental

2.1 Raw material

Bamboo (*D. asper*) was collected from a local bamboo furniture factory. The raw material was cut, milled and sieved to particle sizes below 300 μm. It was subsequently air dried to low moisture content (< 5%). All of the materials were stored in plastic bags at room temperature until being further processed to ethanol, as shown in Fig. 1.

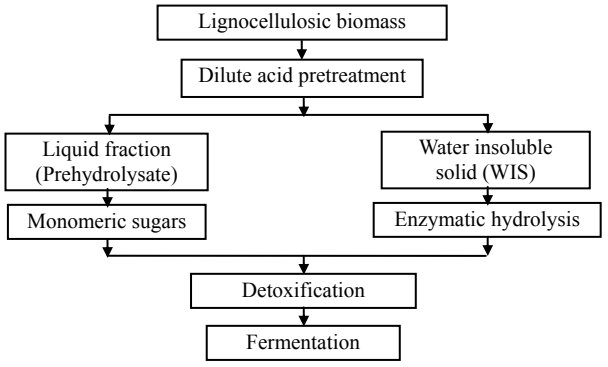

Figure 1. Conversion process of lignocellulosic material to bioethanol.

2.2 Dilute acid pretreatment

A biomass sample of 5 g on a dry basis was used for each pretreatment experiment. In this study, the amount of dry

feedstock solid/liquid loading of 10% w/w was pretreated in an autoclave at a pressure of 1.1 bar, at different temperatures (120, 140°C) with different residence times (30, 60, 90 min) and at different sulfuric acid concentrations (0.6, 0.9, 1.2% w/w). Once the selected temperature and time were reached, allowing a few minutes for the temperature to drop below 40°C, solid and liquid fractions were separated by filtration. The water insoluble solid (WIS) or the pretreated solid residues were washed with deionised water until the pH of the washed water was greater than 5.0. These residues were then used as a substrate in enzymatic hydrolysis process. A portion of washed pretreated solid was stored at -20°C until used. The liquid fraction from pretreatment (prehydrolysate) was analyzed for the monomeric sugar contents by high performance liquid chromatography (HPLC).

2.3 Enzymatic hydrolysis

The washed WIS after dilute acid pretreatment contained a solid loading of about 5%. Enzymatic hydrolysis was carried out in 125-mL Erlenmeyer flasks. Two enzymes used were cellulase (Novo Celluclast 1.5 L) and β-glucosidase (Novozyme188). The β-glucosidase was used to supplement the insufficient β-glucosidase activity in the cellulose. The activity of cellulase and β-glucosidase were 70 FPU/mL and 250 CBU/mL, respectively. Cellulase enzyme loading was 15 FPU/g substrate and β-glucosidase loading was 15 IU/g substrate. Enzymatic hydrolysis was performed in 0.05 M sodium citrate buffer at pH 4.8 at 50°C on a rotary shaker for 72 hr with agitation speed at 140 rpm. Samples were taken at 72 hr for analysis of glucose and total reducing sugars after enzymatic hydrolysis.

2.4 Analytical methods

Wet chemical analysis was used to determine the composition of bamboo samples by the following ASTM standard methods: E1757 and E1758 [9]. The sugar content (arabinose, galactose, glucose and xylose) of the liquid fraction (filtrate) after pretreatment was determined by HPLC in a Waters 2695 liquid chromatograph with refractive index detector. An Aminex HPX-87P carbohydrate analysis column operating at 85°C with ultrapure water as a mobile phase was used. Activities of cellulase and β-glucosidase were measured as FPU and IU, respectively [15]. All analytical determinations were performed in triplicate and average results are shown.

3. Results and Discussion

3.1 Raw material composition

Table 1 shows the composition of the raw materials. The chemical analysis showed that glucan was the dominant component in bamboo, followed by lignin and xylan. Cellulose (glucan) and hemicelluloses (xylan, mannan, arabinan, galactan) accounted for more than 65% of the dry weight, making bamboo a good substrate for ethanol production. This is in similar magnitude to holocellulose, the sum of cellulose and hemicelluloses, as reported by Kamthai [16] for *D. asper*. Cellulose and lignin contents were found to be in the high end of the range reported for grasses [2]. Xylose was the main sugar (89%) for the hemicellulose fraction.

Table 1. Wet chemical analysis of bamboo.

Composition	% wt, dry basis
Cellulose as:	40.7
Glucan	40.7
Hemicelluloses as:	26.5
Xylan	23.6
Mannan	0.6
Arabinan	1.1
Galactan	1.2
Ash	1.2
Lignin	27.1

3.2 Prehydrolysate composition

Table 2 shows the monomeric sugars in the filtrate as per 100 g material after acid pretreatment at different conditions. As far as the substrate material solubilisation is concerned, xylose was found to increase monotonically with increases in sulfuric acid concentration, pretreatment temperature and residence time. The maximum xylose yield in prehydrolysate was 14.92 g/100 g when pretreated at strong pretreatment conditions (140°C, 1.2% sulfuric acid concentration and residence time of 90 min). The minimum xylose yield occurred at mild pretreatment conditions (120°C, 0.6% acid concentration, 30 min). Xylose solubilisation was significantly influenced by acid concentration, pretreatment temperature and residence time. The hemicellose hydrolysate contained monomeric sugars such as arabinose, galactose, mannose and xylose. Xylose is by far the major carbohydrate component. From Table 2, it was observed that at mild pretreatment conditions (30 min, 120°C and 0.6% acid concentration), the amount of xylose was rather small, but at 60 min residence time, the xylose was found to increase. It was clear that mild pretreatment was not sufficient for the solubilisation of the hemicelluloses. Similar results were reported in the literature, such as, Ballesteros et al. [17] who used cardoon as a substrate and found that xylose yield in the liquid fraction was 0.9 g/100 g (about 6% of xylan) when pretreated at 160°C without acid addition, and increased to 13.5 g/100 g (92% of xylan) when pretreated with 0.1-0.2% sulfuric acid concentration at 180°C. Sun and Cheng [18] summarised dilute acid pretreatment of rye straw at strong condition (1.5% sulfuric acid for 90 min at 121°C), showing xylose yield in liquid fraction of 146 mg/g biomass (66% of xylan). However, Torget et al. [19] used sulfuric acid pretreatment of short rotation hardwoods and herbaceous crops, and found that about 92% of the xylan was removed when switch grass and weeping love grass were pretreated by 0.5% sulfuric acid at 140°C for 60 min or 160°C for 10 min. A maximum yield of 80.8% in the depolymerisation of xylan to xylose was obtained in rice straw at a pressure of 15 bar (temperature of about 201°C), 0.5% acid concentration and 10 min retention time. It was not conclusive that the higher severity of the treated condition would result in an increase in xylan removal.

From the dilute acid pretreatment, it was found that the important monomeric sugar to convert to ethanol is glucose, with recovery ranging from 0.75-3.50 g/100 g. Glucose is the monomeric sugar from glucan, which is the major component in cellulose. Glucan was solubilised to glucose at the most severe conditions considered in this study (140°C, 1.2% acid concentration, 90 min residence time), reaching 3.50 g/100 g. However, this was very low, compared to the xylose yield obtained at the same conditions. The yield of glucose decreased with the decreasing severity of pretreatment conditions. It was clearly demonstrated that glucan in cellulose was not significantly affected by dilute acid pretreatment. This finding was in line with that reported in the literature [2,18]. It was pointed out [17] that this solubilised glucose was not degraded but recovered in the prehydrolysate. The major portion of glucan content in the raw material was recovered in the solid residue.

3.3 Enzymatic hydrolysis

Fig. 2 shows total reducing sugars from enzymatic hydrolysis when enzymatic cellulose complex (Celluclast 1.5 L) supplemented with β-glucosidase (Novozym 188) was used. Total sugars from enzymatic hydrolysis rose from 49.2 mg/g at mild pretreatment conditions (120°C, 30 min, 0.6% acid concentration) to 83.9 mg/g at 120°C, 60 min, 1.2% acid concentration. Within these conditions, the mild pretreatment condition offered lower total sugars than the more severe pretreatment conditions did. However, at 120°C and 140°C, the amount of total sugar yields was different. At 120°C, total sugar yields increased for

high pretreatment time and acid concentrations, but at 140°C, when the pretreatment condition became more severe, total sugar yields decreased. The improvements in enzymatic hydrolysis yields were concomitant with the pretreatment temperature, time or acid concentration may be attributed, among other reasons, to the solubilisation of the hemicellulose fraction because enzyme accessibility to cellulose was favoured [20]. According to Table 2, xylose increased with the pretreatment severity. Similar indications seemed to apply to the total reducing sugars in enzymatic hydrolysis. Xylan solubilisation was the main mode of action occurring during sulfuric acid pretreatment, which can be directly related to improvements in enzymatic hydrolysis. In fact, the yield of enzymatic digestion of cellulose in pretreated biomass increased with xylan removal [20]. However, Cara et al. [21] stated that the acid pretreatment of olive tree shows that there is strictly no direct relation between pretreatment hemi-celluloses solubility and enzymatic hydrolysis yield. Laureano-Perez et al. [22] suggested that not only hemi-celluloses solubilisation, but some other factors such as cellulose crystallinity, surface area accessibility or lignin content may also affect enzymatic hydrolysis. Total sugar yields from various lignocellulosic biomass sources are summarized in Table 3. The yields refer to the total amount of sugars available after pretreatment and enzymatic hydrolysis from 100 g of raw material. The data showed that total sugar yield from bamboo was rather smaller than other biomass sources. This may be attributed to some sugar degradation due to acid action. Further improvements in total reducing sugar yield may be necessary to increase the final ethanol yield of the process.

4. Conclusion

The potential use of bamboo as fuel source for bioethanol was investigated with the generation of fermentable sugars from bamboo as the main focus of this study. The chemical characterization of the plant was conducted and its conversion of bamboo by dilute acid pretreatment to produce sugars was analysed to show that bamboo was rich in cellulose and hemi-cellulose fractions which were desirable fuel characteristics for ethanol production.

It was found that soft pretreatment conditions led to a sugar-rich prehydrolysate. The dilute acid pretreatment of bamboo produced digestible residues and solubilised significant amounts of the hemicellulose fraction. The maximum xylose yields could be obtained at severe pretreatment condition where relatively little amounts of glucan were affected. Mild pretreatment condition at 30 min retention time, 0.6% sulfuric acid concen-tration and 120°C did not provide good solubilisation of hemicelluloses. High enzymatic hydrolysis yield of 85 mg/g was achieved with increases in pretreatment time and sulfuric acid concentration, while pretreatment temperature was not found to play an important role. Total sugar yields were relatively lower than other mainstream biomass feedstocks. Further improvements of increased total reducing sugar yields may be necessary to increase final ethanol yields of the process.

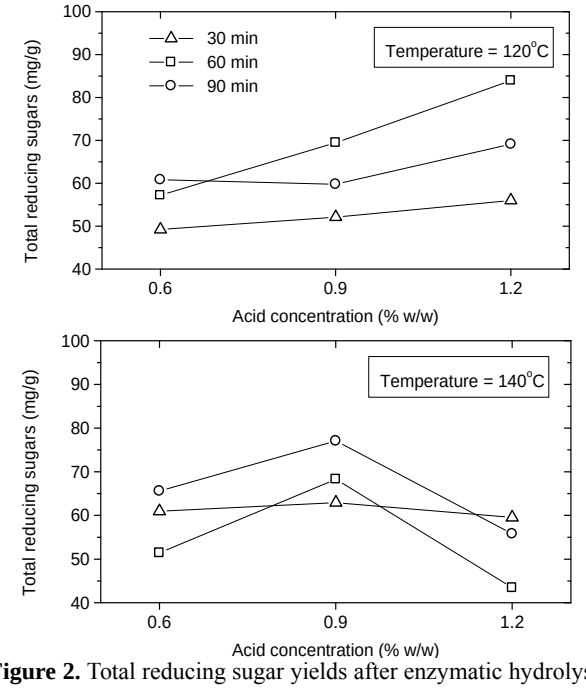

Figure 2. Total reducing sugar yields after enzymatic hydrolysis at different conditions.

Table 2. Sugars in pr5ehydrolysate from dilute acid pretreatment of bamboo at different conditions.

Temperature (°C)	Residence time (s)	Acid concentration (%w/w)	Glucose (g/100g)	Xylose (g/100g)	Other sugars* (g/100g)	pH
120	30	0.6	0.75	3.10	nd	1.18
		0.9	0.82	6.65	nd	0.98
		1.2	0.87	6.16	nd	0.70
	60	0.6	0.91	4.00	0.28	1.24
		0.9	1.65	8.50	0.38	1.01
		1.2	1.14	9.97	nd	0.89
	90	0.6	0.82	9.56	nd	1.04
		0.9	1.30	10.91	nd	0.96
		1.2	1.61	12.46	0.10	0.69
140	30	0.6	1.09	9.49	nd	1.01
		0.9	1.24	11.48	nd	0.80
		1.2	1.56	13.31	nd	0.72
	60	0.6	1.06	10.83	nd	1.12
		0.9	1.32	12.24	nd	0.89
		1.2	2.13	11.72	nd	0.58
	90	0.6	1.43	12.60	nd	1.06
		0.9	2.60	14.85	nd	0.72
		1.2	3.50	14.92	nd	0.60

*galactose, arabinose and mannose, nd: not detected

Table 3. Sugar yields from enzymatic hydrolysis of dilute acid pretreated lignocellulosic biomass.

Lignocellulosic biomass	Pretreatment conditions			Sugar yield (g/100g)	References
	Temperature (°C)	Acid concentration (% w/w)	Residence time (min)		
Bamboo	120 - 140	0.6 -1.2	30 - 90	4.3 - 8.5	This study
Rye straw	121	0.6 - 1.5	30 - 90	12.5 - 19.7	[18]
Bermuda grass	121	0.6 - 1.5	30 - 90	19.5 - 22.9	[18]
Olive tree	170 - 210	0.2 - 1.4	10	13.6 - 24.5	[21]
Saline biomass	165	1.4	8	28 - 33	[23]

Acknowledgments

This work was supported by the Faculty of Engineering, Chiang Mai University. The authors wish to thank The Departments of Biology, and Agriculture, Chiang Mai University, for technical assistance with analytical facilities.

References

[1] Balat M, Balat H, Oz C, Progress in bioethanol processing, *Progress in Energy and Combustion Science* 34 (2008) 551-573.

[2] Sun Y, Cheng J, Hydrolysis of lignocellulosic materials for ethanol production: a review, *Bioresource Technology* 83 (2002) 1-11.

[3] Hendriks ATWM, Zeeman G, Pretreatments to enhance the digestibility of lignocellulosic biomass, *Bioresource Technology* 100 (2009) 10-18.

[4] Moisier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M, Features of promising technologies for pretreatment of lignocellulosic biomass, *Bioresource Technology* 96 (2005) 673-686.

[5] Campo ID, Alegrıa MZ, Echeverrıa M, Echeverrıa I, Diluted acid hydrolysis pretreatment of agri-food wastes for bioethanol production, *Industrial Crops and Product* 24 (2006) 214–221.

[6] Karimi K, Kheradmandinia S, Taherzadeh MJ Conversion of rice straw to sugars by dilute-acid hydrolysis, *Biomass and Bioenergy* 30 (2006) 247–253.

[7] Saha BC, Iten LB, Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol, *Process Biochemistry* 40 (2005) 3693-3700.

[8] Gaspar M, Kalman G, Corn fiber as a raw material for hemicelluloses and ethanol production, *Process Biochemistry* 42 (2007) 1135-1139.

[9] Scurlock JMO, Dayton DC, Hames B, Bamboo: an overlooked biomass resource?, *Biomass and Bioenergy* 19 (2000) 229-244.

[10] Asada C, Nakamura Y, Kobayashi F, Waste reduction system for production of useful materials from un-utilized bamboo using steam explosion followed by various conversion methods, *Biochemical Engineering Journal* 23 (2005) 131-137.

[11] Zhang XY, Xu CY, Wang HX, Pretreatment of bamboo residues with *Coriolus versicolor* for enzymatic hydrolysis, *Journal of Bioscience and Bioengineering* 104 (2007) 149-151.

[12] Shimokawa T, Ishida M, Yoshida S, Nojiri M, Effect of growth stage on enzymatic saccharification and simultaneous saccharification and fermentation of bamboo shoots for bioethanol production, *Bioresource Technology* 100 (2009) 6651-6654.

[13] Sathitsuksanoh N, Zhu Z, Ho TJ, Bai MD, Zhang YHP, Bamboo saccharification through cellulose solvent-based biomass pretreatment followed by enzymatic hydrolysis at ultra-low cellulose loadings, *Bioresource Technology* (2009) doi:10.1016/j.biortech.2009.09.081

[14] Pungbun Na Ayudhya P, Bamboo resources and utilization in Thailand, in Puangchit L, Thaiutsa B, Thamincha S, eds. *Bamboo 2000 International Symposium* (2000) 6–12, Chiang Mai, Thailand.

[15] Ghose T, Measurement of cellulose activities, *Pure and Applied Chemistry* 59 (1987) 257-268.

[16] Kamthai S, Preliminary study of anthraquinone in sweet bamboo (*Dendrocalamus asper* Backer) alkaline sulfite pulping, *Chiang Mai Journal of Science* 34 (2007) 235-247.

[17] Ballesteros I, Ballesteros M, Manzanares P, Jose Negro M, Dilute acid pretreatment of cardoon for ethanol production, *Biochemical Engineering Journal* 42 (2008) 84-91.

[18] Sun Y, Cheng J, Dilute acid pretreatment of rye straw and bermuda grass for ethanol production, *Bioresource Technology* 96 (2005) 1599-1606.

[19] Torget R, Werdene P, Himmel M, Grohmann K, Dilute sulfuric acid pretreatment of short rotation woody and herbaceous crops, *Applied Biochemistry and Biotechnology* 24-25 (1990) 115-126.

[20] Kabel MA, Bos G, Zeevalking J, Voragen AGJ, Schols HA, Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw, *Bioresource Technology* 98 (2007) 2034–2042.

[21] Cara C, Ruiz E, Oliva JM, Saez F, Castro E, Conversion of olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification, *Bioresource Technology* 99 (2008) 1869–1876.

[22] Laureano-Perez L, Teymouri F, Alizadeh H, Dale BE, Understanding factors that limit enzymatic hydrolysis of biomass, *Applied Biochemistry and Biotechnology* 121-124 (2005) 1081-1099.

[23] Zhang Y, Pan Z, Dilute acid pretreatment and enzymatic hydrolysis of saline biomass for sugar production, American Society of Agricultural and Biological Engineers (2006) ASABE paper number 067003.