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Conversion of Bamboo to Sugars by Dilute Acid and Enzymatic Hydrolysis

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Abstract- Lignocellulosic biomass is an important alternative energy source to be utilized for ethanol production. In this work, bamboo (*Dendrocalamus asper Backer*) was used as biomass feedstock for conversion to fermentable sugars. Pretreatment was carried out with dilute sulfuric acid at concentrations between 0.4 – 1.6% w/w, and residence time between 45 – 135 min at a fixed temperature of 140^{o}C. Prehydrolyzate was later analyzed for total sugars by high performance liquid chromatography. For the conditions considered, maximum glucose and xylose yields after chemical treatment were obtained from bamboo to be 25.8 and 16.9 mg/mL, respectively. Water insoluble solids obtained were subsequently hydrolysed with cellulase *(Trichoderma reesei)* and β-glucosidase (Novozyme 188) for 72 h. It was found that increasing yields were obtained with increasing acid concentrations and residence times. The effects of pretreatment severity and enzymatic hydrolysis greatly increased the sugar concentrations after the hydrolysis.

Keywords- Biomass; lignocellulosic; pretreatment; glucose; ethanol; renewable energy.

1. Introduction

Woody biomass is sustainably available in large quantities in various regions of the World. About 370 million dry tons of woody biomass accounting for 30% of the total biomass is projected to be available [1]. Bamboo is a potential biomass source that is widely distributed in Thailand and Asia. There are more than 1000 types of bamboo in record spreading over 180,000 km2 [2]. Bamboo is of economic and high cultural significance in Asia because it is both lightweight and exceptionally durable. The treated bamboos are used extensively as building materials for houses, construction scaffolding, flooring, bridges, etc. Also, they are extensively used to make furnitures, chopsticks, food steamers, paper pulps. Bamboo is grown as ornamental plants, has high density and can be harvested year round, which eliminates long-term storage. Although bamboo is recognized as a useful resource, its present utilization is rather limited. Further development may be required, especially in bioenergy application [3].

Ethanol is a renewable fuel that can be used as partial gasoline replacement. Demand for ethanol will increase with reduction in petroluem production. Production of fuel ethanol from lignocellulose materials (Fig. 1) such as bamboo is advantageous because of local availability. To take this advantage, it is important to convert the materials into fermentable sugars as much as possible. Pretreatment is a key step in bioconversion process [4]. Chemically, woody biomass has higher lignin content and recalcitrant to microbial than bamboo. Additionally, more energy is required to overcome the recalcitrance of woody biomass through pretreatment for enzymatic saccharification. Bamboo appears to have potential as a lignocellulosic source for ethanol production.

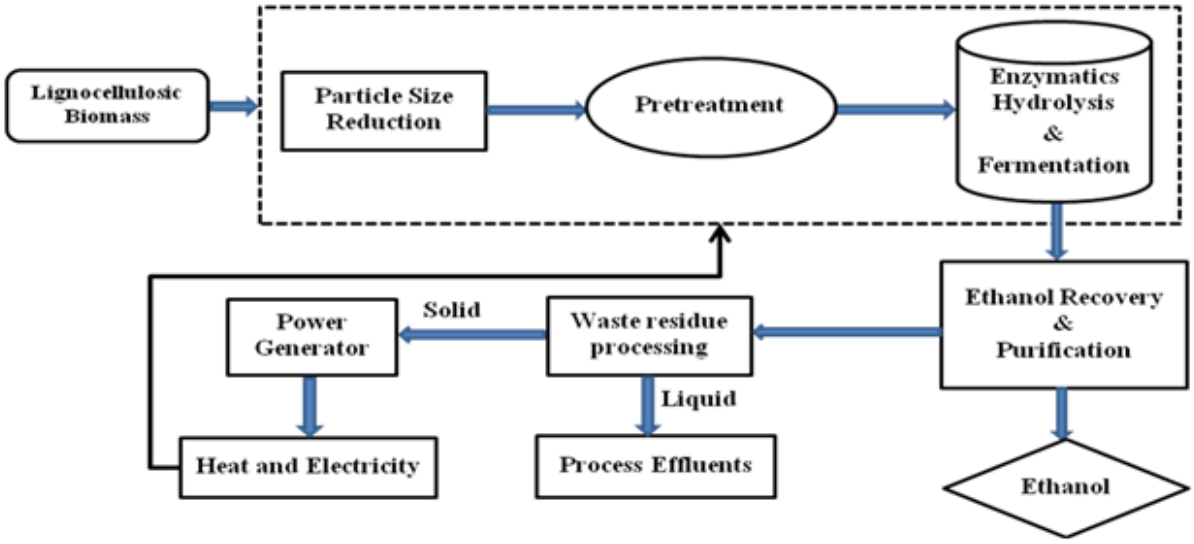

Fig. 1. Process flow of biological conversion of lignocellulosic biomass into ethanol

Specifically, chemical pretreatment is required prior to size reduction and enzymatic cellulose saccharification. Chemical pretreatment can alter the chemical composition and physical structure of lignocellulosic materials by partially removing some cell-wall components such as hemicellulose and lignin. Dilute acid pretreatment has been among the most widely adopted methods becasue it is effective and inexpensive, and it can increase the enzymatic saccharification rates of bamboo, and other lignocellulosic materials [5-9]. Limited studies were carried out to specifically investigate production of fermentable sugars [10, 11].

Although different lignocellulosic materials has been investigated to produce fermentable sugars for ethanol production, report on acid pretreatment and enzymatic hydrolysis of bamboo is still rare. This work may provide useful information on utilization of bamboo for bioenergy application. The objective of this work was to evaluate the effects of dilute acid pretreatment and enzymatic hydrolysis of bamboo on glucose and xylose yields, taking into account variation in acid concentration and residience time.

2. Methodology

2.1. Raw Materials

A full grown bamboo (Dendrocalamus asper Backer) was used as raw material. The sample was air-dried and cut to sizes of about 10 x 50 mm. It was then milled and the fraction containing particles with a size less than 0.25 mm were selected to provide a fine size class and stored in plastic bags at room temperature until further processed. The structural carbohydrate composition of the completely dry biomass was determined using a modified quantitative saccharification. Monomeric sugars were measured by a high performance liquid chromatograph (HPLC) instrument with a Bio-Rad Aminex HPX-87P column. Lignin and ash were measured according to the US national renewable energy labolatory, similar to that in [6]. Table 1 shows the chemical composition of raw material. Bamboo was found to have high cellulose content of over 40%. Lignin and hemicellulose were in simialr range, about 27%. With regards to hemicellulose, xylan appeared to be the major component.

Table 1. Chemical composition of bamboo

Composition	Aged bamboo (% w/w, dry basis)
Cellulose	40.7
Glucan	40.7
Hemicellulose	26.5
Xylan	23.6
Mannan	0.6
Arabinan	1.1
Gallactan	1.2
Ash	1.2
Lignin	27.1

2.2. Dilute Acid Pretreatment

All materials were dried at 103 oC for 72 h to ensure a low moisture content prior to treatment. A biomass sample of 5 g on dry basis was used for each dilute acid treatment experiment. The pretreatment were carried out using 50 mL at acid concentration between 0.4 – 1.6% w/w. The samples were then held at a fixed 140oC for residence times between 45 – 135 min. After allowing the temperature to drop at 40oC, the treated samples were immediately quenched. The cooled samples were then quickly separated into solid and liquid fractions by filtration. The filtrate was sampled in order to determine the concentration of glucose and xylose in the hydrolysates. This was done by HPLC while the pretreated solid residues or water insoluble solid (WIS) fractions were taken to be used in hydrolysis process.

2.3. Enzymatic Hydrolysis

The enzymatic hydrolysis was performed in 100 mL Erlenmeyer flasks using 5% dry matter (w/w) and 50 M sodium citrate buffer (pH 4.8) at 2% (w/v) dry. Pretreatment substrate loading enzyme used was cellulase from Trichoderma reesei with an activity of 65 filter paper unites (FPU)/g, and β-glucosidase (Novozyme 188) activity of 590 IU/g. Enzyme loading was 15 FPU/g of dry pretreated substrate of cellulase and 15 IU/g of dry pretreated substrate of β-glucosidase. It was later analyzed for total sugars by the HPLC. Eq. (1) shows the disintegration process of the cellulose molecules by water and after a process of hydrolysis cellulose degraded into glucose.

$$(C_6H_{12}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6 \qquad (1)$$

2.4. Combined Severity Factor

The combined severity factor (CSF) was used to compare the efficiencies of hydrolysis time, temperature and acid concentration into a single variable [12]. CSF in this study is defined in Eq. (2) as

$$CSF = \log\left[t \cdot \exp\left(\frac{T_H - T_R}{14.75}\right)\right] - pH \qquad (2)$$

where t is hydrolysis time in min, TH is the hydrolysis temperature in oC, TR is the reference temperature (most often 100oC), and pH is the acidity of the aqueous solution in terms of acid concentration.

3. Results and Discussion

3.1. Prehydrolysate

Results of glucose and xylose yields from acid treatment are summarized in Tables 2 and 3. The effects of acid concentration/time combination at stable temperature of 140oC on bamboo was shown, represent glucose recovery and hemicellulose derived sugars recovery. The highest glucose yield of 25.8 mg/mL occurred at 120 min, acid concentration of 1.4% w/w where CSF was 1.7. At the same residence time, glucose yields were found to increase with increasing acid concentration.

Table 2. Total glucose yield for dilute acid pretreatment

Time (min)	Concentration (%w/w)	CSF	Glucose (mg/mL)	Other sugars* (mg/mL)
45	1.0	0.6	16.9	-
60	0.6	0.7	13.9	-
60	1.4	1.0	15.7	-
90	0.4	0.7	15.0	0.22
90	1.0	1.2	13.9	-
90	1.6	1.7	21.2	-
120	0.6	1.3	16.5	-
120	1.4	1.7	25.8	0.38
135	1.0	1.6	17.1	-

* galactose, arabinose and mannose

Table 3. Total xylose yield for dilute acid pretreatment

Time (min)	Concentration (%w/w)	CSF	Xylose (mg/mL)	Other sugars* (mg/mL)
45	1.0	0.6	13.2	-
60	0.6	0.7	11.8	-
60	1.4	1.0	15.4	-
90	0.4	0.7	10.6	0.22
90	1.0	1.2	13.4	-
90	1.6	1.7	16.9	-
120	0.6	1.3	12.5	-
120	1.4	1.7	16.1	0.38
135	1.0	1.6	15.9	-

* galactose, arabinose and mannose

An increase in severity factor did not always produce increasing glucose recovery. With respect to hemicellulose derived sugars, maximum xylose yield was found to be 16.9 mg/mL, occurring at 90 min, acid concentration of 1.6% w/w with CSF = 1.7. The highest combined glucose and xylose yields were measured at a residence time of 90 min and acid concentration of 1.6% w/w. It should be

noted that even though glucan is the most abundant component in bamboo, glucose recovery observed in the prehydrolysate was quite small, comparable to xylose recovery from hemicellulose fraction. This might be due to partial solubilization of the cellulose fraction from dilute acid pretreatment. The main issue in acid treatment was degradation of hemicellulose, while keeping cellulose in the WIS fraction for enzymatic hydrolysis.

3.2.Enzymatic Hydrolysis

To assess the effect of different pretreatment conditions tested on the digestibility of pretreated bamboo (WIS fraction), enzymatic hydrolysis were performed. The enzymatic hydrolysis yields were expressed as glucose and xylose released in 72 h from WIS fraction. Enzymatic hydrolysis yields are shown in Figs. 2 and 3 as a function of acid concentration and residence time, respectively. High glucose recovery was realized at this stage. However, it was surprising to find that increasing the pretreatment time or the acid concentration did not show positive effect on saccharification of pretreated bamboo cellulose fraction. Even at severe pretreatment conditions, there seemed to be a fraction of cellulose recalcitrant to enzymatic hydrolysis.

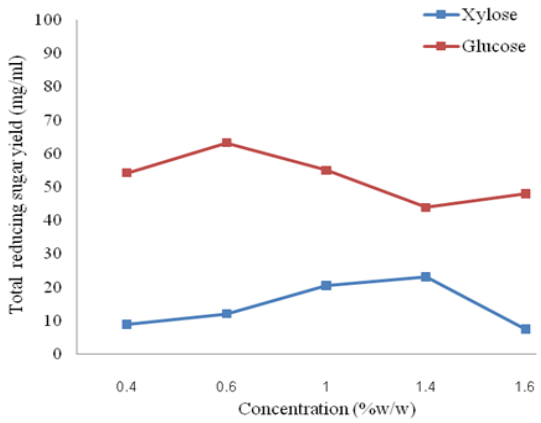

Fig. 2. Sugar yields after enzymatic hydrolysis as a function of acid concentration.

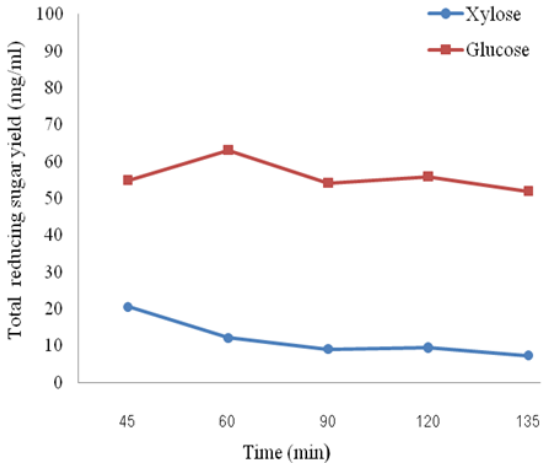

Fig. 3. Sugar yields after enzymatic hydrolysis as a function of residence time.

The total sugar yield was found to slightly increase with the increased degree of treatment severity. This was contributed by solubilization of hemicellulose fraction in the solid residue. Other factors like cellulose structure, surface area accessibility or lignin content may also affect enzymatic hydrolysis.

3.3. Comparison with Literature

Total sugar yields from various lignocelluloses biomass reported in the literature are shown in Table 4. The yields referred to the total amount of sugars available after pretreatment and enzymatic hydrolysis. It can be seen that total sugar yields from bamboo were relatively high, compared to other biomass sources for similar chemicals, temperatures, acid concentrations and residence times used. This was contributed mainly to the fact that more severe conditions were used.

Table 4. Comparison of total sugar recovery from dilute sulfuric acid pretreatment and enzymatic hydrolysis of various lignocellulosic biomass

Biomass	Concentration(%w/w)	Time(min)	Temperature(^{o}C)	Total sugar yield (g/100g)	Reference
Bamboo	0.4-1.6	45-135	140	30.6-46.8	This study
Bamboo	0.6-1.2	30-90	120-140	3.9-18.5	[2]
Rye straw	0.6-1.5	30-90	121	12.5-19.7	[9]
Bermuda grass	0.6-1.5	30-90	121	19.5-22.9	[9]
Olive tree	0.2-1.4	10	170-210	13.6-28.3	[11]
Eucalyptus	0.92-1.84	30	180	34.9	[13]
Pine tree	2.2	15-30	180	39.4	[14]
Corncob	0.5-2	100-140	100-120	29.8	[15]

4. Conclusion

Bamboo biomass is a very important feedstock for the future biofuels. It has composition that was rich in cellulose and hemicelluloses fractions. Conversion by dilute acid treatment and enzymatic hydrolysis to produce sugars was investigated. Acid catalyzed pretreatment of bamboo produced digestible solid residues and solubilised significant amount of the hemicelluloses fraction. Sugar rich prehydrolysate was also obtained. Maximum glucose and xylose yields were obtained at 120 min, 1.4% w/w and 90 min, 1.6% w/w, respectively. Enzymatic hydrolysis offered high yields in glucose recovery from solid fractions from high severity pretreatment conditions.

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