BIOCONVERSION OF BAMBOO TO BIOETHANOL USING THE TWO-STAGE ORGANOSOLV AND ALKALI PRETREATMENT

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Bamboo's ability to grow on nutrient-poor soils, with little requirement of silvicultural management, easy harvesting characteristics, vegetative propagation, fast growth, and a host of other desirable characteristics, make it a good candidate as an energy crop. Energy crops are cultivated solely for use as sources of energy through their conversion into alcohols. This study set out to determine the potential of moso bamboo to be used in the two-stage organosolv and alkali pretreatment for the production of bioethanol. Moso bamboo contains 63.3% (w/w) holocellulose and can serve as a low-cost feedstock for bioethanol production. After organosolv pretreatment (2% w/w H_2SO_4 in 75% w/w ethanol, 160 °C for 30 min), the bamboo was further delignified through pretreatment of sodium hydroxide (10% and 20% w/w) or calcium hydroxide (10% w/w), which resulted in about 96.5% (NaOH) and 85.7% ($Ca(OH)_2$) lignin removal. The enzymatic hydrolysis of delignified cellulosic bamboo substrate with cellulase (15 FPU/g glucan) and β-glucosidase (30 IU/g glucan) showed 80.9% to 95.5% saccharification after 48 h incubation at 50 °C and pH 4.8. Fermentation of enzymatic hydrolysates with *Saccharomyces cerevisiae* resulted in about 89.1% to 92.0% of the corresponding theoretical ethanol yield after 24 h.

Keywords: Bamboo; Bioethanol; Two-stage pretreatment; Enzymatic hydrolysis; Fermentation

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INTRODUCTION

Global warming and the energy crisis are of great concern to governments and people around the world. Greenhouse gases in the Earth's atmosphere, such as carbon dioxide, cause the biosphere to become warmer. Traditional fossil fuels will be used up in a few decades. Therefore, biofuel is an option for resolving both the energy crisis and global warming. Biofuels converted from biomass are renewable energy and mitigate global warming by recycling carbon dioxide from the atmosphere. Plants absorb carbon dioxide and release oxygen back into the atmosphere. Carbon becomes stored in a plant's cellulose, hemicellulose, starches, sugars, and oils. Then, plants are processed into biofuels used in transportation. Biofuels are combusted and converted back to carbon dioxide when people drive their cars, trucks, and other modes of transportation (Szulczyk 2010).

Bamboo is the key biomass material for the balance of oxygen and carbon dioxide in the atmosphere. Its carbon dioxide storage rate per unit area of planation is four times that of hardwood, and the release of oxygen is 35% higher than that of trees (Southern Metropolis Daily Mark 2012). Meanwhile, its mesh-like roots can prevent soil erosion. Bamboo's ability to grow on nutrient-poor soils, requiring little silvicultural management, with easy harvesting characteristics, vegetative propagation, fast growth, and a host of other desirable characteristics make it a good candidate as an energy crop (Dora 2008).

Energy crops are cultivated solely for use as sources of energy through their conversion into alcohols.

There are usually four steps involved in the conversion of plants, including bamboo, to bioethanol. These are pretreatment, enzymatic hydrolysis, fermentation, and ethanol purification. Organosolv pretreatment has been evaluated as an effective pretreatment method for high-lignin lignocellulosic biomass (Chum *et al.* 1990; Pan *et al.* 2006). It can break down internal lignin and hemicellulose bonds and thus remove almost all of the lignin from biomass (Holtzapple and Humphrey 1984). Through the removal of the lignin, pore-volume and surface area increased, causing an increase in substrate enzymatic digestibility. Alkaline pretreatment using sodium, potassium, calcium, or ammonium hydroxide is another category of pretreatment technology. In general, alkalis are more effective in lignin solubilization, but they cause less cellulose and hemicellulose hydrolysis and solubilization than acid processes. Alkalis can swell cellulose, increase the internal surface of cellulose, and decrease the degree of polymerization and crystallinity, which consequently benefits lignin disruption (Taherzadeh and Karimi 2008). In general, alkaline pretreatment is more effective on agricultural residues than on wood materials (Kumar and Wyman 2009).

Bamboo is the vernacular or common term for members of a particular taxonomic group of large woody grasses (subfamily Bambusoideae). It has both woody and grass characteristics (Jiang 2008). Recently, we reported an organosolv pretreatment of moso bamboo for robust conversion to sugars (Li *et al.* 2012a). Bamboo was reacted with a solution of 2%(w/w) H_2SO_4 in 75% ethanol at 160 to 180 °C for about 30 to 60 min, and it yielded a solid fraction containing 83.4% cellulose in the organosolv-pretreated substrate. The cellulose conversion to glucose yield reached 77.1% to 83.4% after enzymatic hydrolysis. But the cellulose conversion to glucose yield was lower than wood and agricultural waste, which can reach higher than 95%. The fermentation of the hydrolysate was not evaluated then. For base pretreatment of bamboo, the effect was limited. The glucose yield just increased from 2.4% to 20.9% after enzymatic hydrolysis (Li *et al.* 2012b). The object of this work was to provide a preliminary assessment of the effect of combination pretreatments (organosolv and alkali) on the chemical composition and enzymatic hydrolysis of bamboo. The fermentation of the enzymatic hydrolysates using *Saccharomyces cerevisiae* yeast was also conducted here.

EXPERIMENTAL

Materials

Moso bamboo (*Phyllostachys heterocycla*) was comminuted by the combination of chipping and milling to attain a powder of 1 mm using a laboratory mill. The moisture content of the powder samples was measured under oven-dried (OD) conditions for 24 h at 105±2 °C. Commercial enzymes, cellulase, and β-glucosidase, were purchased from Sigma. *Saccharomyces cerevisiae* yeast was purchased from Angel Yeast Co., Ltd., China. The yeast is sold for ethanol fermentation. All chemical reagents used in this study were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd., China.

Pretreatments

Bamboo samples were first pretreated by organosolv pretreatment and then by alkali pretreatment. The general process was as follows: The organosolv pretreatment

was carried out in a microwave accelerated reaction system made by CEM Corporation (Model MARS, USA). Aqueous ethanol of 75% (w/w) and 2% of sulfuric acid (w/w bamboo) were used for the organosolv pretreatment with certain pretreated temperature (160 °C), time period (30 min), and 16% (w/v) solid content. The organosolv-pretreated substrate and spent liquor were then separated by vacuum filtration. The solid substrate was washed thoroughly with water. After its weight loss and chemical composition were determined, the substrate was treated by 10% (w/w) $Ca(OH)_2$, 10%, and 20% (w/w) NaOH solutions, respectively. The alkali pretreatment was carried out at 50 °C for 70 h. The substrate residue was separated by vacuum filtration and washed with water, too. Then it was stored in 4 °C for enzymatic hydrolysis.

Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out in 100 mL plastic jars at 50 °C on a shaking incubator (KYC-100C, Shanghai Fuma Laboratory Equipment Co. Ltd. China) at 220 rpm. Bamboo substrate equivalent to 0.8 g glucan was loaded with 40 mL of 0.05 M sodium acetate buffer (pH 4.8). Approximately 1.5 mg of tetracycline chloride was added to control the growth of microorganisms and prevent consumption of liberated sugars. Two enzymes, cellulase (15 Filter Paper Units (FPU) per gram glucan) and β-glucosidase (30 International Unit (IU) per gram glucan), were loaded. Hydrolysates were sampled periodically at 1, 3, 6, 12, 24, and 48 hour to analyze glucose concentration. The hydrolysis was conducted in duplicate for each substrate; the average was reported here.

Ethanol Fermentation

Preculture preparation of *Saccharomyces cerevisiae* yeast was as follows: *Saccharomyces cerevisiae* yeast powder was added into a 100 mL glass flask with 40 mL of 2% glucose solution. The flask was placed in a water bath at 38 °C for 15 to 20 min. Then the mixture was kept at 33 °C for another 1.5 h.

The hydrolyzates and glucose (as a reference) solutions were supplemented with (g/L): peptone, 5.0; KH_2PO_4, 2.0; $MgSO_4$, 1.0; and $CaCl_2$, 0.25. The solutions were then autoclaved at 121 °C, 20 min for sterilization. The pH of hydrolyzates was adjusted to 5.5±0.1 using either 0.6 M sodium hydroxide or 6% sulfuric acid. The precultured *Saccharomyces cerevisiae* yeast solution was inoculated into the hydrolyzates for continuous ethanol fermentation. Then the flasks were placed in a shaker and incubated at 150 rpm, at 37 °C for 24 h. In order to provide the anaerobic conditions, the flasks were sealed with plastic wrap. The samples were stored in a freezer before analysis.

Crystallinity Analysis

The crystallinity of bamboo before and after pretreatment was measured by X-ray diffraction (XRD) using a diffractometer with Cu Kα radiation at 40 kV and 30 mA (Panalytical Corporation, Almelo, The Netherlands). The samples were scanned and the intensity was recorded in a 2θ range from 5° to 45°. The crystallinity of each sample was expressed in terms of a crystallinity index (CrI) using the following Eq. (Segal *et al.* 1959),

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

where I_{002} is the overall intensity of the peak at 2θ at about 22° and I_{am} is the intensity of the baseline at 2θ at about 18°.

Analytical Methods

The carbohydrate and lignin (acid-soluble and insoluble) of the untreated and all pretreated bamboo substrates were analyzed according to National Energy Laboratory (NREL) Analytical Procedure: Determination of Structural Carbohydrates and Lignin in Biomass (with modifications) (Sluiter *et al.* 2008). The method was based on degradation of carbohydrates to monomeric sugars by a two-stage sulfuric acid hydrolysis and determined the sugars by HPLC. Acid-soluble lignin was measured at 205 nm on a UV-Visible spectrophotometer (Dence 1992). The acid-insoluble lignin contents were determined by drying the acid-treated samples in a vacuum oven at 60 °C for 10 h.

The liquid samples were analyzed by HPLC, which was equipped with refractive index detector (Waters Corporation, Milford, Massachusetts, USA). The detection of sugars including glucose, xylose, mannose, galactose, and arabinose in the hydrolyzates and carbohydrate analyses were performed using an anion exchange column (Aminex HPX-87P, Bio-Rad, USA) at 85 °C with 0.6 mL/min flow of water.

The enzymatic hydrolytic reactions of each pretreated substrates and the ethanol fermentation reactions were monitored by measuring the time-dependent glucose and ethanol concentrations in the reaction solutions. For fast analysis, glucose and ethanol in the solutions was determined by a commercial Biosensor Analyzer (SBA-40E, Shandong Academy of Science, Shandong Province, China). The instrument precision is about 2% based on manufacturer specifications. The average of duplicate runs was reported here.

RESULTS AND DISCUSSION

Comparison of Cell Wall Components of Pretreated Substrates

The cell wall chemical composition can provide some indications of the effect of chemical pretreatment on bamboo chemical structure. Chemical components of untreated bamboo and pretreated bamboo substrates are listed in Table 1. The untreated bamboo exhibited glucose, xylose, and lignin contents of about 41.34%, 21.96%, and 24.29%, respectively. There were low contents of arabinose (1.10%), galactose (0.34%), and mannose (0.59%) in untreated bamboo.

Table 1. Chemical Analyses of Untreated and Pretreated Bamboo Substrates

Substrate (%)	Arabinose	Galactose	Glucose	Xylose	Mannose	Acid-insoluble lignin	Acid-soluble lignin	Solid recovery
Untreated Bamboo	1.10±0.10	0.34±0.00	41.34±0.44	21.96±1.03	0.59±0.04	22.84±0.22	1.45±0.01	-
Organosolv	0.16±0.02	0.04±0.00	70.37±3.52	17.11±0.74	ND	9.91±0.43	1.91±0.11	55.20
Organosolv + NaOH(10%)	0.18±0.01	0.13±0.01	80.37±4.10	16.66±0.68	ND	0.64±0.04	1.68±0.10	48.30
Organosolv + NaOH(20%)	0.21±0.04	0.12±0.01	84.41±4.55	15.24±0.60	ND	0.04±0.00	1.11±0.15	43.92
Organosolv + $Ca(OH)_2$(10%)	0.20±0.03	0.12±0.01	76.73±3.10	17.68±0.78	ND	5.28±0.50	3.97±0.25	49.67

The organosolv pretreatments with acid catalyst were very effective in removing hemicellulose and lignin. As a result, cellulose was enriched in the organosolv-pretreated substrates as high as 73.37%. This suggested that the organosolv pretreatment with sulfuric acid could minimize the loss of cellulose, which served as the main resource in bioethanol production. The addition of acid to the liquid mixture played a very important

role in catalyzing the removal of hemicellulose and lignin. For two-stage organosolv and alkaline pretreatment, more hemicellulose and lignin were removed than organosolv pretreatment alone. Especially for lignin removal, there was only 0.04% acid-insoluble lignin left in the organosolv and 20% NaOH pretreated substrate. The cellulose was enriched in the substrates to 80.37%, 84.41%, and 76.73%, respectively.

The organosolv spent liquor was mixed with three volumes of water to precipitate the dissolved lignin. Based on the 24.29 g lignin in 100 g raw original bamboo, the amount of precipitated lignin was 67.9%. The sugars and soluble lignin in the combined liquor of filtrate and water washes are listed in Table 2. The concentration was calculated on original spent liquors. No mannose was detected in the spent liquors. Only the second alkali pretreatment data are listed in Table 2 for two-stage pretreatments. The concentrations of sugar and lignin in the organosolv pretreatment stage were almost the same. As discussed above, an increase in the alkali dosage increased the removal of components. Especially for lignin, there were 6.57 g/L acid-soluble lignin detected in 20% NaOH pretreated spent liquors compared with 2.03 g/L (10% NaOH) and 0.51 g/L ($Ca(OH)_2$).

Table 2. Chemical Analyses of Pretreated Spent Liquors

Spent Liquor/ (g/L)	Arabinose	Galactose	Glucose	Xylose	Acid-soluble lignin
Organosolv	1.99±0.54	0.11±0.00	1.03±0.31	10.58±0.72	5.12±0.53
Organosolv + NaOH(10%)	0.17±0.02	0.02±0.00	0.36±0.04	2.27±0.54	2.03±0.30
Organosolv + NaOH(20%)	0.18±0.04	0.04±0.00	0.44±0.05	3.24±0.59	6.57±0.48
Organosolv + $Ca(OH)_2$(10%)	0.09±0.01	0.02±0.00	0.21±0.03	0.96±0.28	0.51±0.10

Mass Balance of Sugars During Pretreatments

For an ideal pretreatment, it should provide readily enzymatic digestible substrates. But that is not enough; it should also maximally recover all the components of original biomass. As shown in Table 3, sugars and lignin in solid substrates were calculated based on 100 g untreated raw bamboo. As discussed above in Table 1, it contained 41.34 g glucose, 22.00 g xylose, and 24.29 g lignin from 100 g oven-dry bamboo. The total sugars and lignin recovery was 55.20 g for organosolv without alkali pretreatment. Pretreatment with alkali pretreated addition resulted in lower recovery that ranged from 43.92 to 49.67 g. Low substrate yield from the pretreatment was due in part to the dissolution of more cellulose, hemicellulose, and lignin.

Table 3. Mass Balance of 100 g Raw Oven-dry Bamboo during Pretreatment

Substrate	Solid recovery (%)	Glucose (g)	Xylose (g)	Lignin (g)
Untreated Bamboo	-	41.34±0.44	21.96±1.03	24.29±0.21
Organosolv	55.20±4.10	38.84±3.51	9.44±0.72	6.52±0.44
Organosolv + NaOH(10%)	48.30±4.00	38.82±2.90	8.05±0.60	1.12±0.10
Organosolv + NaOH(20%)	43.92±5.90	37.07±3.78	6.69±0.760	0.50±0.05
Organosolv + $Ca(OH)_2$(10%)	49.67±3.50	38.11±3.10	8.78±0.70	4.59±0.70

Enzymatic Hydrolyzability of Pretreated Bamboo Substrates

The enzymatic hydrolyzability of organosolv pretreated bamboo substrates were shown in Fig. 1. Untreated original bamboo was used as a comparison for the enzymatic digestibility. The enzymes loading were 15 FPU (Filter Paper Units) cellulase and 30 IU (International Units) β-glucosidase per gram cellulose for all enzymatic hydrolysis. The cellulose-to-glucose conversion yield of untreated raw bamboo after a 48 h hydrolysis was only 2.4%. The cellulose-to-glucose conversion yield of organosolv pretreatment with sulfuric acid pretreatment significantly improved the enzymatic digestibility of the bamboo (69.4%). Meanwhile, with the second alkali pretreatment, the cellulose-to-glucose conversion yield was significantly increased. The two stages of organosolv with 20% NaOH pretreatment substrate even reached 95.5% cellulose-to-glucose yield. The sugar content in the hydrolyzates increased sharply in the first 12 h and gradually continued until 48 h. Based on comparison among the pretreatments, the cellulose-to-glucose conversion yields were increasing with decreasing crystallinity index of cellulose. The removal of hemicellulose and lignin increased cellulose susceptibility to enzymes.

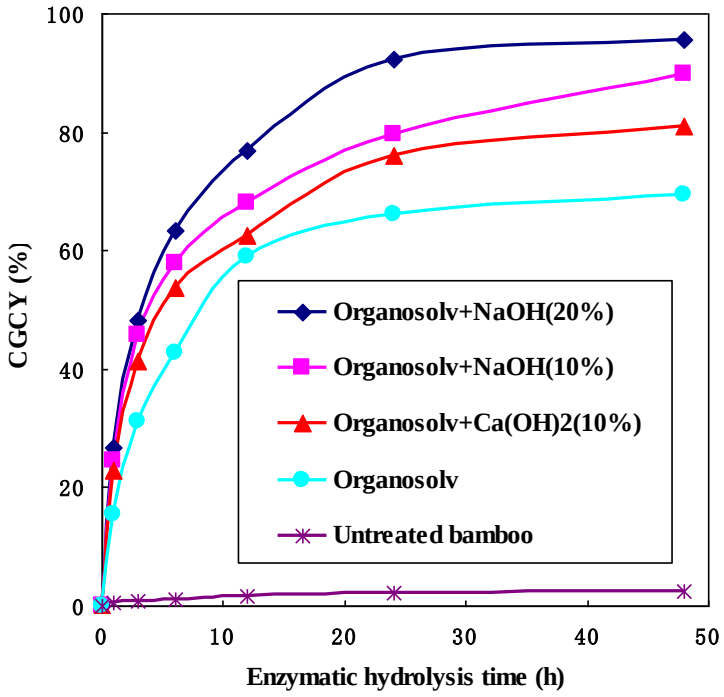

Fig. 1. Comparison of enzymatic hydrolyzability of different pretreated bamboo substrates with an enzyme loading of 15 FPU cellulase and 30 IU β-glucosidase per gram of cellulose, 50 ^{o}C, pH 4.8, and on a 220 rpm shaker. CGCY: Cellulose-to-Glucose Conversion Yield

Effect of Cellulose Crystallinity Index on Enzymatic Hydrolysis

The crystallinity of cellulose, representing its accessible surface area protected by lignin and hemicellulose, is believed to have a significant effect on enzymatic saccharification of glucan (Zhang and Lynd 2004). X-ray diffraction analysis indicated that the crystallinity of bamboo increased after the pretreatments (Table 4). Compared with the organosolv pretreatment, the crystallinity of two-stage organosolv and alkali pretreated bamboo decreased, indicating there was a breakdown of the crystalline

cellulose region and also an increase in the amorphous regions. This result suggests that the alkali pretreatment was very effective in reducing cellulose crystallinity. On the other hand, removing hemicellulose and lignin from the bamboo may lead to deformation of crystallites.

Enzymatic hydrolysis of each substrate was done with cellulase and β-glucosidase. As shown in Fig. 1, the cellulose-to-glucose conversion yield (at 48 h of reaction) increased with the reduction in crystallinity, suggesting that the crystalline structure of bamboo inhibits the enzymatic hydrolysis of cellulose. The initial rate of cellulose hydrolysis (at 3 h of reaction) also increased with decreasing crystallinity. This reflects the fact that cellulase hydrolyzes amorphous cellulose faster than crystalline cellulose.

Table 4. Crystallinity Analysis of Untreated and Pretreated Bamboo Substrates

Substrate	Crystallinity Index (CrI)
Organosolv	60.19±2.10
Organosolv + NaOH(10%)	58.58±3.09
Organosolv + NaOH(20%)	58.03±2.58
Organosolv + $Ca(OH)_2$(10%)	60.04±4.10
Untreated Bamboo	52.48±3.56

Ethanol Fermentation of Enzymatic Hydrolyzates

The enzymatic hydrolysis and fermentation can be performed with different processes. Separated hydrolysis and fermentation (SHF) is the strategy in which the process can be performed at the optimum operation conditions of both hydrolysis and fermentation.

The optimum temperature for cellulase is typically in the range of 45 to 50 °C, while it is between 30 and 37 °C for most of the ethanol fermentation micro-organisms (Taherzadeh and Karimi 2007). In this process, the hydrolyzate can be separated from the solid residuals, *e.g.*, lignin and the clear hydrolyzate can be subjected to fermentation.

Anaerobic cultivation of bamboo substrates enzymatic hydrolyzates to bio-ethanol by *Saccharomyces cerevisiae* yeast was investigated, and the result is shown in Fig. 2. Pure glucose was selected as a reference in fermentation. Cultivation of hydrolyzates resulted in rapid sugar consumption. Complete glucose assimilation by the yeast was observed within less than 24 h.

The results showed that the different pretreated methods significantly improved the ethanol production of the theoretical yield for bamboo. According to Fig. 2, the ethanol production yield of pure glucose was 87.0%. Organosolv pretreatment improved the ethanol production yield to 89.1%. However, alkali pretreatment assisted the process and enhanced this efficiency up to 89.2 to 92.0%, which was the best result for ethanol production among all the applied pretreatment techniques. Furthermore, the results showed a strong effect of alkaline (NaOH) pretreatment on ethanol yield (90.3% and 92.0%), while the effect of $Ca(OH)_2$ was not significant (89.2%).

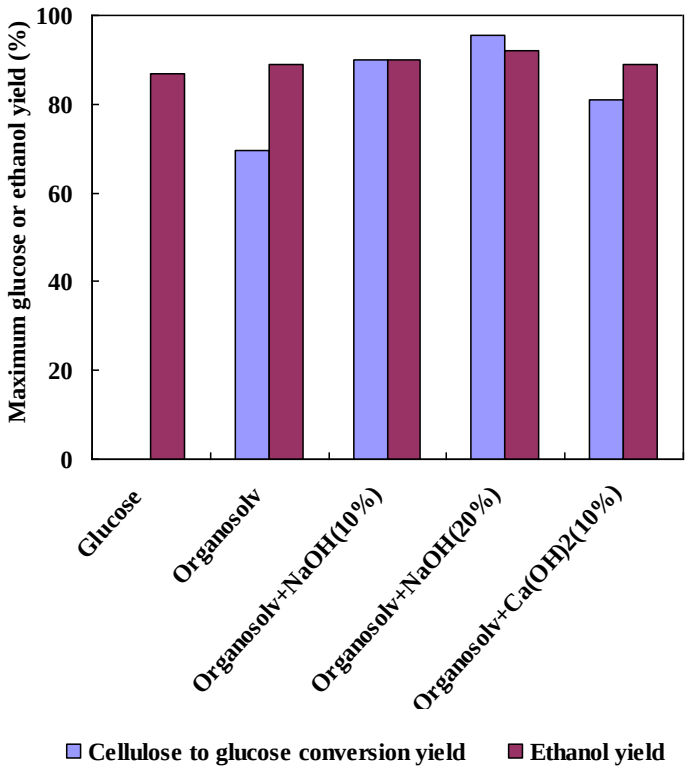

Fig. 2. Glucose and ethanol yield (% theoretical yield) after enzymatic hydrolysis and fermentation

CONCLUSIONS

Ethanol can be successfully produced from bamboo with *Saccharomyces cerevisiae* yeast. In order to achieve a high yield of ethanol from the bamboo, an efficient pretreatment process is necessary. A two-stage pretreatment, organosolv followed by sodium hydroxide pretreatment, was shown to be an efficient alternative for pretreatment of bamboo before enzymatic hydrolysis.

The pretreatment was able to almost completely delignify (97.94%) the biomass, enriching the cellulose to more than 80% in the substrate, and reducing the crystallinity index of the cellulose. The resulting liquid, consisting of the enzymatic hydrolyzates supplemented with *Saccharomyces cerevisiae* yeast and mineral nutrients, was subjected to ethanol fermentation. As a result, a high ethanol fermentation yield of 92.0% (based on theoretical yield) was achieved.

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REFERENCES CITED

Chum, H. L., Johnson, D. K., and Black, S. K. (1990). "Organosolv pretreatment for enzymic hydrolysis of poplars. 2. Catalyst effects and the combined severity parameter," *Ind. Eng. Chem. Res.* 29, 156-162.

Dence, C. W. (1992). "The determination of lignin," In: Dence, C. W. (ed.), *Methods in Lignin Chemistry*, Springer-Verlag, Berlin, 33-61.

Dora, S. (2008). "Bamboo as a source of bioenergy feedstock in Ghana," College of Agric and Natural Resources, 13-Jul-2008. Kwame Nkrumah University of Science & Technology, Ghana.

Holtzapple, M. T., and Humphrey, A. E. (1984). "The effect of organosolv pretreatment on the enzymatic hydrolysis of poplar," *Biotechnol. Bioeng.* 26, 670-676.

Jiang, Z., H. (2008). *Bamboo and Rattan in the World*, Chinese Forestry Press, Beijing.

Kumar, R., and Wyman, C. E. (2009). "Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies," *Biotechnology Progress* 25, 302-314.

Li, Z., Jiang, Z., Fei, B., Pan, X., Cai, Z., Liu, X., and Yu, Y. (2012a). "Ethanol organosolv pretreatment of bamboo for efficient enzymatic saccharification," *BioResources* 7(3), 3452-3462.

Li, Z., Jiang, Z., Fei, B., Yu, Y., and Cai, Z. (2012b). "Effective of microwave-KOH pretreatment on enzymatic hydrolysis of bamboo," *Asia-Pacific Power and Energy Engineering Conference(APPEEC 2012).* China, Shanghai, 27-Mar-2012.

Pan, X. J., Gilkes, N., Kadla, J., Pye, K., Saka, S., Gregg, D., Ehara, K., Xie, D., Lam, D., and Saddler, J. (2006). "Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: Optimization of process yields," *Biotechnology and Bioengineering* 94(5), 851-861.

Segal, L., Creely, J. J., Martin, A. E., and Conrad, C. M. (1959). "An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer," *Text. Res. J*. 29, 786-794.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). "Laboratory analytical procedure (LAP): Determination of structural carbohydrates and lignin in biomass," *Technical Report NREL/TP-510-42618*. http://www.nrel.gov/biomass/analytical_procedures.html

Southern Metropolis Daily Mark. (2012). "Bamboo: An ultimate green material," Jan. 8, http://gcontent.oeeee.com/8/18/818f4654ed39a1c1/Blog/df2/752aa2.html. (Chinese)

Szulczyk, K. R. (2010). "Which is a better transportation fuel – butanol or ethanol?" *International Journal of Energy and Environment* 1(3), 501-512.

Taherzadeh, M. J., Karimi, K. (2007). "Acid based hydrolysis processes for ethanol from lignocellulosic materials: A review," *BioResources* 2(3), 472-499.

Taherzadeh, M. J., Karimi, K. (2008). "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review," *International Journal of Molecule Sciences* 9, 1621-1651.

Zhang, Y. H., and Lynd, L. R. (2004). "Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulose systems," *Biotechnol. Bioeng.* 88, 797-824.

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