Appl Biochem Biotechnol (2011) 163:658–668
DOI 10.1007/s12010-010-9071-4

Dilute Acid Pretreatment of Corncob for Efficient Sugar Production

G. S. Wang · Jae-Won Lee · J. Y. Zhu ·
Thomas W. Jeffries

Received: 3 May 2010 / Accepted: 16 August 2010 /

Abstract Aqueous dilute acid pretreatments of corncob were conducted using cylindrical pressure vessels in an oil bath. Pretreatments were conducted in a temperature range of 160–190 °C with acid-solution-to-solid-corncob ratio of 2. The acid concentration (v/v) in the pretreatment solution was varied from 0% to 0.7%, depending on temperature. This gives acid charge on ovendry-weight corncob of 0–2.58%. It was found that optimal pretreatment temperature is between 160 and 170 °C based on total xylose and glucose yields and thermal energy consumption in pretreatment. At 170 °C and acid charge of 2.2% on cob, total glucose yield and xylose recovery were 97% and 75%, respectively, which resulted in an overall monomeric sugar recovery of about 88%. Xylose concentration in the hydrolysate was about 12%, with xylose-to-acetic-acid ratio of 8 and to furan (furfural and hydroxymethylfurfural) of about 15.

Keywords Pretreatment · Cellulosic ethanol · Enzymatic hydrolysis · Corncob · Inhibitors

Introduction

Corncob is a by-product in corn (maize) production. Traditionally, it has been used as animal beddings or returned to the field [6]. As a lignocellulose biomass, corncob has an

---

This work was conducted on official government time of Zhu and Jeffries while Wang and Lee were visiting scientists at the USDA Forest Service, Forest Products Laboratory. The work is in public domain in the USA.

G. S. Wang
TianJin Key Laboratory of Pulp and Paper, TianJin University of Science and Technology,
TianJin, China

J.-W. Lee
Department of Forest Products and Technology (BK 21 Program) and Bio-Energy Research Institute,
Chonnam National University, Gwangju 500-757, South Korea

J. Y. Zhu (✉) · T. W. Jeffries (✉)
Forest Products Laboratory, US Forest Service, Madison, WI, USA
e-mail: jzhu@fs.fed.us
e-mail: twjeffri@wisc.edu

energy content of about 18.5 MJ/kg [5], slightly higher than that of corn stover (17 MJ/kg) and switchgrass (18 MJ/kg). However, it has an energy density of about 5,000 MJ/m^3 [5], about two times that of corn stover and switchgrass, a significant advantage over these feedstocks for reducing transportation cost in biofuel production. About 50 million metric tons of corncob are available annually based on the 12.1 billion tons corn production in 2008 [11] in the USA; therefore, corncob could be a significant source of lignocellulose biomass for biofuel production by using effective and economical collection strategies. Because of its low lignin content (≈10%) and high carbohydrate content (≈70%), corncob has low recalcitrance to enzyme and microbial activity. Therefore, biochemical conversion of corncob to ethanol through enzymatic saccharification and fermentation is a viable technology pathway.

Limited studies have been reported on ethanol production from corncob, probably because corncob has only recently been considered as a viable source of biomass. Unlike stover, commercial processes are available for its collection. Furthermore, the high xylose content (30%) of this material presents a challenge. Early work on the use of corncob for ethanol production includes a study of *Escherichia coli* B to ferment hydrolysate from sulfuric acid pretreatment of corncob [1] and demonstration that *Saccharomyces cerevisiae* 1400 (pLNH33) will ferment hydrolysate following ammonia pretreatment and saccharification of the substrate [2]. Recently, recombinant *S. cerevisiae* [10] and *Kluyveromyces marxianus* [12] have also been shown to ferment xylose from corncob. Chen and others [3] used *S. cerevisiae* 316 to ferment a dilute acid pretreatment of corncob solid substrate. Fed-batch studies were also conducted for high solids simultaneous saccharification and fermentation of pretreated corncob solid substrates [9, 12]. However, limited studies were carried out on pretreatment optimization for maximal fermentable sugar production from corncob. Lee and others [8] used oxalic acid to pretreat corncob and *Pichia stipitis* CBS 6054 for fermentation. The pretreatment used impregnation and steam pretreatment steps. The xylose was recovered by washing. The study is focused on fermentation, and ethanol yield on unit ton of corncob was not reported. None of these studies provided a thorough examination of maximal sugar production from both the pretreatment hydrolysate and the cellulosic substrate, pretreatment energy consumption, and materials balance to calculate final ethanol production from corncob. As a result, the potential of corncob for ethanol production cannot be objectively evaluated.

The objective of this study is to provide a complete examination of fermentable sugar production along with fermentation inhibitor formation from corncob following acid hydrolysis. Dilute sulfuric acid pretreatment is used because sulfuric acid is a low-cost and readily available chemical for industrial applications, with low safety and environmental concerns. Aqueous pretreatment of corncob with a low acid-solution-to-cob-solid ratio was used, which eliminates impregnation for process simplification. The low pretreatment liquid-to-solid ratio (L/S) significantly reduces thermal energy consumption for pretreatment and ensures a hydrolysate with high xylose concentration. The fermentable sugar data and inhibitor profiles obtained in a range of pretreatment conditions conducted provide readers enough information for process optimization for cellulosic ethanol production.

Experimental

Materials

Standardized corncob pellets (Pestell, New Hamburg, Canada) with a moisture content of 10% were used. Sulfuric acid was used as received from Sigma-Aldrich (St. Louis, MO, USA).

Commercial enzymes, Celluclast 1.5 L (cellulase) and Novozyme 188 (β-glucosidase), were generously provided by Novozymes (Franklinton, NC, USA).

Substrate Production from Corncob

All pretreatments were conducted in 100-mL cylindrical stainless steel reaction vessels (Parr, Moline, IL, USA) submerged in a 75-L oil bath (Blue M Electric, Blue Island, IL, USA, 4.4 kW, model MW-1155C). Polyethylene glycol was used as the heating medium in the oil bath. First, 25 g ovendry (od) corncob was added to a vessel. Then, a 50-mL solution of sulfuric acid/deionized (DI) water, at the desired concentration, was added. The acid-solution-to-od-cob-solid ratio (L/S) of 2 was used for all experiments. Depending on the pretreatment temperature, acid concentrations of the solutions ranged from 0% to 0.7% (v/v), or equivalently, acid charges of 0–2.576% (w/w) based on od corncob. The oil bath was electrically heated to the desired pretreatment temperatures, which ranged between 160 and 190 °C. The loaded vessels were then placed into a rotating cage inside the oil bath. Four digesters were used for each batch run. The rotating speed was 6 rpm to ensure good mixing of the corncob and acid solution inside the vessel. A digital display thermocouple probe (Omega, model HH-51) was used to monitor the oil temperature and to make fine adjustments to the mechanical thermostat controls of the oil bath. After a preset pretreatment time of 30 min was reached, the digesters were taken out and quickly cooled to room temperature using tap water. The pretreatment hydrolysate and substrate were separated through vacuum filtration using fast draining qualitative filter paper (Ahlstrom grade 617). The hydrolysates were saved for sugar analysis and fermentation. The solid cellulosic substrates were washed thoroughly using fast draining qualitative filter paper (Ahlstrom grade 617). After dewatering, the solid substrates were weighed and saved for subsequent enzymatic hydrolysis. The moisture contents of the dewatered substrates were measured gravimetrically by drying subsets of the samples overnight in an oven at 105 °C. Therefore, solids yields from pretreatments could be determined for mass balance and final ethanol yield analysis.

Enzymatic Hydrolysis

Enzymatic hydrolyses of the pretreated corncob substrates were carried out at a solids consistency of 2% (w/v) in 50-mL sodium acetate buffer (pH 4.8) at 50 °C using a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA, USA) at 200 rpm. A mixture of Celluclast 1.5 L with an activity loading of 7.5 FPU/g substrate and Novozyme 188 with an activity loading of 11.25 CBU/g substrate was used. Hydrolysates were sampled periodically for glucose analysis. Each data point was the average of duplicate experiments. The relative standard deviation of duplicate enzymatic hydrolysis measurements was 2.1%

Determination of Sugars

Concentrations of glucose, xylose, arabinose, and ethanol were determined after separation by high-performance liquid chromatography (HPLC) using a BioRad (Hercules, CA, USA) Aminex HPX-87H column (300×7.8 mm) at 55 °C with 5 mM H_2SO_4 as eluant, at a flow rate of 0.3 mL/min and an injection volume of 20 μL. A refractive index detector (Hitachi High-Technologies, model L-2490, Japan) was used to quantify the products. Samples were appropriately diluted in DI water and then filtered through PrepSEP C18 (Fisher Scientific) filters prior to injection. The relative standard deviation based on duplicate experiments was about 5%.

Analysis of Fermentation Inhibitors

The amounts of hydroxymethylfurfural (HMF) and furfural in the hydrolysates were analyzed using a HP series II 1090 HPLC equipped with a C18(2) 5 μ, 250×4.6 mm column (Phenomenex, Torrance, CA, USA) at a flow rate of 0.8 mL/min (isocratic). Mobile phase was composed of acetonitrile 100 mL, DI water 800 mL, and acetic acid 1%. Acetic acid in the pretreatment hydrolysate was measured by the same HPLC for sugar analysis. Results were obtained from the average of duplicate measurements. The standard deviation from duplicate measurements was 7%.

Results and Discussion

Substrate Solid Yield and Composition

For the pretreatment conditions carried out in this study, solid substrate yield ranged from 50% to 66% (Table 1). In general, solid yield decreases with the increase of pretreatment combined severity factor (CSF) [4]. CSFs shown in Table 1 were calculated based on the initial liquor pH and a pretreatment time of 30 min. Xylan removals varied from about 50% to 90%, and glucan losses were minimal of about 4–11% for all the experiments conducted. As expected, xylan removal increased with an increase in pretreatment temperature and acid concentration except for two anomalous data points (underlined). Lignin measurements from some substrates (underlined) are higher than that in the corncob sample. This type of measurement uncertainty is the limit of current analytical methods. Similar problems were reported by the National Renewable Energy Laboratory (private communications, Dan Schell).

Xylose Yield

Xylose yields from pretreatments were calculated from the measured xylose concentrations in the hydrolysates and the L/S of 2. Although xylan removal increases linearly with an increase in pretreatment temperature and acid charge, not all removed xylan was converted to xylose. As shown in Fig. 1, maximal xylose yield occurred at an acid charge on od cob of about 1.4% at pretreatment temperature 180 °C. Xylose yield decreased when the acid charge was further increased. Under any constant acid charge, xylose yield is highest at about 170 °C among the four pretreatment temperatures studied. Furthermore, maximum xylose yield was 23.6 wt% of od cob achieved at 170 °C and 2.2% acid charge, which is equivalent to 75% theoretical xylose recovery based on xylan content in cob. The observed decreases in xylose yield under high acid charges and temperatures (Fig. 1) are probably due to the formation of furfural and other by products (discussed later).

Enzymatic Cellulose Conversion and Glucose Yield

Enzymatic conversion of the cellulose substrate was calculated based on measured glucose concentration in the enzymatic hydrolysate and substrate glucan content. Under the current cellulase loadings of 7.5 FPU/g substrate, excellent cellulose conversions were achieved in about 12–24 h for most pretreatments (Fig. 2). For comparison and pretreatment optimization, we used the data obtained from 12 h of enzymatic hydrolysis to calculate enzymatic glucose and total sugar yields in the following discussions. Hydrolysis rate increases as pretreatment acid charge increases at a given temperature (170 °C in Fig. 2)

Table 1 Weights of corncob components after aqueous sulfuric acid pretreatments for 30 min

T (°C)	Acid charge on cob (%)	Initial liquor pH	CSF[a]	Component weight (g)				
				Klason lignin	Arabinan	Glucan	Xylan	Solid yield (g)
Untreated sample				0.139	0.219	0.370	0.278	1.00
160	1.472	1.33	1.91	0.1297	0.0074	0.3489	0.1000	0.6630
	1.840	1.22	2.02	0.1229	0.0063	0.3457	0.0776	0.6214
	2.208	1.14	2.10	0.1267	0.0058	0.3379	0.0589	0.5901
	2.576	1.12	2.12	0.1321	0.0056	0.3429	0.0504	0.5901
170	1.104	1.51	2.03	0.1270	0.0052	0.3496	0.0714	0.6166
	1.472	1.45	2.09	0.1268	0.0048	0.3469	0.0605	0.5981
	1.840	1.39	2.15	0.1383	0.0039	0.3363	0.0387	0.5662
	2.208	1.36	2.18	0.1356	0.0041	0.3384	0.0357	0.5605
180	0	6.42	−2.59	*0.2436*	0.0025	0.4656	0.0153	0.7797
	0.184	2.07	1.76	0.1266	0.0049	0.3497	0.0952	0.6387
	0.368	1.86	1.97	0.1260	0.0037	0.3556	0.0635	0.6002
	0.736	1.58	2.25	0.1226	0.0046	0.3474	0.0864	0.6321
	0.736	1.63	2.20	0.1326	0.0029	0.3374	0.0426	0.5622
	1.104	1.55	2.28	0.1370	0.0027	0.3380	0.0324	0.5487
	1.472	1.46	2.37	0.1180	0.0076	0.3462	*0.1448*	*0.7122*
	1.840	1.20	2.63	*0.0756*	0.0126	0.2036	*0.1640*	0.5513
	2.208	1.16	2.67	0.1384	0.0030	0.3434	0.0192	0.5367
	2.576	1.10	2.73	0.1464	0.0023	0.3136	0.0162	0.5151
190	0.368	2.04	2.09	0.1533	0.0014	0.3469	0.0319	0.5850
	0.736	1.87	2.26	0.1629	0.0015	0.3353	0.0311	0.5862
	1.104	1.77	2.36	0.1592	0.0011	0.3403	0.0209	0.5658
	1.472	1.71	2.42	0.1553	0.0018	0.3402	0.0219	0.5663
RSTD (%)[b]				0.80	1.60	1.00	1.40	

[a] CSF [4] $= \log\left[t \cdot \exp\left(\frac{T-100}{14.75}\right)\right] - \mathrm{pH}$

[b] Relative Standard Deviation

due primarily to the increase in the amount of xylan removal. As shown in Fig. 3, enzymatic hydrolysis glucose yield (EHGY) after 12 h of hydrolysis was maximal for the substrate pretreated at 180 °C with an acid charge on od cob of about 1.8%. EHGY decreased in a manner similar to the xylose yield from pretreatment hydrolysate when the acid charge was further increased. EHGYs from substrates produced at 160 °C were low, occurring at about 34 wt% cob. This was probably due to insufficient removal of xylan, thereby resulting in recalcitrance and low enzymatic cellulose conversion. Even so, substrate yields were higher compared to those pretreated under different temperatures with a similar acid charge. Increasing pretreatment temperature beyond 170 °C did not further increase EHGY because solid yield loss increased at higher temperatures. The maximum EHGY was about 37.5 wt% of od cob achieved under several conditions: 170 °C and acid charge of 2.2%, or 180 °C at an acid charge of 1.6%, or 190 °C at an acid charge 1.5%.

Glucan lost during pretreatment dissolved in the hydrolysate and could be recovered through fermentation. The amounts of dissolved glucan recovered as glucose in the

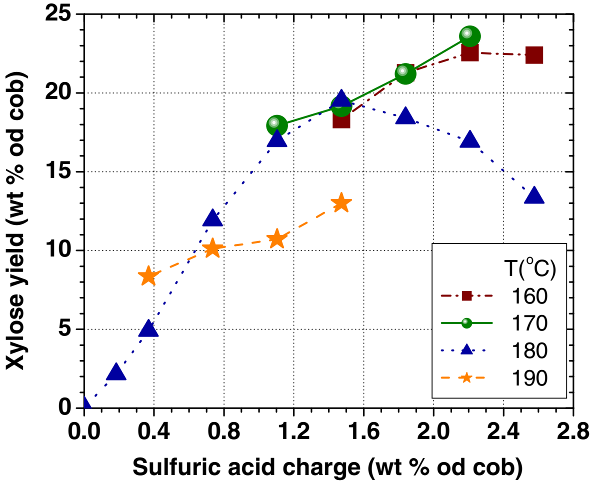


Fig. 1 Effects of pretreatment temperature and sulfuric acid charge on xylose yield from pretreatment hydrolysate

hydrolysates are shown in Fig. 4. Under pretreatment conditions for maximal EHGY, glucose yields from hydrolysates are 2.0 wt% od cob.

Total Monomeric Sugar Yield

Total monomeric sugar yield from pretreatment is the summation of EHGY from the substrate, xylose, and glucose yield from pretreatment hydrolysate. The arabinose yields from the pretreatment hydrolysate at maximal EHGY were about 1.2 wt% od cob and can be neglected. The maximum xylose and glucose yield was 63 wt% cob achieved at 170 °C and acid charge of 2.2% for the experimental conditions examined in this study (Fig. 5). This is equivalent to 87.5% conversion of the glucan and xylan from corncob. These findings suggest that excellent total monomeric sugar yield could be achieved at 170 °C with an acid charge of 2.2%. Total sugar yield curves for 170 and 190 °C shown in Fig. 5

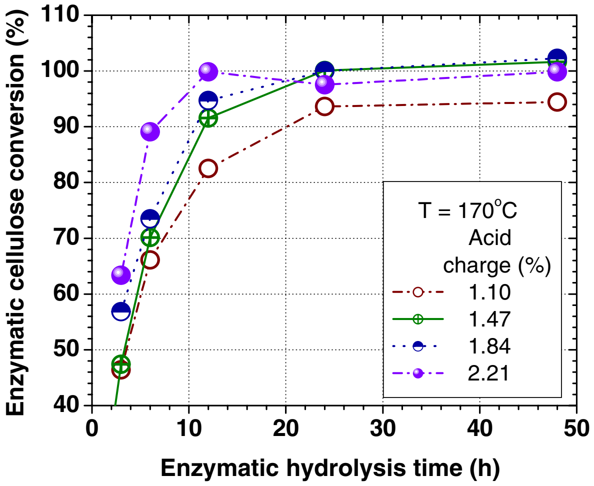
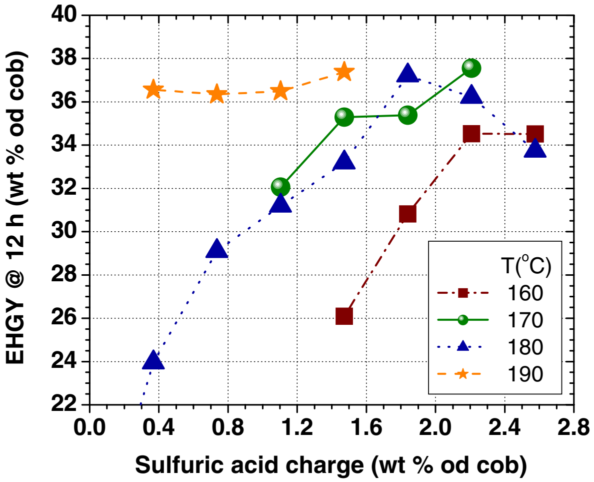


Fig. 2 Time-dependent enzymatic hydrolysis cellulose conversion of substrates produced at 170 °C and various sulfuric acid charges

Fig. 3 Effects of pretreatment temperature and sulfuric acid charge on EHGY after 12 h of enzyme actions



have not achieved a maximum. However, the low xylose recovery of only about 10–13 wt% of cob at 190 °C compared to over 20 wt% of cob at 170 °C (Fig. 1) and low substrate solid yield at 190 °C suggests that total sugar yield will not exceed 63% when acid charge is further increased above 1.5%, which was the maximal acid charged at 190 °C in this study. Therefore, a pretreatment temperature of 170 °C is probably optimal to maximize total sugar yield.

A preliminary mass balance analysis was carried out for the pretreatment run achieved maximal total sugar yield at 170 °C and an acid charge of 2.2% (Fig. 6). For 1 tonne (1,000 kg) of untreated corncob, the total yield of monomeric sugar in the pretreatment spent hydrolysate was 268 kg, which includes 12 and 236 kg, respectively, of monomeric arabinose and xylose and an additional 20 kg of glucose, mainly from the dissolved glucan by acidic hydrolysis and glucose residues in hemicelluloses during acid pretreatment. These results correspond to overall sugar recoveries (including 375 kg glucose from enzymatic hydrolysis) of the major saccharides (arabinose, xylose, and glucose) of about 50%, 75%, and 97%, respectively, of the corresponding polysaccharides in untreated corncob.

Fig. 4 Effects of pretreatment temperature and sulfuric acid charge on glucose recovery from pretreatment hydrolysate

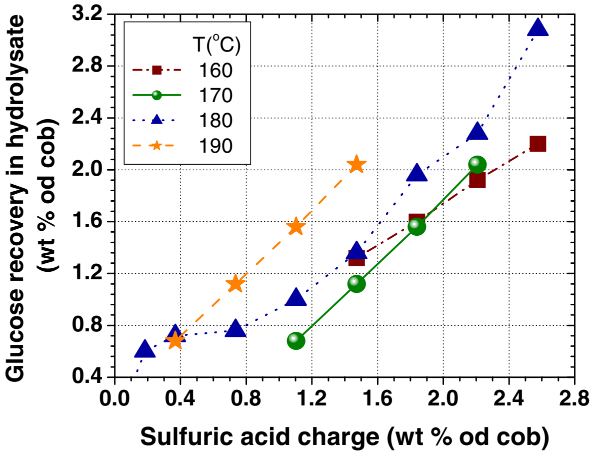
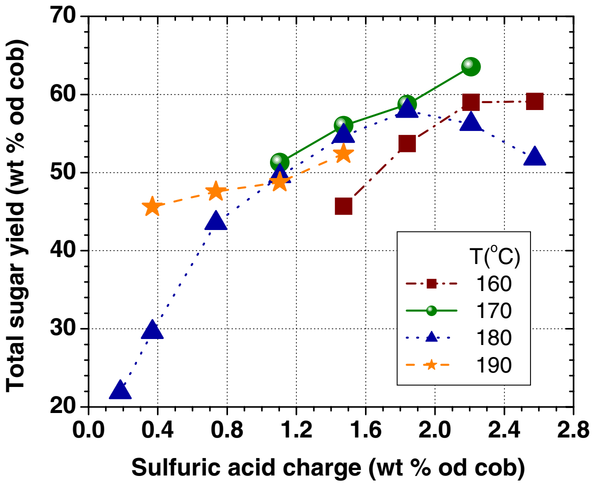


Fig. 5 Effects of pretreatment temperature and sulfuric acid charge on total xylose and glucose yield



Pretreatment Energy Efficiency

We compared maximal sugar production energy efficiency at different pretreatment temperatures. We define pretreatment energy efficiency as total sugar yield divided by pretreatment energy consumption [13], that is, enthalpy of the cob suspension at the pretreatment temperature. Total sugar yields used in the calculation were from runs at acid charges of 2.6%, 2.2%, 1.8%, and 1.5% at 160, 170, 180, and 190 °C, respectively. We assume that 50% of the thermal energy used in pretreatment can be recovered. As shown in Fig. 7, results indicate that pretreatment energy efficiencies are highest, about 0.73 kg/MJ, at both 160 and 170 °C, suggesting that both energy efficiency and total sugar yield are maximized at 170 °C and an acid charge of 2.2%. Therefore, it is safe to say that the optimal pretreatment temperature should be around 170 °C.

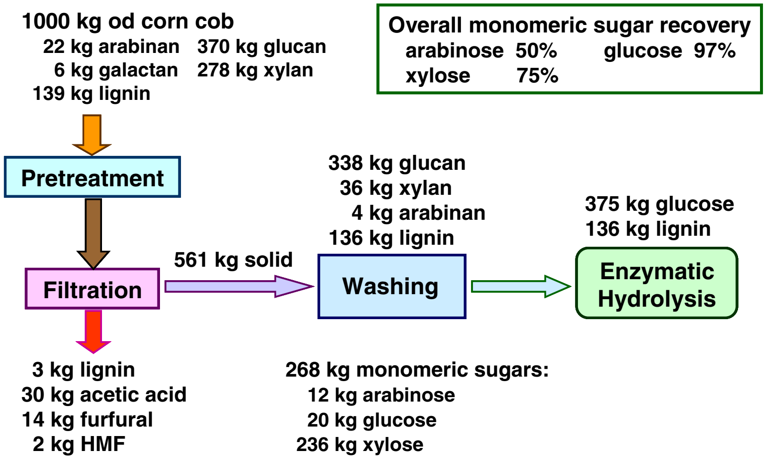
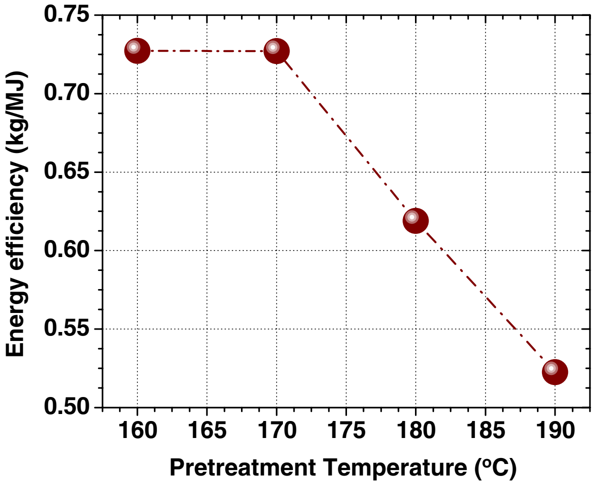


Fig. 6 Preliminary mass balance of a corncob pretreatment at 170 °C and sulfuric acid charge on od cob of 2.2%

Fig. 7 Maximal energy efficiency for total monomeric sugar production at various pretreatment temperatures



Formation of Fermentation Inhibitors

Formation of fermentation inhibitors during pretreatment is one of the major barriers in production of ethanol from pretreatment hydrolysate (the hemicellulosic sugar stream). Acetic acid is formed during pretreatment due to the presence of acetyl groups in the corncob. Some of the acetic acid produced can be consumed during pretreatment. The amount of acetic acid in the final hydrolysate first increases as the sulfuric acid charge increases and then reaches an asymptotic value of about 3 wt% od cob (or 15 g/L) (Fig. 8). Temperature has a small effect on acetic acid content in the hydrolysate unless it is increased to 190 °C. At 160 and 170 °C, the xylose-to-acetic ratios are between 7 and 8, with a maximal ratio of 8, and are not significantly affected by the sulfuric acid charge. Therefore, it is advantageous to conduct pretreatment between 160 and 170 °C to reduce acetic acid formation.

Fig. 8 Effects of pretreatment temperature and sulfuric acid charge on amount of acetic acid present in the hydrolysate

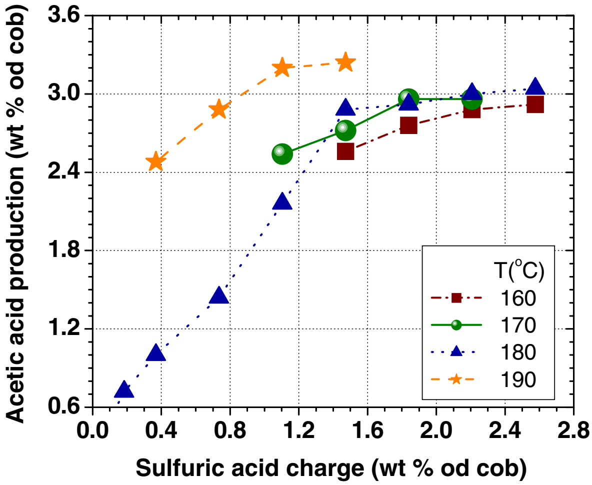
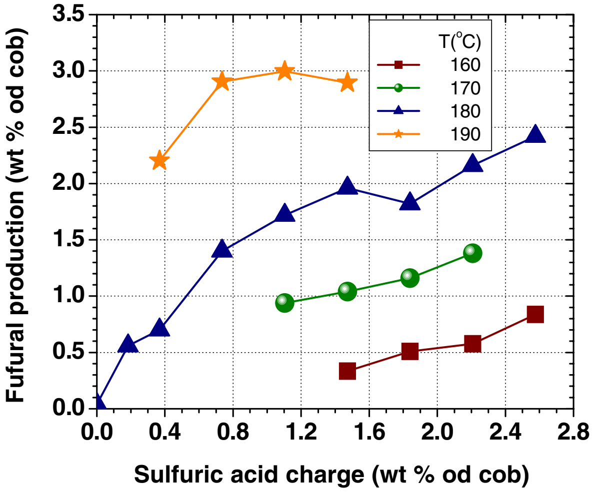
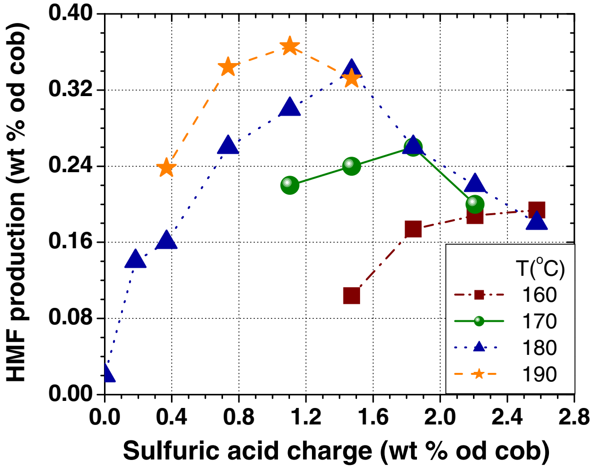


Fig. 9 Effects of pretreatment temperature and sulfuric acid charge on furfural formation



Under acidic conditions at high temperatures, furfural and HMF will be generated from hexosans and pentosans, respectively [7]. Fufural formation increases as both pretreatment temperature and acid charge increase (Fig. 9). Amounts of fufural formed are between 1.0 and 1.5 wt% of corncob (or 5–7.5 g/L) at a temperature 170 °C for the range of acid charge of between 1.1% and 2.2%. Maximal fufural formation occurs at about 3 wt% od cob (15 g/L) and 190 °C. HMF formation also increases as pretreatment temperature increases. However, HMF formation first increases then decreases as acid charge increases (Fig. 10). Maximal HMF formation at 170 °C is about 0.3 wt% od cob (or 1.5 g/L). The results shown in Figs. 9 and 10 suggest that the formation of furfural and HMF is strongly dependent on temperature. To facilitate fermentation of xylose in the pretretament hydrolysate, a low temperature may be preferred to reduce the formation of furan—for example, 120 °C at sulfuric acid concentration of 0.75% (v/v) [10]. Therefore, optimization needs to integrate with fermentation for a given yeast strain.

Fig. 10 Effects of pretreatment temperature and sulfuric acid charge on HMF formation



Conclusions

Aqueous pretreatment of corncob using dilute sulfuric acid can result in excellent monomeric sugar recovery for bioethanol production. A liquid-to-corncob-solid ratio as low as 2 can result in efficient sugar production through aqueous pretreatment. Optimal pretreatment temperature is between 160 and 170 °C based on xylose yield, EHGY, and monomeric sugar yield on unit thermal energy consumption, and the formation of fermentation inhibitors (acetic acid, furfural, and HMF). Optimal acid charge should be above 2.2 and 1.8% for pretreatment conducted at 160 and 170 °C, respectively. At 170 °C and an acid charge 2.2%, we achieved a total glucose yield of 97% and xylose recovery of 75%, which was equivalent to an overall monomeric sugar recovery of about 88%. We produced a hydrolysate with xylose concentration of about 12% with xylose-to-acetic-acid ratio of 8 and xylose-to-the-sum-of-furfural-and-HMF ratio of about 15. To reduce the formation of furan to facilitate fermentation of pretreatment hydrolysate, mild pretreatment using low temperature and acid dosage needs to be studied in detail.

Acknowledgments This work was supported by the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (Project No. 2010-0020141) to J.W. Lee and by EdeniQ under CRADA 08-RD-11111126-007 with the Forest Products Laboratory. The Tianjin Education Commission of China and the USDA Forest Service provided financial support for Wang's visiting appointment at the Forest Products Laboratory. The authors thank Frederick J. Matt of the Analytical Chemistry and Microscopy Laboratory of the USDA Forest Products Laboratory (FPL) for carrying out the carbohydrate determination and Diane Dietrich (FPL) for conducting HMF and furfural analysis.

References

1. Becall, D. S., & Ingram, L. O. (1992). Conversion of hydrolysate of corn cobs and hulls into ethanol by recombinant *Escherichia coli* B containing integrated genes for ethanol production. *Biotechnology Letters, 14*(9), 857–862.
2. Cao, N. J., Krishnan, M. S., Du, J. X., Gong, C. S., Ho, N. W. Y., Chen, Z. D., et al. (1996). Ethanol production from corn cob pretreated by the ammonia steeping process using genetically engineered yeast. *Biotechnology Letters, 18*(9), 1013–1018.
3. Chen, M., Xia, L., & Xue, P. (2007). Enzymatic hydrolysis of corncob and ethanol production from cellulosic hydrolysate. *International Biodeterioration and Biodegradation, 59*, 85–89.
4. Chum, H. L., Johnson, D. K., Black, S. K., & Overend, R. P. (1990). Pretreatment-catalyst effects of the combined severity parameter. *Applied Biochemistry and Biotechnology, 24*(25), 1–14.
5. Foley, K. (1978). *Physical properties, chemical properties and use of the Anderson's corbcob products*. Maumee: The Andersons.
6. Inglett, G. E. (1970). *Corn: Culture, processing and products*. Westport: AVI.
7. Larsson, S., Palmqvist, E., Hahn-Hagerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., et al. (1999). The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme and Microbial Technology, 24*, 151–159.
8. Lee, J.-W., Rodrigues, R. C. L. B., & Jeffries, T. W. (2009). Simultaneous saccharification and ethanol fermentation of corn cob pretreated with oxalic acid using response surface methodology. *Bioresource Technology, 100*, 6307–6311.
9. Liu, K., Lin, L., Yue, J., Li, X., Fang, X., Zhu, M., et al. (2010). High concentration ethanol production from corncob residues by fed-batch strategy. *Bioresource Technology, 101*(13), 4952–4958.
10. Shen, Y., Zhang, Y., Ma, T., Bao, X., Du, F., Zhuang, G., et al. (2008). Simultaneous saccharification and fermentation of acid-pretretaed corncobs with recombinant *Saccharomyces cerevisiae* expressing beta-glucosidase. *Bioresource Technology, 99*, 5099–5103.
11. USDA. (2009). *Crop production 2008 summary*. Washington: National Agricultural Statistics Service.
12. Zhang, M., Wang, F., Su, R., Qi, W., & He, Z. (2010). Ethanol production from high dry matter corncob using fed-batch simultaneous saccharification and fermentation after combined pretreatment. *Bioresource Technology, 101*(13), 4959–4964.
13. Zhu, J. Y., & Pan, X. J. (2010). Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation. *Bioresource Technology, 101*, 4992–5002.