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Pretreatment of Corn Stover by Soaking in Aqueous Ammonia at Moderate Temperatures

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Abstract

Soaking in aqueous ammonia at moderate temperatures was investigated as a method of pretreatment for enzymatic hydrolysis as well as simultaneous saccharification and cofermentation (SSCF) of corn stover. The method involves batch treatment of the feedstock with aqueous ammonia (15–30 wt%) at 40–90°C for 6–24 h. The optimum treatment conditions were found to be 15 wt% of NH_3, 60°C, 1 : 6 of solid-to-liquid ratio, and 12 h of treatment time. The treated corn stover retained 100% glucan and 85% of xylan, but removed 62% of lignin. The enzymatic digestibility of the glucan content increased from 17 to 85% with 15 FPU/g-glucan enzyme loading, whereas the digestibility of the xylan content increased to 78%. The treated corn stover was also subjected to SSCF test using Spezyme-CP and recombinant *Escherichia coli* (KO11). The SSCF of the soaking in aqueous ammonia treated corn stover resulted in an ethanol concentration of 19.2 g/L from 3% (w/v) glucan loading, which corresponds to 77% of the maximum theoretical yield based on glucan and xylan.

Index Entries: Biofuel; bioethanol; biomass conversion; simultaneous saccharification and cofermentation; hemicellulose; lignin.

Introduction

Hemicellulose is the second largest carbohydrate source in the lignocellulosic biomass. Effective utilization of it is a necessary element in biomass conversion process. In most known process schemes of biomass conversion, the hemicellulose fraction is recovered in liquid during the pretreatment stage. It is so because most pretreatment methods apply temperatures high enough to solubilize the hemicellulose. These conventional pretreatment methods generate hydrolyzates containing a mixture of sugars, lignin, and various decomposed products, which are inhibitor to enzymatic hydrolysis and toxic to bioconversion processes *(1–6)*. A previous study indicates that hemicellulose removal becomes significant at temperatures above 130°C *(7)*. In order to utilize the soluble hemicellulose in the pretreatment hydrolyzate, it must be detoxified before

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it is subjected to bioprocessing. Detoxification is not an established process at this time, but it is a significant cost factor in the overall bioconversion scheme.

In our previous work on pretreatment, corn stover was treated in aqueous ammonia at room temperature (soaking in aqueous ammonia [SAA]). We found that with proper operation of this process, one can remove 74% of the lignin, but retain nearly 100% of glucan and more than 85% of xylan *(8)*. It is one of the few pretreatment methods wherein both glucan and xylan are retained. The treated corn stover was found to be highly digestible by cellulase. As the hemicellulose fraction mostly remains intact along with cellulose fraction, they can be hydrolyzed by cellulase enzyme to give glucose and xylose. It is to be noted that most commercial "cellulose" enzymes do exhibit xylanase activity as well as glucanase activity. Such enzyme hydrolyzate is nontoxic, therefore, can be put through a subsequent microbial conversion without detoxification. Elimination of detoxification is a significant cost-saving measure. Retention of hemicellulose in solid during pretreatment is a desirable feature because the overall bioconversion can be carried out without separate recovery and processing of xylose from the pretreatment liquid *(8)*.

In previous SAA at room temperature, a problem arose that the reaction time in the range of 10 d is required to treat the feedstock properly by this method. In this study, SAA with elevated temperatures was investigated to see if the reaction time can be reduced and still retain the hemicellulose fraction. The focus of this work is to evaluate the overall effectiveness of the SAA at moderate temperature as a pretreatment process. The effects of reaction parameters on the composition and the digestibility of the remaining glucan and xylan were investigated. The reaction parameters of interest were solid-to-liquid ratio, reaction time, and ammonia concentration. A recombinant *Escherichia coli*, strain KO11 (American Type Culture Collection (ATCC®) 55124, Manassas, VA) is reported to utilize hexose as well as pentose and convert them into ethanol efficiently *(9,10)*. A simultaneous saccharification and cofermentation (SSCF) using this strain and a cellulase enzyme was used in this work, to evaluate the SAA and the overall conversion scheme as the bioprocess to produce ethanol from lignocellulosic biomass.

Materials and Methods

Materials

Air-dried ground corn stover was supplied by the National Renewable Energy Laboratory (NREL, Golden, CO). The corn stover was screened to a nominal size of 9–35 mesh. The initial composition of the corn stover, as determined by NREL, was: 36.1 wt% glucan, 21.4 wt% xylan, 3.5 wt% arabinan, 1.8 wt% mannan, 2.5 wt% galactan, 17.2 wt% Klason lignin, 7.1 wt% ash, 3.2 wt% acetyl group, 4.0 wt% protein, and 3.6 wt% uronic acid. α-Cellulose was purchased from Sigma (Cat. No. C-8200, St. Louis, MO Lot No. 11K0246). Cellulase enzyme, Spezyme CP (Genencor, Palo Alto, CH, Lot No. 301-00348-257) was obtained from NREL. The average activity and

the protein content of the enzyme, as determined by NREL were: 31.2 filter paper unit (FPU)/mL and 106.6 mg/mL, respectively. Activity of β-glucosidase (Novozyme 188 from Novo Inc., Sigma Cat. No. C-6150, Lot No. 11K1088) was 750 CBU/mL. Recombinant *E. coli* ATCC 55124 (KO11) was used for the SSCF tests. LB medium (Sigma Cat. No. L-3152) was used for the growth of KO11, which contained 1% tryptone, 0.5% yeast extract, 1% NaCl, and 40 mg/L chloroamphenicol.

Experimental Setup and Operation

Corn stover was treated with 15–30 wt% of aqueous ammonia in screw-capped laboratory bottles at 40–90°C for 6–24 h. Solid-to-liquid ratios ranging 1 : 2–1 : 10 were applied. After soaking, the solids were separated by filtering, washed with DI water until pH reached 7.0, and subjected to the enzymatic digestibility tests. Klason lignin, carbohydrate content, and digestibility were determined by NREL Chemical Analysis and Testing Standard Procedure *(11)*.

Digestibility Test

The enzymatic digestibility of corn stover was determined in duplicates following the procedure of the NREL Chemical Analysis and Testing Standard Procedure *(11)*. The conditions of the enzymatic digestibility tests are 50°C and pH 4.8 (0.05 *M* sodium citrate buffer). Enzyme loadings were: 15 and 60 FPU of Spezyme CP/g-glucan, supplemented with 30 CBU of β-glucosidase (Novozyme 188)/g-glucan. The initial glucan concentration was 1% (w/v). One hundred mL of total liquid was used in the digestibility test. Screw-capped Erlenmeyer flasks (250 mL) containing the enzyme hydrolysis preparations were placed in an incubator shaker (New Brunswick Scientific, Innova-4080). Samples were taken periodically and analyzed for glucose, xylose, and cellobiose content using high-performance liquid chromatography (HPLC). Total released glucose after 72 h of hydrolysis was used to calculate the enzymatic digestibility. α-Cellulose and untreated corn stover were put through the same procedure as a reference and control.

Simultaneous Saccharification and Cofermentation

A 250-mL Erlenmeyer flask was used as the bioreactor. It was shaken in the incubator (New Brunswick Scientific, Innova-4080) at 38°C and 150 rpm (0.64*g*). Into a 100 mL working volume of liquid, treated corn stover sample was introduced to reach 3% (w/v) glucan content in the reactor. α-Cellulose was put through the same procedure as the control. The SSCF runs were performed with buffer without external pH control, starting at pH 7.0 at the beginning of the fermentation and gradually decreasing to pH 6.0 at the end. The loading of cellulase enzyme (Spezyme CP) was 15 FPU/g-glucan, and that of β-glucosidase (Novozyme 188) was 30 CBU/g-glucan. The ethanol yield in SSCF test was calculated as follows:

$$\text{Theoretical maximum ethanol yield (\%)} = \frac{\text{Ethanol produced (g) in reactor}}{\text{Initial sugar (g) in reactor} \times 0.511} \times 100$$

Note: Sugar is interpreted as glucose plus xylose in the SSCF work.

Analytical Methods

The solid samples, such as treated/untreated corn stover, α-cellulose, and so on, were analyzed for sugar and Klason lignin following NREL Chemical Analysis and Testing Standard Procedures *(11)*. Each sample was analyzed in duplicates. Sugars were determined by HPLC using a Bio-Rad Aminex HPX-87P column (BioRad Laboratories, Hercules, CA). For the SSCF tests, HPX-87P and 87H columns were used to measure the sugar content and ethanol, respectively. An YSI 2300 Glucose/Lactate analyzer (YSI Incorporated, Yellow Springs, OH) was used for rapid analysis of glucose during inoculums preparation. A refractive index detector was used for HPLC analysis.

Scanning Electron Microscope

Untreated and treated corn stover samples were freeze-dried before observation through a scanning electron microscope (ZEISS, Thornwood, NY Model-DSM940).

Results and Discussion

Effect of Reaction Temperature and Ammonia Concentration

The effects of reaction temperature and ammonia concentration in SAA were investigated. The results are summarized in Table 1. Two different ammonia concentrations and three different temperatures were applied at each concentration. As seen in Table 1, the major compositional changes are in the lignin. Delignification increased from 50 to 77% as temperature was increased from 40°C to 90°C (Table 1). Increase of ammonia concentration from 15 to 30% showed little effect on delignification. Treatment at 60°C with 15–30 wt% of ammonia achieves 67–71% of delignification. The glucan contents were well preserved over the entire range of treatment condition. About 80% of the xylan is preserved in the SAA. Xylan loss of 20% is much lower than those observed from other treatment methods using aqueous ammonia *(8,12,13)*. Increasing of the ammonia concentration from 15 wt% to 30 wt% resulted in slightly improved enzymatic digestibility of the treated samples. The condition of 15 wt% of ammonia at 60°C carries a special meaning because the system pressure under this condition equals the atmospheric pressure. The SAA can thus be carried out without the use of pressure vessel, a significant cost benefit.

Table 1
Effect of Reaction Temperatures and Ammonia Concentrations on the Compositions and the Enzymatic Digestibility in SAA-Treated Corn Stover[a]

NH3 concentration (wt%)	Temperature (°C)	S.R. (%)[b]	Lignin (%)[c]	Delignification	Solid (%)		Enzymatic digestibility (%)[d]	
					Glucan	Xylan	Glucan	Xylan
Untreated	–	–	17.2	–	36.1	21.4	17.2	12.5
	–	–	± 0.4	–	± 0.3	± 0.2	± 1.5	± 1.2
30	40	77.4	8.4	51.2	36.1	17.7	86.5	75.4
	–	± 2.5	± 0.2	± 1.2	± 0.5	± 0.3	± 2.5	± 1.8
	60	69.6	5.1	70.4	35.9	17.5	90.3	82.2
	–	± 1.1	± 0.4	± 2.5	± 0.2	± 0.4	± 1.6	± 1.7
	90	68.1	3.9	77.3	35.6	16.0	98.0	85.2
	–	± 2.0	± 0.3	± 1.6	± 0.4	± 0.1	± 2.1	± 2.2
15	40	76.0	8.6	50.0	36.9	17.9	80.0	72.5
	–	± 1.6	± 0.6	± 3.7	± 0.3	± 0.6	± 1.5	± 1.3
	60	71.4	5.6	67.4	36.1	17.2	90.1	79.8
	–	± 2.1	± 0.5	± 2.9	± 0.7	± 0.4	± 2.7	± 1.9
	90	67.3	3.9	77.3	35.8	16.5	93.4	81.5
	–	± 1.0	± 0.2	± 0.9	± 0.6	± 0.5	± 1.5	± 1.1

[a]Data in the table are based on the oven-dry untreated biomass. Values are expressed as mean and standard deviation ($n = 2$ for the composition analysis, $n = 4$ for the enzymatic digestibility). Pretreatment conditions: 15–30 wt% of ammonia concentration, 40–90°C of reaction temperature, 24 h of reaction time, and 1 : 10 (based on wt) of solid : liquid ratio.

[b]S.R. stands for solid remaining after reaction.

[c]Klason lignin.

[d]Digestibility at 72 h, enzymatic hydrolysis conditions: 15 FPU/g-glucan, pH 4.8, digestibility at 50°C and 150 rpm. Digestibility (%) = ([grams of glucan or xylan digested by enzyme]/[grams of glucan or xylan added]) × 100.

Most of the subsequent experiments were therefore carried out under this condition.

Effect of Reaction Time and Solid-to-Liquid Ratio

In order to study the effect of reaction time, three different reaction times (6, 12, and 24 h) were applied keeping the reaction temperature at 60°C and the ammonia concentration at 15 wt%, and the solid : liquid ratio at 1 : 6. The composition data and enzymatic digestibility after these treatments are summarized in Table 2. The data indicate that the xylan and lignin remaining in the solids generally decrease as reaction time increase. Solubilization of xylan is 17–19%, and lignin removal was in the range of 47–69% with 6–24 h of retention time. However, increase beyond 12 h of treatment was insignificant.

The effect of solid-to-liquid ratio was tested at 60°C. The results are summarized in Table 3. Lignin removal and enzymatic digestibility increased

Table 2
Effect of Reaction Time On The Compositions and Enzymatic Digestibility in SAA-Treated Corn Stover[a]

Time (h)	S.R. (%)[b]	Lignin (%)[c]	Delignification (%)	Solid (%)		Enzymatic digestibility (%)[d]	
				Glucan	Xylan	Glucan	Xylan
Untreated	–	17.2	–	36.1	21.4	17.2	12.5
	–	± 0.4	–	± 0.3	± 0.2	± 1.5	± 1.2
6	75.2	9.1	47.1	36.1	18.8	79.7	71.9
	± 2.4	± 0.6	± 3.5	± 0.2	± 0.3	± 1.5	± 0.5
12	71.3	6.4	62.6	36.1	17.8	85.0	77.9
	± 1.5	± 0.3	± 1.8	± 0.0	± 0.5	± 1.2	± 2.2
24	71.1	5.3	69.3	35.9	17.4	86.4	78.4
	± 1.8	± 0.2	± 1.0	± 0.4	± 0.2	± 1.1	± 17

[a]Data in the table are based on the oven-dry untreated biomass. Values are expressed as mean and standard deviation ($n = 2$ for the composition analysis, $n = 4$ for the enzymatic digestibility). Pretreatment conditions: 15 wt% of ammonia concentration, 60°C of reaction temperature, and 1 : 6 of solid : liquid ratio (based on wt).

[b]S.R. stands for solid remaining after reaction.

[c]Klason lignin.

[d]Digestibility at 72 h, enzymatic hydrolysis conditions: 15 FPU/g-glucan, pH 4.8, digestibility at 50°C and 150 rpm. Digestibility (%) = ([grams of glucan or xylan digested by enzyme]/[grams of glucan or xylan added]) × 100.

when the solid-to-liquid ratio was increased from 1 : 2 to 1 : 10. Delignification increased from 38 to 67%. The digestibility (with 15 FPU/g-glucan) also increased steadily from 74 to 91%. However, Xylan removal stayed relatively constant at 17–18%. The overall view is that a 1 : 6 ratio of solid/liquid is near the optimum level as it represents the minimum S : L ratio that can give a satisfactory pretreatment effect (85% glucan digestibility at 72-h). On the basis of the collective experimental data of delignification, xylan remaining, and digestibility we determined the optimum operating condition of the SAA to be: 60°C, 12 h of reaction time, and 1 : 6 of S : L ratio.

The enzymatic digestibility profile of a representative SAA-treated corn stover is shown in Fig. 1. The digestibilities at 72 h with 15 FPU/g-glucan are 85% and 78% for glucan and xylan, respectively. In the initial phase of profile, hydrolysis rate of SAA-treated corn stover is much higher than that of α-cellulose, perhaps owing to the presence of less crystalline cellulose in the treated corn stover. As indicated by the 78% of xylan digestibility, Spezyme CP obviously has a substantial amount of xylanase activity as well as glucanase activity. Judging from the digestibility values, the xylanase activity of Spezyme CP measured against the SAA-treated corn stover is somewhat lower than glucanase activity.

Table 3
Effect of Solid-to-Liquid Ratio on the Compositions and Enzymatic Digestibility in SAA-Treated Corn Stover[a]

Solid-to-liquid	S.R. (%)[b]	Lignin (%)[c]	Delignification (%)	Solid (%)		Enzymatic digestibility (%)[d]	
				Glucan	Xylan	Glucan	Xylan
Untreated	–	17.2	–	36.1	21.4	17.2	12.5
	–	± 0.4	–	± 0.3	± 0.2	± 1.5	± 1.2
1 : 2	79.0	10.7	37.7	36.1	18.1	74.0	66.2
	± 0.9	± 0.7	± 4.1	± 0.1	± 0.2	± 1.1	± 1.2
1 : 4	74.4	8.2	52.6	36.1	17.4	81.3	73.6
	± 10.4	± 0.4	± 2.2	± 0.3	± 0.4	± 0.5	± 2.2
1 : 6	71.3	6.4	62.6	36.1	17.8	85.0	77.9
	± 1.5	± 0.3	± 1.8	± 0.0	± 0.5	± 1.2	± 2.2
1 : 8	71.6	6.1	64.8	35.3	18.4	87.1	77.8
	± 0.8	± 0.1	± 0.8	± 0.2	± 0.1	± 0.2	± 1.7
1 : 10	71.4	5.6	67.2	36.1	17.2	90.1	79.8
	± 2.1	± 0.5	± 2.9	± 0.7	± 0.4	± 2.7	± 1.9

[a]Data in the table are based on the oven-dry untreated biomass. Values are expressed as mean and standard deviation (n = 2 for the composition analysis, n = 4 for the enzymatic digestibility). Pretreatment conditions: 15 wt% of ammonia concentration, 60°C of reaction temperature, 1 : 2–1 : 10 of solid : liquid ratio (based on wt), and 12 h of reaction time.

[b]S.R. stands for solid remaining after reaction.

[c]Klason lignin.

[d]Digestibility at 72 h, enzymatic hydrolysis conditions: 15 FPU/g-glucan, pH 4.8, digestibility at 50°C and 150 rpm. Digestibility (%) = ([grams of glucan or xylan digested by enzyme]/[grams of glucan or xylan added]) × 100.

Selectivity of Lignin Removal Over Xylan Removal

Soluble lignin and its derivatives are toxic to microorganism and they also inhibit the enzymatic hydrolysis. The lignin content is, therefore, one of the major factors hindering the SSCF process *(1–6,14)*. Our previous study has also shown that removal of lignin form biomass substrates, which improves the microbial activity and the enzyme efficiency *(8,12,13,15,16)*. Lignin removal and xylan retention are two major factors in the SAA. The main purpose of the SAA is to maximize these factors with a constraint that we attain acceptable level of digestibility. We paid close attention to these reactions, especially the ratio of the lignin removal reaction over xylan removal reaction. We now introduce a selectivity defined as follows:

$$\text{Selectivity} = \frac{m_{\text{Lignin}}}{m_{\text{Xylan}}}$$

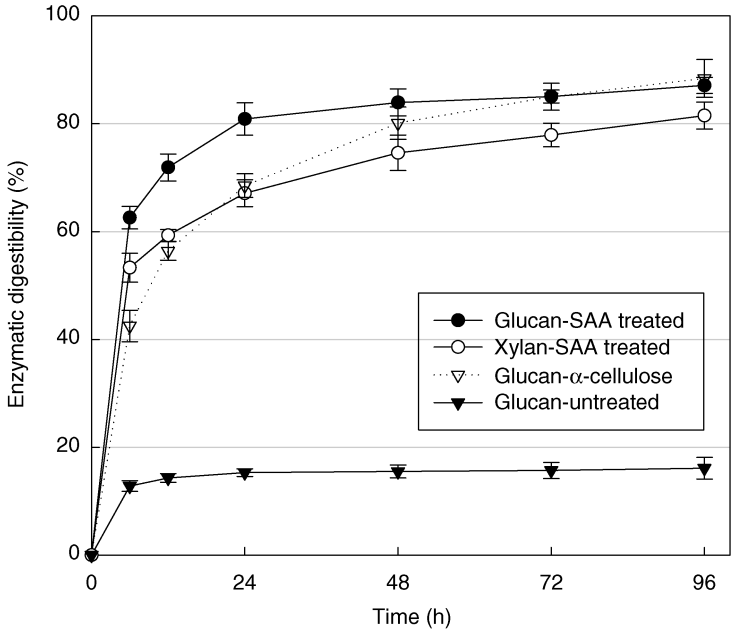

Fig. 1. Comparison of enzymatic hydrolysis between untreated and SAA-treated samples (*Note:* 15 FPU of cellulase/g-glucan, 30 CBU of β-glucosidase/g-glucan 50°C, 150 rpm, pretreatment conditions: 15 wt% of ammonia concentration, 60°C of reaction temperature, 12 h of reaction time, and 1 : 6 (based on wt) of solid : liquid ratio. Digestibility (%) = ([grams of glucan or xylan digested by enzyme/grams of glucan or xylan added]) × 100. The data in the figure show the mean value [$n = 4$]).

Where, m_{Lignin} and m_{Xylan} are the mass loss rate of lignin and xylan from the solid. The temperature effects on experimentally determined selectivity are presented in Fig. 2. The data clearly show that the selectivity attains maximum at 60°C for the two levels of ammonia concentrations (15 and 30 wt%). The aforementioned optimum temperature of 60°C for SAA is therefore reaffirmed from the standpoint of selectivity as well.

Scanning Electron Microscope

Physical changes because of SAA were observed in the scanning electron microscope pictures of treated and untreated samples. Figure 3 shows that SAA treatment altered the biomass structure significantly. The untreated sample shows rigid, ordered fibrils, and connected structure (Fig. 3A). In the treated samples, the fibers are somewhat separated and exposed. A large amount of mass appears to have been removed from the initial connected structure (Fig. 3B). Pinholes and gaps are also visible in the treated corn stover, leading to a speculation that the surface area and the porosity have also increased.

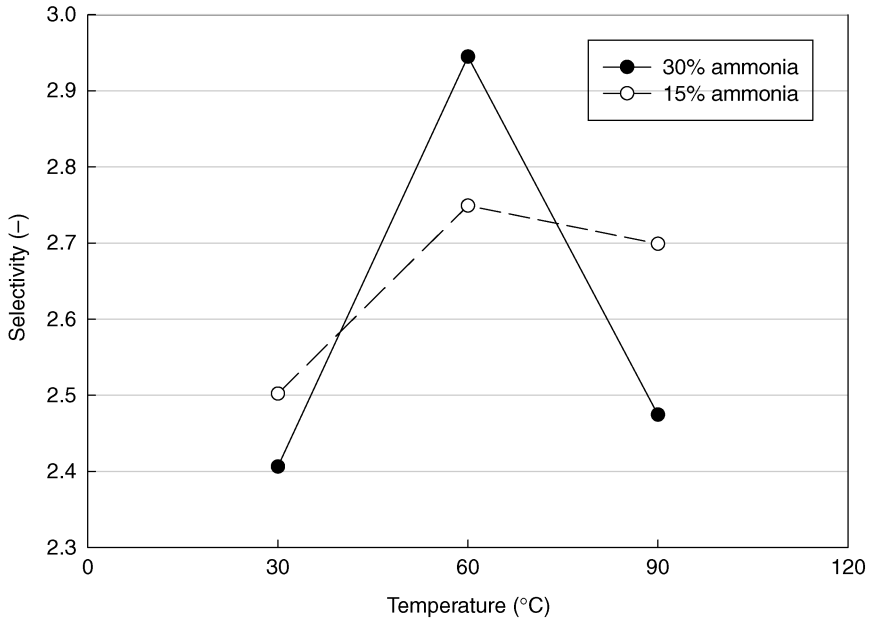

Fig. 2. Selectivity plot of the SAA-treated corn stover on various temperature and ammonia concentrations (*Note:* 1. Data in the figure are based on the oven-dry untreated biomass; Pretreatment conditions: 24 h of reaction time, 1 : 10 [based on wt] of solid : liquid ratio, 15 or 30 wt% of ammonia concentration, 40–90°C of reaction temperature. 2. Selectivity = m_{Lignin}/m_{Xylan} [where, m_{Lignin} and m_{Xylan} are the mass loss rate of lignin and xylan in the solid, respectively]. The data in the figure show the mean value [$n = 2$; standard deviation < 0.35]).

Simultaneous Saccharification and Cofermentation

SSCF of SAA-treated corn stover and α-cellulose was performed using recombinant *E. coli* ATCC 55124 (KO11) and Spezyme CP. The SAA conditions for the corn stover were: 15 wt% ammonia, 60°C, 12 h of treatment time, and 1 : 6 of solid-to-liquid ratio. Figures 4 and 5 present ethanol and sugar concentrations in the SSCF performed over an extended period. With initial feed of corn stover equivalent to 3 g of glucan/100 mL, the maximum ethanol concentration reached 19.2 g/L. It was attained after 96 h. This represents 77% of the maximum theoretical yield based on glucan and xylan. The same ethanol yield from SSCF is interpreted as 113% on the basis of glucan alone, a clear indication that both the xylan and glucan were converted to ethanol by the SSCF. The ethanol yield from the treated corn stover is substantially higher than that of α-cellulose performed with same glucan loading. The sugar profiles of Fig. 4 further indicate that glucan and xylan are consumed concurrently by *E. coli* (KO11), an important feature from a process viewpoint. The separate yield of ethanol from glucan and that from xylan were not identifiable in this work. The main purpose of the

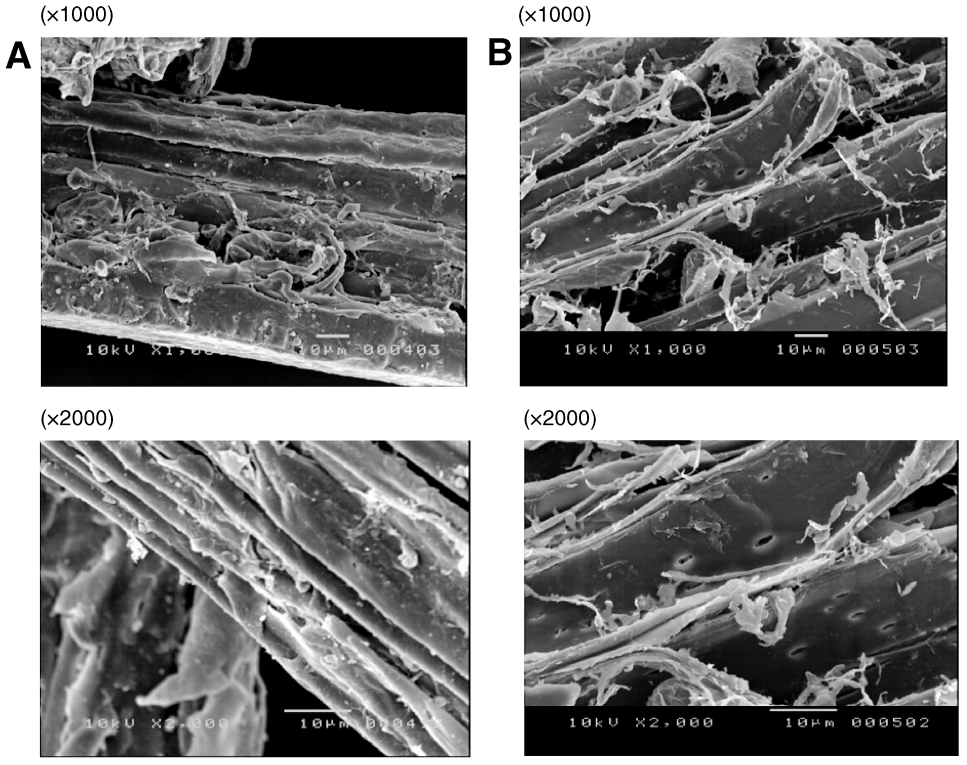

Fig. 3. Scanning electron micrographs of treated **(A)** and untreated corn stover **(B)** (*Note:* Pretreatment conditions: 15 wt% of ammonia concentration, 60°C of reaction temperature, 12 h of reaction time, and 1 : 6 [based on wt] of solid : liquid ratio).

SSCF is to convert both hexose and pentose in a single reactor. The SSCF described in this work serves that purpose well.

Conclusion

SAA at moderate temperatures is a pretreatment method suitable for corn stover. This process is simple and requires low process energy. In this method, most of the xylan and all of glucan are retained after the treatment. SAA operated at 60°C reduces the reaction time from 10 d of room temperature operation to 12 h. The optimum operating conditions for the SAA at moderate temperature are 15 wt% ammonia, 60°C, 12 h, and 1 : 6 of solid : liquid ratio.

The treated corn stover exhibited enzymatic digestibilities of 85% and 78% for glucan and xylan, respectively, with enzyme loading of 15 FPU/g-glucan. The SAA-treated corn stover was subjected to a SSCF test using Spezyme CP (Genencor cellulase) and recombinant *E. coli* (strain KO11). The ethanol yield in the SSCF was 77% of maximum theoretical yield based on glucan and xylan of the treated sample. The same yield is

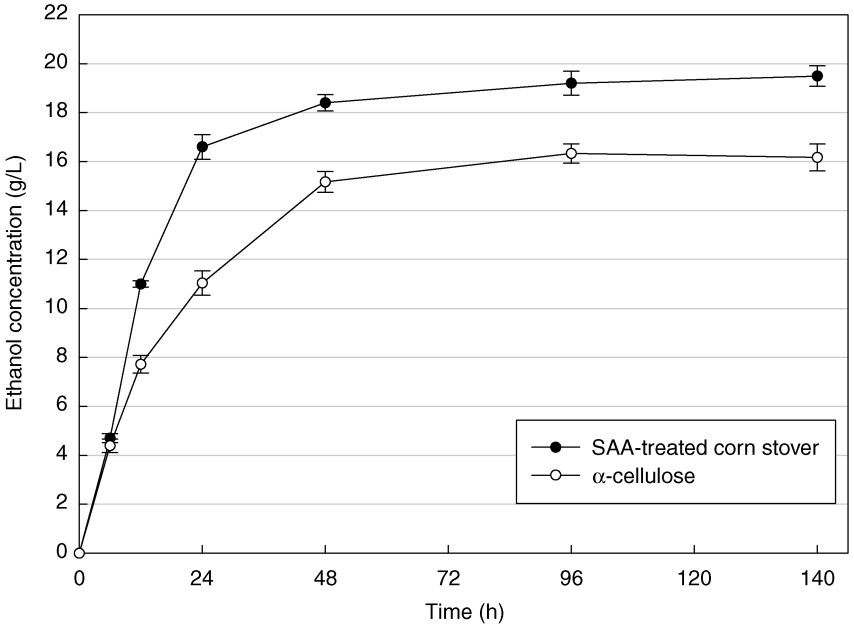

Fig. 4. SSCF of SAA-treated corn stover by recombinant *E. coli* (KO11) (*Note:* Microorganism: *E. coli* ATCC 55124; substrate: 3% [w/v] glucan loading/100 mL working volume; SAA-treated corn stover [15 wt% of ammonia concentration, 60°C of reaction temperature, 12 h of reaction time, and 1 : 6 of solid : liquid ratio]; SSCF: 15 FPU of Spezyme CP/g-glucan; 30 CBU of Novozyme 188/g-glucan; LB medium [0.5% of yeast extract and 1% of tryptone]; anaerobic condition; 38°C, 150 rpm. The data in the figure show the mean value and standard deviation [$n = 4$]).

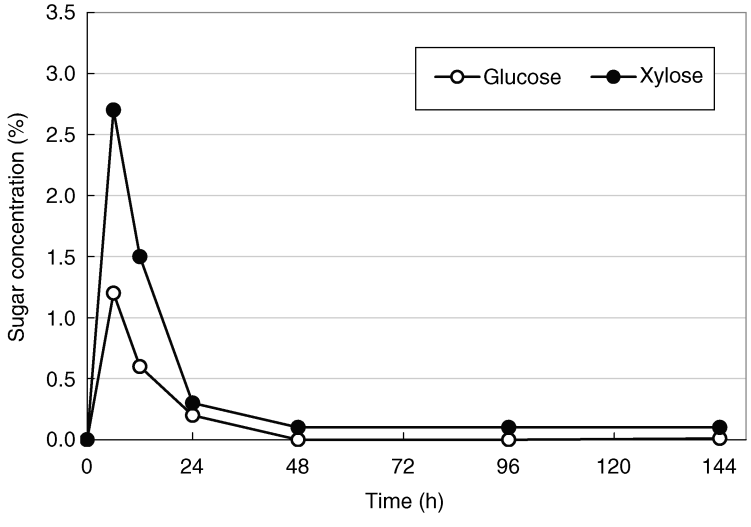

Fig. 5. Sugar concentration profiles in SSCF (*Note:* Microorganism: *E. coli* ATCC 55124; substrate: 3% [w/v] glucan loading/100 mL reactor; SAA-treated corn stover [15 wt% of ammonia concentration, 60°C of reaction temperature, 12 h of reaction time, and 1 : 6 of solid : liquid ratio]; SSCF: 15 FPU of Spezyme CP/g-glucan; 30 CBU of Novozyme 188/g-glucan; LB medium [0.5% of yeast extract and 1% of tryptone]; anaerobic condition; 38°C, 150 rpm. The data in the figure show the mean value [$n = 4$; standard deviation <0.7]).

calculated to be 113% on the basis of glucan alone, a clear indication that the SSCF converts both glucan and xylan into ethanol. The SSCF of SAA-treated corn stove proves to be an efficient one-step bioprocess scheme that can convert both the glucan and xylan in biomass into ethanol.

Acknowledgments

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