Fast Acidogenic Fermentation of Corn Stover Through a Two-Step Method: Nitric Acid Hydrolysis Combined with the Fermentation of Hydrolysate

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This study investigated a novel route for fast acidogenic fermentation of corn stover through a two-step method. Corn stover was first hydrolyzed by nitric acid, and the hydrolysate was then used for acidogenic fermentation. The results showed that the yields of soluble xylose, arabinose, and glucose in hydrolysate obtained from HNO_3 hydrolysis of corn stover at the optimum condition achieved 96.86% of initial xylose, 98.54% of initial arabinose, and 18.58% of initial glucose, respectively. Acidogenic fermentation of hydrolysate proved to be effective, compared to acidogenic fermentation of raw corn stover; the production of volatile fatty acids (VFAs) increased by 54.8%, fermentation time decreased by 50%, and the distribution of VFAs was more suitable for subsequent methanogenic fermentation. This research provided an effective, suitable, and economical method for biogas production from corn stover.

Keywords: Corn stover; Hydrolysis; Acidogenic fermentation; Biogas

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INTRODUCTION

The rapid increase in the demand for alternative energy sources has triggered a renewed interest in producing fuels (such as biodiesel, bioethanol, biogas, and biohydrogen) from biomass (Marzialetti *et al*. 2008; Wang *et al*. 2012a). Governments around the world are seeking the use of indigenous biomass that is readily available and renewable, not only for supplying energy, but also for improving environmental quality and supporting local economies (Marzialetti *et al*. 2008; Isci and Demirer 2006). Corn stover, one of the most efficient biomasses in terms of its high carbohydrate content, is abundant and readily available in China. There are about 0.1 billion tons of corn stover generated from corn annually in China (Zheng *et al*. 2009; Wang *et al*. 2007). Although there are a few methods available for corn stover reutilization, over 50% of corn stover remains unused and causes serious environmental problems due to open field burning and dumped stacking (Zheng *et al*. 2009; Pang *et al*. 2008). Two-thirds of the Chinese population are farmers living in rural areas; they consume about 60% of the total energy usage in China yearly (Zheng *et al*. 2009); thus, it is a challenge to provide enough energy for farmers. Using corn stover to produce biofuels could be a good option for solving the aforementioned challenge, as it not only can supply sustainable energy for famers, but it can also reduce environmental pollution due to energy usage.

As one kind of biomass material, corn stover can be converted to bioenergy sources such as bioethanol and biogas through biological processes. However, the cost of ethanol production is relatively high when using currently accessible technology (Zhang *et al*. 2011a). Therefore, the industrialization of ethanol production from lignocellulosic materials such as corn stover is not economically feasible. The conversion of corn stover into biogas through anaerobic fermentation may be a promising and economical alternative, resulting in the elimination of waste and production of clean biofuel. However, anaerobic fermentation of lignocellulosic materials is a slow process (Yadvika and Sreekrishnan 2004). The main reason for this is that lignocellulosic materials such as corn stover are solid, consisting mainly of cellulose, hemicellulose, and lignin, which are closely associated and are not readily accessible to microorganisms (Lu *et al*. 2008). Many pretreatments have been used prior to anaerobic fermentation to break down the linkages between cellulose, hemicellulose, and lignin, which makes cellulose and hemicellulose accessible to microorganisms and also improves the production of biogas (Zheng *et al*. 2009; Zhao *et al*. 2010). However, the anaerobic fermentation of pretreated lignocellulosic materials is still slow, as the substrate is solid. A new method was proposed that accelerates the anaerobic fermentation process and improves gas production. The principle is that the hydrolysate is primarily composed of small molecule sugars that are more accessible to microorganisms. Thus, the proposal involves transferring as many of the effective components (mainly sugars) of the lignocellulosic material as possible into the liquid phase (by hydrolyzing them) and then anaerobically fermenting the resulting hydrolysate. Therefore, an efficient pretreatment that will not inhibit the subsequent fermentation process is required for obtaining hydrolysate consisting of as much of the fermentable sugars as possible.

A number of pretreatment methods have been investigated, including dilute acid pretreatment, alkali, inorganic salt, liquid hot water, steam explosion, enzymatic pretreatment, and other methods (Pingali *et al*. 2010; Yang *et al*. 2012; Mao *et al*. 2012; Gullon *et al*. 2010; Kaushik and Singh 2011; Lin *et al*. 2010; Ormsby *et al*. 2012). Among all the methods, dilute acid pretreatment is considered to be the most promising because it effectively converts hemicellulose into fermentable sugars. The most widely used and tested approaches are based on dilute sulfuric acid. Another strong acid, nitric acid has been studied by a few researchers. Rodríguez-Chong *et al.* (2004) used nitric acid to pretreat sugar cane bagasse and confirmed that nitric acid pretreatment was more efficient than sulfuric acid and hydrochloric acid, as nitric acid took a shorter time to ensure a high fermentable sugar concentration and a low concentration of inhibitors. Zhang *et al.* (2011b) reported that nitric acid was an effective catalyst for hemicellulose hydrolysis in corn stover, and the maximum yields of monomeric xylose and monomeric arabinose in the liquid fraction reached 96.1% of initial xylose and 100% of initial arabinose, respectively. These results show that nitric acid is effective for hydrolysis of lignocellulosic materials. Furthermore, for methane production, nitric acid pretreatment is more suitable than sulfuric acid pretreatment because sulfate could inhibit subsequent fermentation processes, and the production of H_2S from reducing sulfate is toxic for microorganisms (McCartney and Oleszkiewicz 1993; Oude Elferink *et al*. 1994). Subsequent fermentation processes are not inhibited by nitrate, and the production of N_2 from reducing nitrate is environmentally friendly. Therefore, nitric acid pretreatment is a favorable method for the conversion of lignocellulosic materials into biogas. However, nitric acid is more expensive than sulfuric acid. To improve the economic feasibility of using it, the amount of nitric acid must be reduced. If more fermentable sugars can be obtained with low acid consumption and a short reaction time, the economic interest in hydrolysis of lignocellulose materials with nitric acid would be enhanced; therefore, fast acidogenic fermentation of corn stover through a two-step method could be efficient and economical.

So far, whereas most researchers have reported the enhancement of biogas production from the pretreated corn stover, few studies have discussed the improvement of biogas production of corn stover by transferring as many of the effective components (mainly sugars) of the corn stover as possible into the liquid phase (by hydrolyzing them) and then anaerobically fermenting the resulting hydrolysate. There is need for studies on the pretreatment of corn stover and the acidogenic fermentation of hydrolysate, which could accelerate the fermentation of corn stover. In this work, a two-step acidogenic fermentation method was investigated. The effectiveness of nitric acid pretreatment for corn stover was evaluated based on the fermentable sugar yield in hydrolysate, which can be fermented by microorganisms to biogas. In addition, the anaerobic acidogenic fermentation of hydrolysate was studied to evaluate the feasibility of subsequent methanogenic fermentation.

EXPERIMENTAL

Materials

Corn stover collected at a local farm (Tangshan, China) was used as the raw material. The corn stover was air-dried, milled, screened to select the fraction of particles with a size lower than 0.5 mm, homogenized in a single lot, and stored until needed.

Methods

Dilute acid hydrolysis

The hydrolysis experiments were performed in glass media bottles at 80 ^oC and 120 ^oC, and in a laboratory-scale pure titanium reactor with a total volume of 500 mL at 150 ^oC and 180 ^oC. The glass media bottles were heated to 80 ^oC and 120 ^oC with a water bath and autoclave, respectively, and the reaction times were 10, 30, 60, 90, and 120 min. The pure titanium reactor was heated to 150 ^oC and 180 ^oC with an electric heater, and the reaction times were 2, 5, 10, 30, and 60 min. Nitric acid concentrations were 0.05, 0.1, 0.2, 0.4, and 0.6% (w/w). Each experiment was performed using a liquid-to-solid ratio of 9:1 (w/w). After the reaction was complete, the reactors were cooled to below 50 ^oC with room-temperature water; the wet materials were then filtrated using a vacuum pump, obtaining a solid phase and a liquid phase for further analysis.

Anaerobic acidification fermentation of corn stover hydrolysate

The seed sludge was obtained from a municipal sewage treatment plant located in Tianjin, China. Six 1000-mL continuously stirred tank reactors (CSTR) with an effective volume of 800 mL were used to acclimatize seed sludge. At the beginning of the experiments, each reactor was placed in a water bath at a controlled temperature of 37 ^oC. The seed sludge was acclimated with glucose and xylose. During the start-up stage, the experiments were conducted in semi-continuous mode, in which 30 mL feeding solution was added to each reactor periodically. The composition and concentration of the feeding solutions are shown in Table 1. After acclimatizing the acidogenic bacteria for approx.-imately 15 days, the substrates in the six reactors were mixed together and stored at 4 ^oC. Table 2 shows the composition of the cultured seed sludge.

The acidogenic fermentation of the hydrolysate was performed in 250-mL bottles filled with 50 mL hydrolysate and 150 mL cultured seed sludge. Before acidogenic fermentation, the hydrolysate was diluted to meet the feed to microbes ratio (F/M ratio) of 1.0 g-COD/gVSS determined to be effective by other researchers (Lin *et al*. 2006, 2008). To compare with the acidogenic fermentation of hydrolysate, the acidogenic fermentation of untreated corn stover was performed with the same F/M ratio in an identical reactor containing 1.3 g of untreated corn stover and 200 mL of cultured seed sludge. Assay with seed sludge alone was performed as a control. VFAs produced from acidogenic fermentation of the hydrolysate and untreated corn stover was subtracted from the assay. The pH of each reactor was adjusted to 7.0 using 2 mol/L HCl solution or 4 mol/L NaOH solution. In all experiments, the mixed liquor was first purged with nitrogen for 5 to 10 min to ensure an anaerobic condition. Reactors were placed on a rotary shaker (150 rpm) and incubated at 37 ^{o}C (±1 ^{o}C). During the course of the experiments, the pH and VFA values were monitored and determined at designated time intervals. All experiments were performed in triplicate.

Table 1. Components of Nutrient Solution

Composition	Concentration (g/L)	Composition	Concentration (g/L)
Glucose	10	$ZnCl_2$	0.05
Xylose	20	$CuCl_2$	0.03
NH_4Cl	2.88	$MnSO_4\bullet H_2O$	0.05
KH_2PO_4	0.66	$(NH_4)_6MoO_24\bullet H_2O$	0.05
$CaCl_2\bullet 2H_2O$	0.03	$AlCl_3$	0.05
$MgSO_4\bullet 7H_2O$	0.025	$CoCl_2\bullet H_2O$	0.05
$FeSO_4\bullet 7H_2O$	0.02	$NiCl_2$	0.05
H_3BO_3	0.05		

Table 2. Components of the Cultured Seed Sludge

Parameters	Units	Average Values
pH	-	4.50
Total Suspended Solids (TSS)	mg/L	6170.00
Volatile Suspended Solids (VSS)	mg/L	4000.00
Acetic acid	mg/L	1086.81
Propionic acid	mg/L	1108.58
Butyric acid	mg/L	482.77
Valeric acid	mg/L	567.96

Analysis methods

The chemical composition of the raw material and WIS fraction was determined according to the National Renewable Energy Laboratory analytical methods for biomass (NREL 2006).

Monomeric sugars were determined using HPLC (LabAlliance, USA) with a refractive detector using a column (BioRad Aminex HPX-87H, 300× 7.8 mm) at 65 ^{o}C and 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min. A refractive detector was used for sugar and acetic acid analysis, and a UV detector was used for analysis of furfural and 5-hydroxymethyl-2-furfural (HMF).

Because most of the sugars present in the hydrolysate obtained after the HNO_3 hydrolysis were in oligomeric form, all filtrated hydrolysate samples were autoclaved with 4% (w/w) H_2SO_4 for 60 min at 121 ^{o}C to break the oligomeric sugars down into monomeric sugars, and the concentrations of the sugars were determined before and after autoclaving. Sugar recovery standard (SRS) was measured to determine a correction factor for the hydrolysis treatment. The amount of oligomeric sugars in hydrolysate was calculated as follows:

Oligomeric sugar (g) = Total sugars (g) in the hydrolysate after autoclaving – Monomeric sugar (g) in the hydrolysate before autoclaving.

Percent yield of xylose was calculated as follows,

$$\text{Xylan-to-xylose conversion (\%)} = \frac{Mass_{xylose \cdot in \cdot liquor} \times 0.88}{Mass_{xylan \cdot in \cdot the \cdot pretreated \cdot corn \cdot stover}} \times 100 \qquad (1)$$

where the factor 0.88 corrects for xylan-to-xylose hydration. A similar equation was used to determine percent yield of glucose and arabinose.

The samples from the acidogenic fermentation process were centrifuged at 8000 xg for 5 min and filtrated through 0.45-μm filters before being subjected to HPLC to determine VFA concentrations. The pH was determined using a pH-meter (HORIBA, D-51, Japan).

Combined severity factor

In this study, the effect of the hydrolysis conditions on the concentration of total sugars and inhibitors was evaluated using the combined severity factor (CSF), which combines catalyst concentration, reaction time, and temperature into a single variable (Sun *et al*. 2011; Zhou *et al*. 2011; Hsu *et al*. 2010; Pedersen and Meyer 2010). The combined severity factor (CSF) was defined as,

$$CSF = log\left(t \cdot exp\left(\frac{T - 100}{14.75} \right) \right) - pH \qquad (2)$$

where *t* is the reaction time (min), *T* is the reaction temperature (^{o}C), and pH is the acidity of aqueous solution determined by acid concentration.

RESULTS AND DISCUSSION

Raw Material Composition

The composition obtained for the corn stover was (weight percent on dry basis): glucan, 36.2%; xylan, 19.0%; araban, 2.9%; Klason lignin, 14.0%; ash, 7.4%; and others, 16.1%. These values were in the range of lignocellulosic materials reported by other researchers (Kim *et al*. 2003; Lu *et al*. 2009).

Effect of Nitric Acid Concentration on Xylose Yield

Figure 1 shows the effect of HNO_3 concentration on xylose yield for corn stover. From Fig. 1, it can be concluded that increasing the HNO_3 concentration can increase the xylose yield when hydrolysis is carried out at 150 ^{o}C for 10 min. By increasing the HNO_3 loading to 0.05%, 0.10%, 0.20%, 0.40%, and 0.60% (w/w), the soluble xylose (monomeric and oligomeric xylose in hydrolysate) yields increased to 17.75%, 20.46%, 43.56%, 84.99%, and 91.55% of initial xylose, respectively. However, the total amount of xylose in the hydrolysate and water-insoluble solids decreased from 95.77% to 92.62%

of initial xylose with increasing HNO_3 concentration. These results confirm that an undesirable decomposition reaction of xylose occurred, and the decomposition products of xylose were mainly furfural. More and more xylose would be degraded to furfural if the HNO_3 concentration was increased. To obtain the high xylose yield, 0.60% HNO_3 was thus considered optimal under the present experimental conditions.

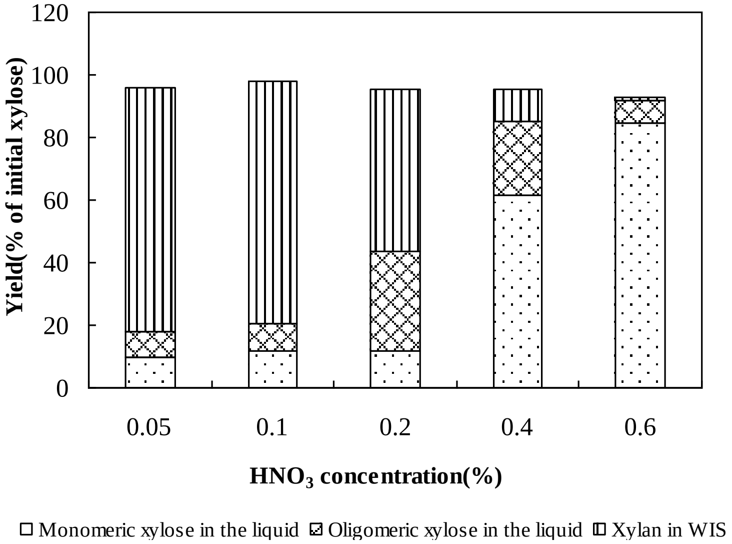

Fig. 1. Yields of xylose in liquids and water-insoluble solids (WISs) from the pretreatments at 150 °C for 10 min with different HNO_3 concentrations

Effect of Temperature and Time on Hemicellulose Hydrolysis

Figure 2 shows the effects of temperature and time on xylose yields. The effects of temperature and time are dramatic. As shown in Fig. 2(A), the amount of xylan in water-insoluble solids decreased slowly with time; meanwhile, the total yield of monomeric and oligomeric xylose in liquid increased with a narrow range (from 10.67% to 16.66% of initial xylose) with increasing pretreatment time at 80 °C. The low yield of xylose in the hydrolysate was most likely due to low temperature, which implies that more energy is required to break down the hemicellulose. If a high yield of xylose is expected, high temperatures are needed.

As the temperature rose to 120 °C, as shown in Fig. 2(B), a significant decrease in the amount of xylan in water-insoluble solids and a remarkable increase in soluble xylose in hydrolysate was found by increasing the temperature, which implies that increasing the temperature can accelerate the degradation of hemicellulose. At a temperature of 120 °C, the yield of oligomeric xylose reached its maximum (35.07% of initial xylose) in the initial 10 min, then decreased as time increased, while the monomeric xylose yield increased with time and the maximum monomeric xylose yield reached 82.71% at 120 min. A similar trend was observed for soluble xylose (monomeric and oligomeric xylose in liquid): the maximum soluble xylose yield (89.87% of initial xylose) was also achieved at 120 min. Although the maximum yield of monomeric and oligomeric xylose in hydrolysate reached 89.87%, the reaction time was too long. If more soluble xylose is to be obtained in a much shorter time, higher temperatures are needed.

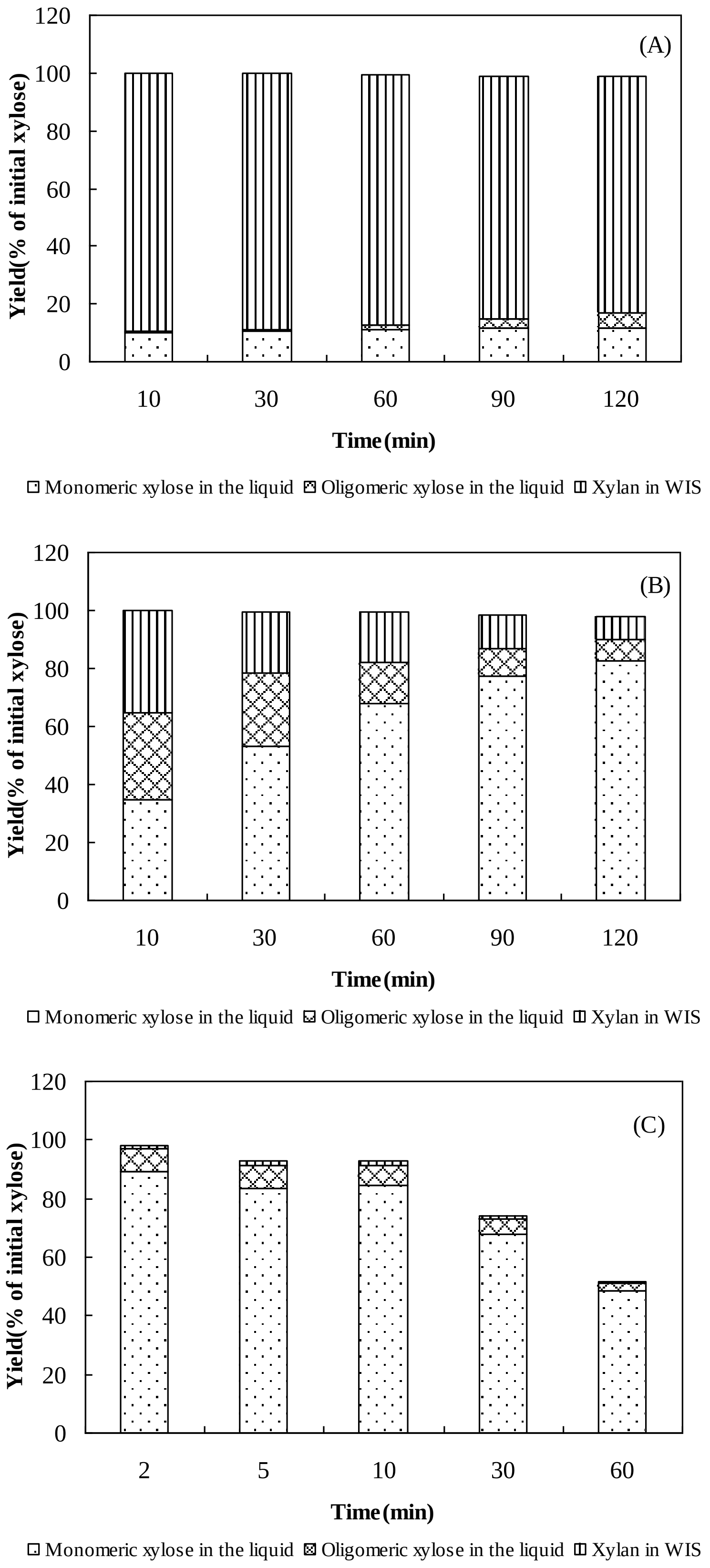

Fig. 2 (a-c). Yield of xylose in liquids and water-insoluble solids (WISs) from the pretreatments with 0.60% HNO_3 at (A) 80^{o}C; (B) 120^{o}C; (C) 150^{o}C; and (D) 180^{o}C

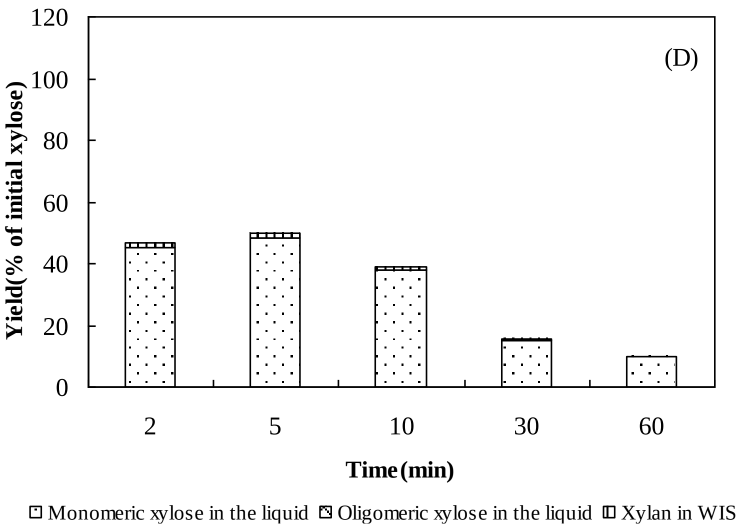

Fig. 2(d). Yield of xylose in liquids and water-insoluble solids (WISs) from the pretreatments with 0.60% HNO_3 at (A) 80°C; (B) 120°C; (C) 150°C; and (D) 180°C

As the temperature rose to 150 °C, as shown in Fig. 2(C), the maximum yields of monomeric xylose and soluble xylose in the hydrolysate reached 89.41% and 96.86% of initial xylose, respectively, a result that was achieved in a much shorter time frame of only 2 min. The maximum soluble xylose yield in this study was higher than that from dilute sulfuric acid pretreatment reported by Liu and Wyman (2004) (90.0%), $FeCl_3$-catalyzed hydrolysis reported by Liu *et al.* (2009b) (87.7%), and $Fe(NO_3)_3$-catalyzed hydrolysis reported by Sun (2011) (91.79%). The higher xylose yield implies that nitric acid is a more efficient catalyst than sulfuric acid, $FeCl_3$, or $Fe(NO_3)_3$. At a temperature of 150 °C, the xylan in water-insoluble solids decreased with time from 1.48% to 0.08% of initial xylose, which indicates that approximately 100% of the hemicellulose was removed. Meanwhile, the yield of monomeric and soluble xylose decreased with time, which can be attributed to the further degradation of xylose to smaller molecular components such as furfural. These results show that increasing the temperature can accelerate the degradation of hemicellulose if the temperature is high enough and that prolonging the reaction time results in the degradation of monomeric xylose.

As shown in Fig. 2(D), although high temperatures can easily remove 100% of the hemicellulose, the yield of soluble xylose was still lower than 49%, which is attributable to the fact that higher temperatures result in a higher degradation of xylose. Obviously, temperatures greater than 180 °C were not a suitable option, as they resulted in a large loss of potential xylose.

A similar trend was found with arabinose (figures are not shown). Increasing temperature can significantly accelerate the degradation of arabinan, and arabinan is not observed in the WIS at 180 °C. Arabinan in the WIS also decreased with time, and its removal reached as high as 100% in 120 min at 120 °C; however, the maximum yield of arabinose was not obtained at this condition because of arabinose degradation. The maximum monomeric arabinose yield of 96.80% of initial arabinose was obtained at the hydrolysis condition of 120 °C for 90 min, which was higher than that of xylose. In addition, minor oligomeric arabinose was found in the hydrolysate. These results indicate that it is much easier to obtain arabinose than xylose.

Effect of Temperature and Time on Cellulose Hydrolysis

Figure 3 illustrates the effects of temperature and time on the glucose yield for nitric acid-hydrolyzed corn stover. The yields of oligomeric glucose ranged from 0.02% to 3.19% of initial glucose. The trends in Fig. 3 suggest that increasing temperature can increase monomeric glucose and soluble glucose yields, while reaction time had little effect on glucose yield. The monomeric glucose and soluble glucose yields increased with increasing reaction time at 80, 120, and 150 ^{o}C, but first increased and then decreased at a high temperature of 180 ^{o}C. The maximum monomeric glucose and soluble glucose yields were found to be 18.54% and 18.58% of initial glucose, respectively, and were obtained at 180 ^{o}C after 10 min. The maximum value was lower than that of $FeCl_3$-catalyzed hydrolysis reported by Liu *et al*. (2009a). The reason the maximum glucose yield was quite slow was that cellulose hydrolysis was difficult because of its high crystallinity (Wang *et al*. 2012a; Pingali *et al*. 2010).

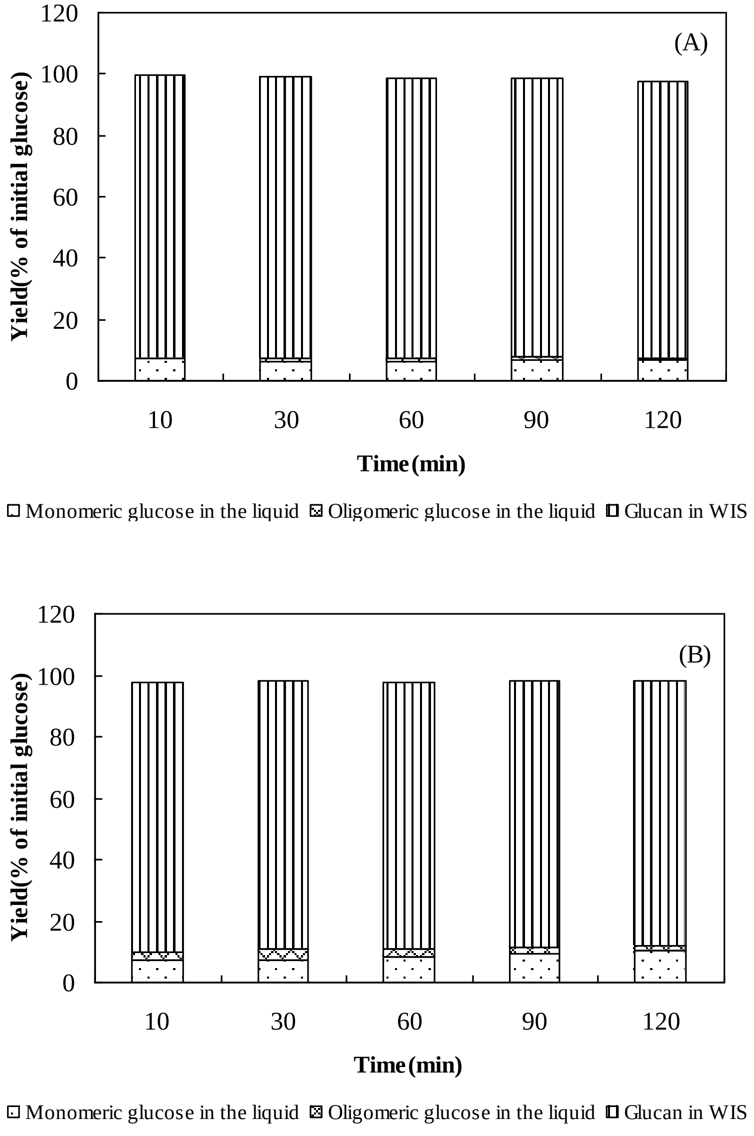

Fig. 3(a-b). Yield of glucose in liquids and water-insoluble solids (WISs) from the pretreatments with 0.60% HNO_3 at (A) 80^{o}C; (B) 120^{o}C; (C) 150^{o}C; and (D) 180^{o}C

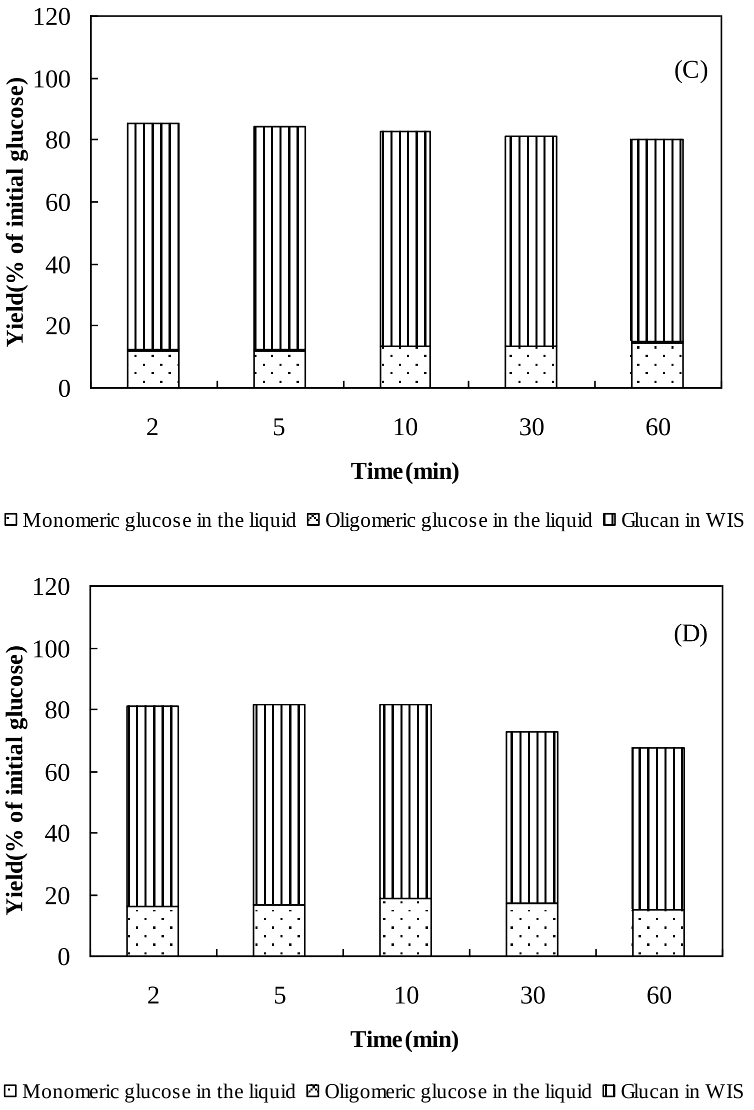

Fig. 3(c-d). Yield of glucose in liquids and water-insoluble solids (WISs) from the pretreatments with 0.60% HNO_3 at (A) 80°C; (B) 120°C; (C) 150°C; and (D) 180°C

Effect of Combined Severity Factor on the Concentration of Total Sugars and Inhibitors

Both hexose and pentose can be used for methane production by a mixture of micro-organisms, which made it necessary to analyze total sugar (including glucose, xylose, and arabinose) yield (Hendriks and Zeeman 2009). However, effective hydrolysis not only releases sugars, but also results in the formation of sugar degradation compounds such as furfural and 5-hydroxymethyl furfural (HMF), which are fermentation inhibitors. Therefore, to fully evaluate the efficiency of hydrolysis conditions, the total concentration of sugars must be offset by the total inhibitor concentration to determine the optimum condition. The concentrations of total sugar and inhibitors as a function of the combined severity factor (CSF) are summarized in Fig. 4. As shown in Fig. 4, the concentration of total sugars increased with an increase in CSF from 0.15 to 0.73, then was maintained at comparatively high levels when the CSF rose from 0.73 to 1.63, dropping significantly when the CSF further increased from 1.63 to

3.09 due to the decomposition of sugars into furfural and HMF. The highest concentration (30.88 g/L) of total sugars was obtained at CSF = 0.73, corresponding to the hydrolysis conditions of 150 °C, 0.6% HNO_3, and a reaction time of 2 min. Figure 4 also shows that the total inhibitor concentration fluctuated at a low level when the CSF increased from 0.15 to 1.62, but then rose sharply when the CSF increased from 1.62 to 3.09. The maximum concentration (20.15 g/L) of total inhibitors appeared at CSF = 3.09, corresponding to the hydrolysis conditions of 180 °C, 0.6% HNO_3, and a reaction time of 60 min.

Above all, a CSF of 0.73 made possible the highest total sugar concentration, but the inhibitor concentration was comparatively low. Therefore, a CSF of 0.73 provided the optimum hydrolysis conditions (nitric acid concentration of 0.6% (w/w), temperature of 150 °C, and reaction time of 2 min) for nitric acid-catalyzed hydrolysis of corn stover. Thus, the hydrolysate obtained under these conditions was selected for the subsequent anaerobic acidogenic fermentation.

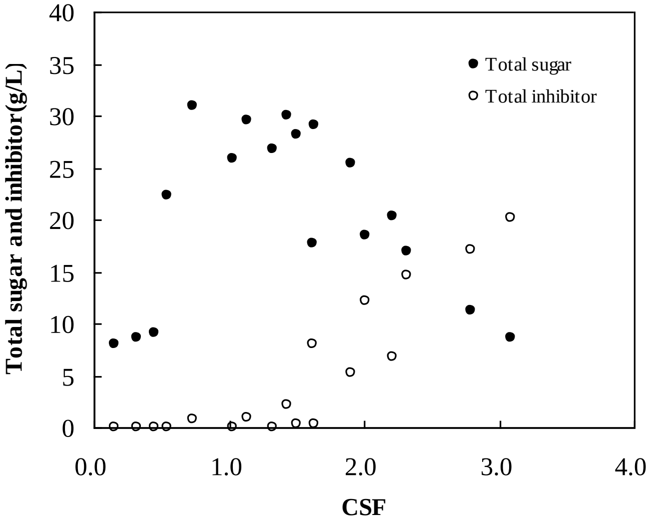

Fig. 4. Concentration of total sugar and inhibitors as a function of combined severity factor (CSF)

Anaerobic Acidification Fermentation of Corn Stover Hydrolysate

Acidogenic fermentation is the second and most critical stage of the methane fermentation process. VFA production and distribution of VFAs in the acidogenic stage can significantly influence the efficiency of subsequent methanogenic stages. Therefore, the acidogenic fermentations of hydrolysate and raw corn stover were investigated to evaluate their potential ability to produce methane.

Figure 5 shows the time-course profiles of VFA production during the acidogenic fermentation of hydrolysate, obtained at optimum hydrolysis conditions, and raw corn stover. As shown in Fig. 5, the concentration of VFAs produced by the acidogenic fermentation of raw corn stover increased slowly with time, and the maximum VFA concentration (1623.22 mgCOD/L) appeared at the 96th hour. However, the VFAs from the acidogenic fermentation of hydrolysate were produced faster than that of raw corn stover, and the maximum VFA concentration (2513.35 mg COD/L), which was more than that of raw corn stover, was obtained at the 48th hour. These results indicate that the acidogenic fermentation of hydrolysate is faster and more efficient than that of raw corn stover due to the hydrolysate being composed mainly of smaller molecule sugars, which are more accessible to microorganisms.

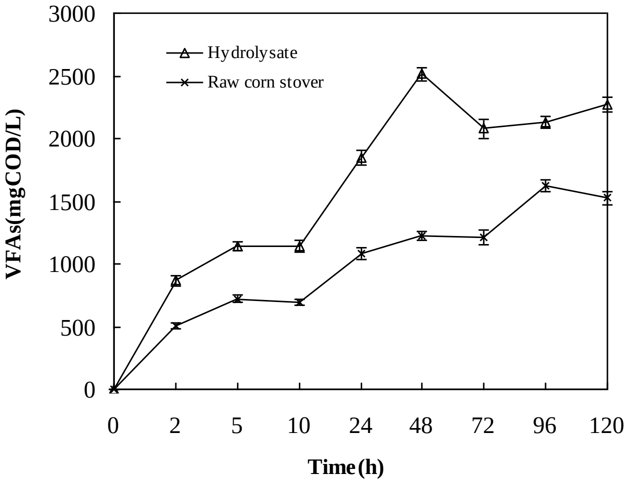

Fig. 5. Time-course profiles of VFA production during the anaerobic acidogenic fermentation of both hydrolysate obtained at optimum hydrolysis conditions and raw corn stover

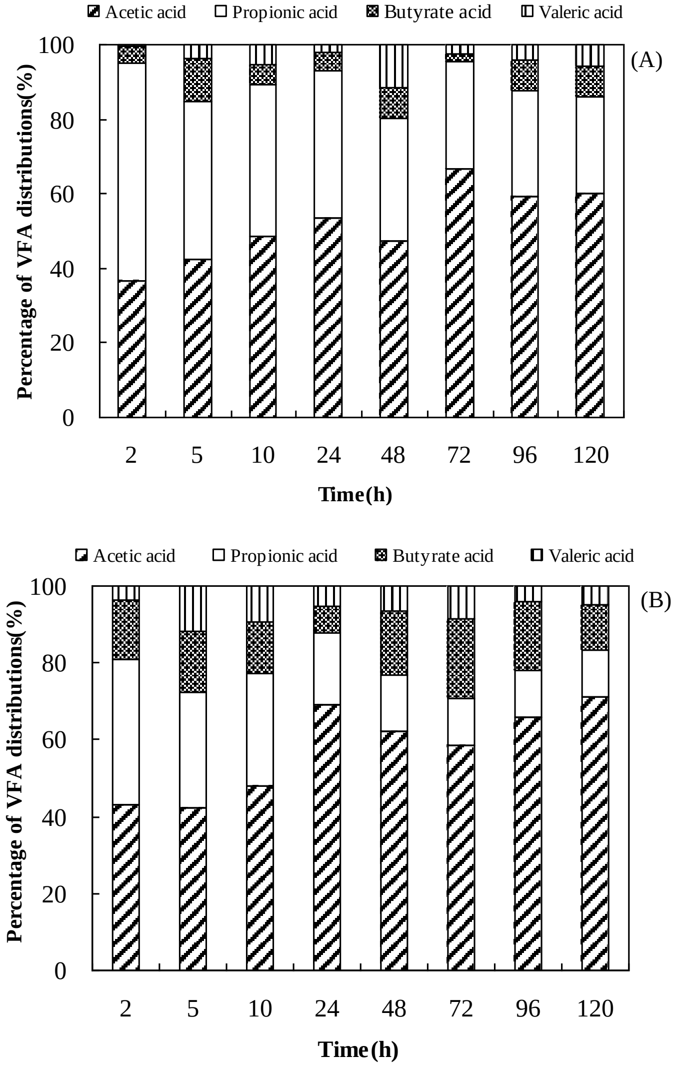

Fig. 6. Distribution of VFAs at the final state of anaerobic acidogenic fermentation of (A) raw corn stover and (B) hydrolysate

The distributions of VFAs are shown in Fig. 6. Acetic acid was the major product, and it can be converted into methane by methanogens. As shown in the figure, acetic acid in the final state produced by hydrolysate and raw corn stover fermentation accounted for 70.95% and 59.91% of VFAs, respectively. In addition, as the second-highest VFA produced, the amount of propionic acid from raw corn stover fermentation was more than that produced by hydrolysate fermentation during the whole acidogenic fermentation process. It has been widely reported that the methanogenesis of propionic acid is slow and that the accumulation of propionic acid causes process failure; thus, propionic acid is an undesirable intermediate product in the methanogenic process (Wang *et al*. 2012b). The VFA distribution of the acidogenic fermentation of hydrolysate was therefore more suitable for subsequent methanogenic fermentation, indicating that hydrolysate was more favorable for anaerobic fermentation. It can thus be concluded that the two-step acidogenic fermentation of corn stover is a feasible method for biogas production.

CONCLUSIONS

1. The results of the present study demonstrate that the two-step acidogenic fermentation of corn stover is an efficient approach.
2. In the first step, the corn stover was hydrolyzed by nitric acid. The results show that nitric acid hydrolysis can be efficient. Under the optimum conditions, the yields of soluble xylose, arabinose, and glucose in the hydrolysate achieved 96.86% of initial xylose, 98.54% of initial arabinose, and 18.58% of initial glucose, respectively.
3. In the second step, the acidogenic fermentation of hydrolysate obtained at optimum condition was performed. Compared with acidogenic fermentation of raw corn stover, the VFA production of the acidogenic fermentation of hydrolysate increased by 54.8% and fermentation time decreased by 50%; in addition, the distribution of VFAs produced by hydrolysate fermentation is more suitable for subsequent methanogenic fermentation. Therefore, the two-step method is a competitive approach to convert corn stover to biogas.

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