

Pretreatment of Corn Stover Silage with $\text{Fe}(\text{NO}_3)_3$ for Fermentable Sugar Production

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Abstract Corn stover silage is an attractive raw material for the production of biofuels and chemicals due to its high content of carbohydrates and easy degradability. The effects of $\text{Fe}(\text{NO}_3)_3$ pretreatment conditions on sugar yields were investigated for corn stover silage. In addition, a combined severity factor was used to evaluate the effect of pretreatment conditions on the concentration of total sugars and inhibitors. Optimum pretreatment condition was obtained at 150 °C for 10 min with 0.05 M $\text{Fe}(\text{NO}_3)_3$, at which the yields of soluble xylose and glucose in liquid achieved 91.80% of initial xylose, 96.74% of initial arabinose and 19.09% of initial glucose, respectively, meanwhile, 91.84% of initial xylose, 98.24% of initial arabinose, and 19.91% of initial glucose were removed. In addition, a severity analysis showed that the maximum sugar concentration of 33.48 g/l was achieved at combined severity parameter value of 0.62, while the inhibitor concentration was only 0.03 g/l. $\text{Fe}(\text{NO}_3)_3$ is an effective catalyst to enhance hemicellulose hydrolysis in corn stover silage, the yields of monomeric xylose in the liquid fraction reached as high as 91.06% of initial xylose and 96.22% of initial arabinose, respectively.

Keywords $\text{Fe}(\text{NO}_3)_3$ · Corn stover silage · Saccharification · Pretreatment · Inhibitor

Introduction

Lignocellulosic materials are the largest sources of polysaccharides including hexose and pentose with potential use for the production of biofuels, xylitol, organic acid, and other chemicals [1]. Meanwhile, economic interest in bioenergies and chemicals production can be enhanced if the needed reducing sugars solution can be obtained from the hydrolysis of low-cost lignocellulosic wastes. At present, corn stover silage is mainly used as animal fodder; however, it has a great potential to become a highly efficient material for production of biofuels and chemicals [2]. Various studies have reported that maize silage could be degraded more easily than raw corn stover [2, 3]. Therefore, corn stover silage is an attractive raw material for bioenergies and chemicals production.

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Although lignocellulosic biomass provides cheap raw material, the prehydrolysis requires to obtain reducing sugars are cost-intensive [4]. Therefore, an efficient pretreatment is required for the conversion of polysaccharides in lignocellulose to reducing sugars [5]. Several pretreatment technologies have been widely investigated, but process optimization is still needed to give overall cost reductions and high sugar yields, which will allow biofuels and chemicals obtained from lignocellulose to be competitive with conventional methods [4]. In addition, pretreatment technology usually combines a catalyst in water with thermal treatment [6]. Dilute acids were the most commonly used catalysts for hydrolysis of lignocellulosic materials, meanwhile, previous studies revealed that dilute acids pretreatment had shown to be effective in removing hemicellulose [7–10]. However, dilute acids pretreatment suffered from their corrosion, which required expensive corrosion-resistant materials and elevated the cost of the pretreatment [11].

In addition to dilute acids pretreatment approaches, inorganic salt pretreatment as a novel method has been investigated and has shown some particular advantages, such as high reaction rate, less corrosive and recyclable. Nguyen and Tucker [12] reported that using a mixture containing aqueous solutions of a dilute acid and metal salt could obtain higher overall fermentable sugar yields than that using dilute acid alone for hydrolyzing cellulose and hemicellulose in lignocellulosic biomass. Meanwhile, inorganic salt pretreatment was also proved to be an efficient pretreatment method to remove the hemicellulose from raw material. Furthermore, FeCl_3 showed a particularly strong effect on the hemicellulose removal, reaching as high as 100%, and the yield of monomeric and oligomeric xylose in the liquid fraction reached as high as 90% [11]. Since inorganic salt is less corrosive than acids and can avoid further over liming to neutralize the hydrolysate, it was more attractive to be used in the pretreatment of lignocellulosic biomass.

In this study, the effectiveness of $\text{Fe}(\text{NO}_3)_3$ pretreatment for corn stover silage was evaluated based on sugars yield in hydrolysate which can be fermented by a number of microorganisms to biogas, hydrogen and organic acids [13–15]. This work also investigated the effects of catalyst concentration, temperature, and time on the removals of hemicellulose and cellulose. In addition, the effects of pretreatment conditions on the total sugar and inhibitor concentrations were evaluated with the combined severity.

Experimental

Raw Material

Maize samples were harvested at the dough stage of maturity, then the plants were separated into ears, leaves and stovers, and the stovers were chopped into approximately 2 cm pieces to serve as raw material for ensilage. Corn stover silage was treated with 1% ammonia water (10 g/kg fresh corn stover). Three parallel silages were prepared for each group and stored in 25 kg capacity plastic containers for 2 months with a temperature ranging of 15–25 °C.

At the end of the incubation period, chemical analyses were conducted for silages. An aqueous extract was obtained by blending 25.00 g of wet silage sample with 100 ml distilled water for 10 min in a blender and squeezing the mixture through cheesecloth, and the pH was determined using digital pH meter [16]. The filtrate was filtered through filter paper, and stored for organic acid analysis. The dry matter (DM), total carbohydrate, lignin, and ash analysis of silage samples were determined based on the laboratory analytical procedure [17]. The remaining silage samples were dried at 60 °C in a fan-assisted oven, milled into fine particles and screened into fractions (10–20 mesh) in preparation for

pretreatment. The chemical composition, pH, and organic acid content of corn stover silage were showed in Table 1.

Experimental Setup

Pretreatment was performed in a laboratory scale pure titanium reactor (Songling/PCF-TA2-0.2, Shandong, China) with a total volume of 200 mL. The reactor was able to withstand a pressure of 9.6 MPa and a temperature of 300 °C, with an electric heater. It included a magnetic force agitation and a thermocouple, regulated by the controller and agitation was set at 200 rpm. Each dry silage sample (7.00 g) mixed with 63.00 g $\text{Fe}(\text{NO}_3)_3$ solution was loaded into the reactor. The pretreatment temperatures were 140, 150, 160, and 170 °C, reaction times were 5, 10, 20, 30, and 40 min, and $\text{Fe}(\text{NO}_3)_3$ concentrations were at 0.01, 0.03, 0.05, and 0.10 M. After treatment, the reactor was removed from the heating jacket and then placed in an ice bath to halt the reaction. The reactor was kept sealed, until the reactor was cooled to about 50 °C. Then the wet material was filtered by vacuum pump, obtaining a solid phase and a liquid phase for further analysis.

Analytical Methods

Sugars were determined by HPLC (LabAlliance) with a refractive index detector by using a column (BioRad Aminex HPX-87H, 300×7.8 mm) at 65 °C and 5 mM H_2SO_4 as mobile phase at a flow rate of 0.6 mL/min. Furfural, HMF and organic acids in hydrolysate were measured by HPLC with an UV detector.

The pH was measured using a PHB-4 pH meter (Shanghai Leici Instrument Inc., China) at room temperature.

Combined Severity Parameter

Many studies employed a severity analysis for comparison of the results over a range of pretreatment severities which associated with reaction time and temperature. The severity factor was defined as follow [18]:

$$R_0 = t \cdot \exp[(T - 100)/14.75] \quad (1)$$

Where t is the reaction time (min), and T is the reaction temperature (°C).

Table 1 pH and composition of the corn stover silage

	Corn stover silage
Glucose (g/100 g DM)	39.73
Xylose (g/100 g DM)	18.96
Arabinose (g/100 g DM)	1.75
Lactic acid (g/100 g DM)	0.84
Acetic acid (g/100 g DM)	0.63
Lignin (g/100 g DM)	14.86
Ash (g/100 g DM)	3.84
Dry matter (g/100 g)	16.23
pH	6.19

In order to couple the pretreatment conditions of time, temperature, and reagent concentration into one parameter, a combined severity (CS) parameter is defined [7, 11, 19, 20] and is calculated from Eq. 1 as follows:

$$CS = \log(R_0) - pH \quad (2)$$

R_0 is the severity factor. In this study, a combined severity factor was used to evaluate the effect of pretreatment conditions on the concentration of total sugars and inhibitors.

Scanning Electron Microscopy of Corn Stover

Samples of corn stover silage before and after pretreatment were mounted on the specimen stubs and sputter-coated with C prior to imaging with a PHILIPS XL-30TMP scanning electron microscope (SEM) using 20 KV accelerating voltage.

Results and Discussion

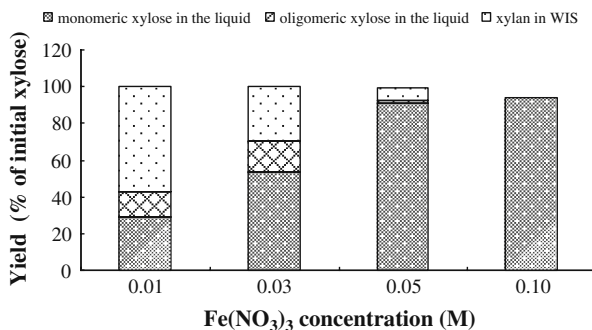
Sample pH

Sample pH was the same for all samples pretreatment at the same $\text{Fe}(\text{NO}_3)_3$ concentration. The measured pH values were 3.84, 2.16, 2.00, and 1.75 for the reaction medium after 0.01, 0.03, 0.05, and 0.10 M $\text{Fe}(\text{NO}_3)_3$ addition, respectively. After pretreatment, the pH values increased significantly for all reaction conditions which were in the range of 2.06–4.67.

Effect of $\text{Fe}(\text{NO}_3)_3$ Concentration on the Xylose Yield

The effects of $\text{Fe}(\text{NO}_3)_3$ concentrations on xylose yields for corn stover silage were showed in Fig. 1. From Fig. 1, it could be concluded that increasing $\text{Fe}(\text{NO}_3)_3$ concentration could increase the xylose yield when pretreatments carried out at 150 °C, 20 min. Increasing $\text{Fe}(\text{NO}_3)_3$ loading to 0.01, 0.03, 0.05, and 0.10 M, the monomeric xylose yields increased to 28.68%, 53.24%, 91.06%, and 94.26% of initial xylose, respectively. The results suggested that increasing $\text{Fe}(\text{NO}_3)_3$ concentration from 0.01 to 0.05 M could cause in a doubling of soluble xylose yield (monomeric and oligomeric xylose in liquid). However, only 1.70% soluble xylose yield improvements could be achieved by increasing the catalyst concentration by 0.05 to 0.10 M. Taking these diminishing returns (xylose) for increased cost ($\text{Fe}(\text{NO}_3)_3$) into account, using 0.05 M $\text{Fe}(\text{NO}_3)_3$ solution as catalyst was optimal.

Fig. 1 Yields of xylose in liquids and water-insoluble solids (WISs) from the pretreatments at 150 °C, 20 min with different $\text{Fe}(\text{NO}_3)_3$ concentrations



Effects of Temperature and Time on the Hemicellulose Hydrolysis

The effects of the combination of temperature and time on xylose yields were shown in Fig. 2. The yields of oligomeric xylose decreased with the increase of time at all experimental temperature. A significant drop in oligomeric xylose yield was found by increasing temperature. The maximum oligomeric xylose yield of 21.53% of initial xylose was obtained at 140 °C. Monomeric xylose yields initially increased with time, but then decreased at lower temperature of 140 and 150 °C, while it gradually decreased with increasing time at higher temperature of 160 and 170 °C. There were a similar profile between soluble xylose (monomeric and oligomeric xylose in liquid) and monomeric xylose. In addition, the experimental data indicated that higher monomer xylose and soluble xylose yields were achieved at temperature of 150 °C, and the maximum monomeric xylose and soluble yields reached 91.06% and 91.79% of initial xylose, respectively. The maximum soluble xylose yield in this study was higher than dilute sulfuric acid pretreatment reported by Schell et al. [21] (77%) and FeCl_3 catalyzed hydrolysis reported by Liu et al. [11] (89%). The higher xylose yield implied that $\text{Fe}(\text{NO}_3)_3$ was a more efficient catalyst than FeCl_3 and sulfuric acid for hemicellulose hydrolysis. The maximum monomeric xylose was also higher than that (77.74%) for control silage (no silage additive) at the same pretreatment condition reported by Sun et al. [22], which suggested that this silage could be degraded more easily than control silage.

As shown in Fig. 2, increasing temperature could accelerate the degradation of hemicellulose. Xylan in water-insoluble solid (WIS) decreased with time, and the xylan removal of corn stover silage were over 90% of initial xylose at 150, 160, and 170 °C. Though higher xylan removal rates were attained at 160 and 170 °C, the maximum yields of soluble xylose were lower than those at 140 and 150 °C, which due to higher temperatures resulted in more xylose degradation than those at lower temperatures.

The results showed that the pretreatment temperature had a great effect on the solubilization of arabinan in corn stover silage. Increasing temperature could significantly accelerate the removal of arabinan, and arabinan could not be observed in the WIS at 160 and 170 °C. Arabinan in the WIS decreased with time, which meant that arabinan removal increased with time, and it reached as high as 100% in 30 min at 140 °C.

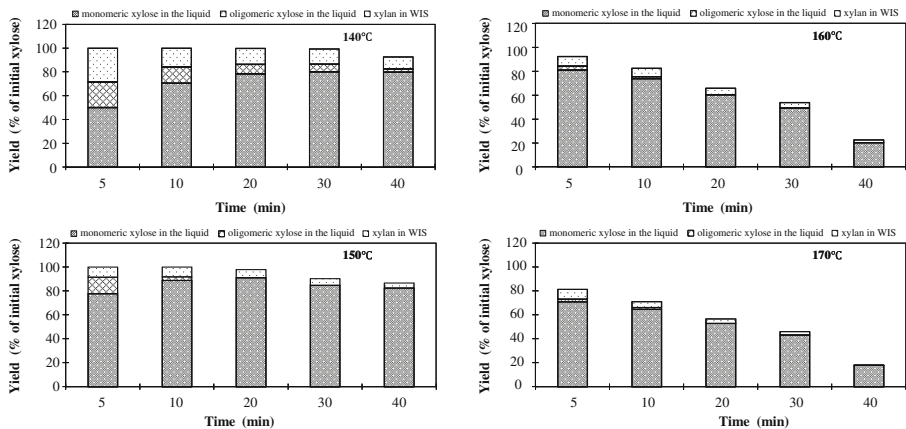


Fig. 2 Yield of xylose in liquids and water-insoluble solids (WISs) from the pretreatments at different temperatures with 0.05 M $\text{Fe}(\text{NO}_3)_3$

The trend is similar between xylose and arabinose. The maximum monomeric arabinose yield 96.22% of initial arabinose was obtained at operating condition of 150 °C for 10 min. Minor oligomeric arabinose was found in liquid fraction, and the maximum yield was only 8.80% of initial arabinose. In addition, the arabinose was quickly converted into the unwanted by-products with the increase of temperature and time which caused in a large loss of arabinose.

Effects of Temperature and Time on the Cellulose Hydrolysis

The main component of corn stover silage is glucan, in the form of cellulose [23]. However, cellulose hydrolysis was more difficult than hemicellulose at the same conditions because of their different structures [15]. Figure 3 illustrated the overall effects of temperature and time on the glucose yields for $\text{Fe}(\text{NO}_3)_3$ pretreatment to the corn stover silage. The yields of oligomeric glucose were from 0.34% to 4.60% of initial glucose. The trends in Fig. 3 suggested that prolonging reaction time could increase the monomeric glucose and soluble glucose yields at 140 and 150 °C. The monomeric glucose and soluble glucose yields first increased and then decreased with increasing time at higher temperatures of 160 and 170 °C. The maximum monomeric glucose yield was found to be 18.47% of initial glucose which was obtained at 150 °C after 40 min, while the maximum soluble glucose yield was 20.63% of initial glucose which was obtained at 160 °C after 10 min.

As shown in Fig. 3, cellulose solubilization increased with time for all reaction temperature. Increasing temperature also accelerated the solubilization of cellulose, the maximum value varied from 20.61% to 34.95% of initial glucose. The maximum value obtained at 140 °C was lower than that (24%) of FeCl_3 catalyzed hydrolysis for corn stover reported by Liu et al. [11].

Effect of Temperature and time on Total Sugar Yield in Liquid

The total sugar yield of xylose, arabinose, and glucose (including monomeric and oligomeric) obtained from liquid fraction was shown in Fig. 4. The total sugar yield had a

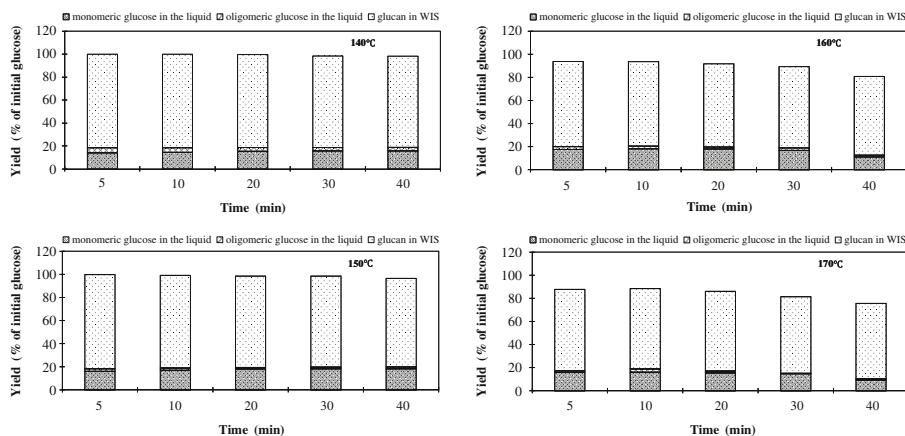


Fig. 3 Yield of glucose in liquids and water-insoluble solids (WISs) from the pretreatments at different temperatures with 0.05 M $\text{Fe}(\text{NO}_3)_3$

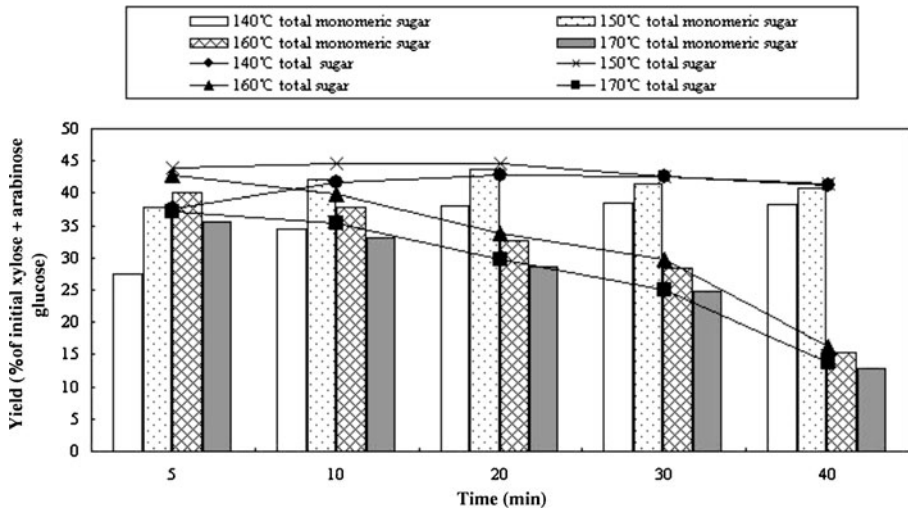


Fig. 4 Total sugar yield in liquids for corn stover silage pretreated with 0.05 M $\text{Fe}(\text{NO}_3)_3$ at different temperatures

similar fashion to that of xylose yield, which was due to the total sugar consisting mainly of xylose. It was observed that a maximum total sugar yield of 42.72% of initial sugar was obtained after 20 min at a low temperature of 140 °C. As the temperature was increased to 150, 160, and finally to 170 °C the maximum yield 44.52%, 42.68%, and 37.09% of initial sugar were obtained, respectively. The maximum total sugar yield for $\text{Fe}(\text{NO}_3)_3$ catalyzed hydrolysis was higher than the reported by Lloyd and Wyman [7] (38.3%). However, the maximum total sugar yields remained quite low, less than 50% of initial sugar, for all reaction conditions analyzed for this work. One factor to be considered was that corn stover silage was mostly composed of cellulose, while cellulose hydrolysis was difficult under these experiment conditions in this work.

Effect of Temperature and time on Inhibitor Production

Effective $\text{Fe}(\text{NO}_3)_3$ pretreatment not only released xylose, arabinose and glucose, but also resulted in the formation of sugar degradation compounds such as furfural and 5-hydroxymethyl furfural (HMF), while these inhibitors were broken down further into formic, levulinic acid, 2-furoic acid and acetone which were also inhibitors. The quantity of toxic compounds depended on pretreatment conditions (temperature, time and catalyst concentration). Figure 5 showed the effects of temperature and time on the inhibitors concentrations. In this work, the inhibitors obtained from hydrolysate liquor to focus on the main contributors which included furfural, HMF, formic and levulinic acid, of which furfural and HMF constituted the majority of total inhibitors. It was evident from Fig. 5, that temperature had a remarkable influence on the concentration of inhibitor. Increasing temperature from 140 to 170 °C could lead to a significant elevation of inhibitor concentration, and the maximum concentrations were obtained from 1.28 to 11.82 g/l for furfural, from 0.58 to 6.28 g/l for HMF, from 0 to 1.41 g/l and 0 to 1.78 g/l, respectively. In addition, each of the inhibitors increased with the increase of time at all pretreatment temperatures.

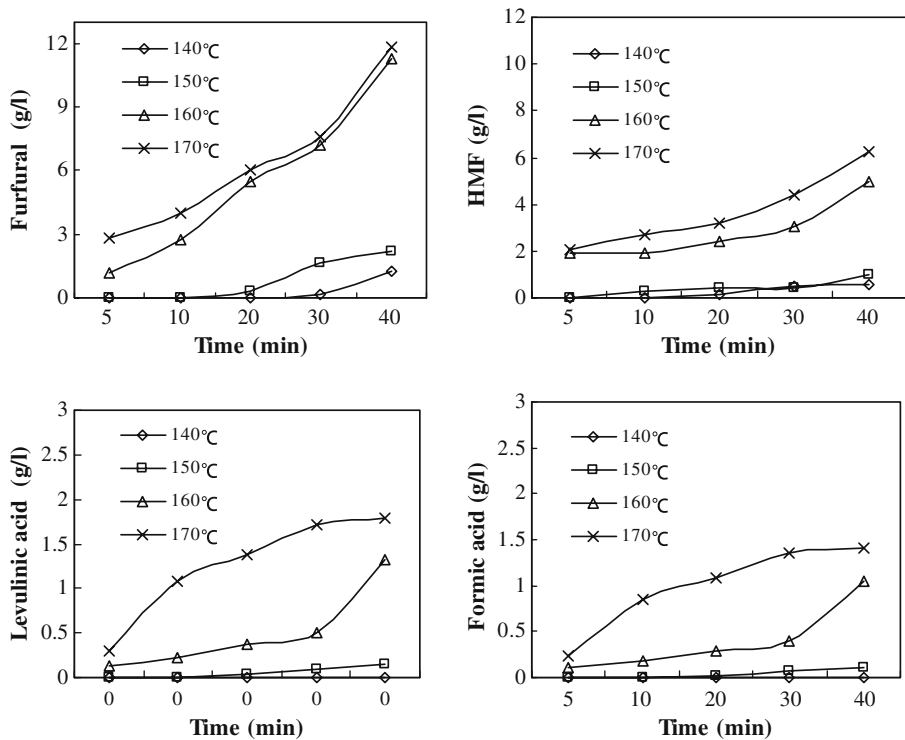


Fig. 5 The effect of temperature and time on the concentration of inhibitors (furfural, HMF, levulinic, and formic acid)

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

In order to fully evaluate the efficiency of a pretreatment condition, the total concentration of sugars must be offset by the total inhibitor concentration to find the true optimum conditions. Figure 6 revealed how the combined severity parameter affected the concentration of total sugars and inhibitors. As showed in Fig. 6, the concentration of total sugars increased when the combined severity rose from 0.03 to 0.32, then maintained at comparatively high levels when the severity rose from 0.32 to 1.22, but dropped swiftly when the combined severity further increased from 1.22 to 1.81 which due to the degradation of sugars into inhibitors, mainly furfural and HMF. The highest total sugar concentration of 33.48 g/l appeared at a $\ln(\text{CS})$ of 0.92. Figure 7 also showed the trend for total inhibitor concentration during pretreatment as a gradual increase with increasing combined severity. Furthermore, the total inhibitor concentration fluctuated at a low level when the combined severity varied from 0.03 to 1.10, but then rose sharply when the combined severity rose from 1.10 to 1.81. The maximum total inhibitor concentration of 15.02 g/l was obtained at a $\ln(\text{CS})$ of 5.44.

Overall, at the moderate combined severity of about 0.32 could obtained higher total sugar concentration and the inhibitor concentration was at a low level. As showed by the observation, a combined severity of 0.32 provided the optimum pretreatment condition for $\text{Fe}(\text{NO}_3)_3$ catalyzed hydrolysis of corn stover silage.

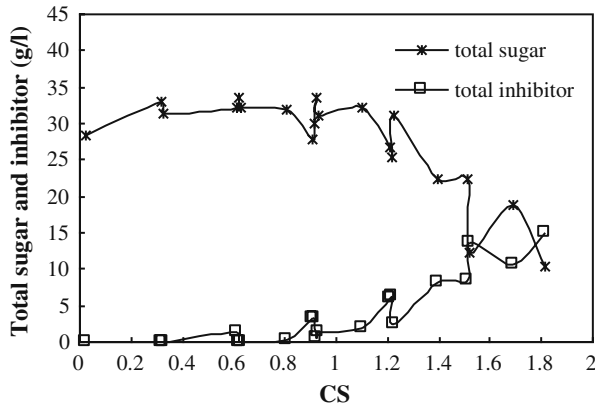


Fig. 6 The effect of combined severity parameter (CS) on the concentration of total sugars and inhibitors for pretreatment of corn stover silage with 0.05 M $\text{Fe}(\text{NO}_3)_3$ at 140–170 °C for 5–40 min

Physical Surface Changes to Corn Stover Silage Due to Pretreatment

$\text{Fe}(\text{NO}_3)_3$ pretreatment not only affected the chemical composition of corn stover silage, but also affected the physical appearance of the corn silage at the microscopic level. SEM images of untreated and $\text{Fe}(\text{NO}_3)_3$ pretreated were taken at $\times 1,000$ and $\times 8,000$ magnification (Fig. 7). The surface of the corn stover silage was undamaged by the grinding which was smooth and contiguous. The long chain of cellulose could be seen clearly by SEM, while hemicellulose appeared a random and amorphous structure with little

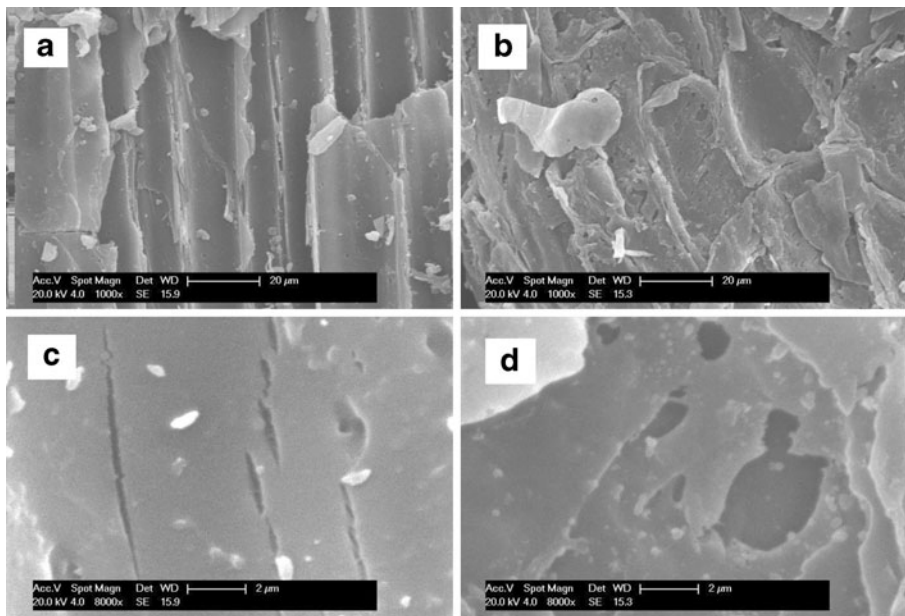


Fig. 7 Scanning electron micrograph of untreated corn stover silage (a) and pretreated (b) at $\times 1,000$ magnification; and untreated (c) and pretreated corn stover silage (d) at $\times 8,000$ magnification

strength, showed as irregular clumps on the surface and were interlaced with cellulose and lignin. The structure of corn stover silage also explained why hemicellulose degradation was easier than cellulose at the same conditions. After pretreatment, the smooth and continuous surface of corn stover silage was destroyed, the long chain of cellulose was cut and almost entirely hemicellulose was removed. The pretreatment also increased the external surface area and the porosity of corn stover silage.

Conclusions

$\text{Fe}(\text{NO}_3)_3$ pretreatment showed a great potential as a novel pretreatment method. This work investigated the effect of $\text{Fe}(\text{NO}_3)_3$ catalyzed hydrolysis on sugar yields for corn stover silage. The results indicated that $\text{Fe}(\text{NO}_3)_3$ was an effective catalyst for hemicellulose hydrolysis in corn stover silage, the yields of monomeric xylose and monomeric arabinose in the liquid fraction reached as high as 91.06% of initial xylose and 96.22% of initial arabinose, respectively. The optimal pretreatment condition was suggested to be at 150 °C with 0.05 M $\text{Fe}(\text{NO}_3)_3$ solution for 10 min, from which the total sugar and inhibitor concentration were 33.48 and 0.03 g/l, respectively, which were higher sugar concentration and lower inhibitor level. The work also indicated that the use of corn stover silage was a feasible option as a feedstock for production of sugars for biofuels and chemicals synthesis, due to its low cost and high sugar yields.

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