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Pretreatment and Fractionation of Corn Stover by Soaking In Ethanol and Aqueous Ammonia

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Abstract A new process for pretreatment of lignocellulosic biomass, designated the soaking in ethanol and aqueous ammonia (SEAA) process, was developed to improve hemicellulose preservation in solid form. In the SEAA process, an aqueous ammonia solution containing ethanol is used. Corn stover was treated with 15 wt.% ammonia at 1:9 solid–liquid ratio (by weight) at 60 °C for 24 h with ethanol added at 1, 5, 20, and 49 wt.% (balance was water). The extents by which xylan was solubilized with no ethanol and with ethanol added at 1, 5, 20, and 49 wt.% of the total liquid were 17.2%, 16.7%, 14.5%, 10.4%, and 6.3% of the original xylan, respectively. Thus, at the highest ethanol concentration used the loss of hemicellulose to the liquid phase was reduced by 63%. The digestibility of glucan and xylan in the pretreated corn stover samples by cellulase was not affected by ethanol addition of up to 20 wt.%. The enzymatic digestibility of the corn stover treated with 49 wt.% ethanol added was lower than the digestibility of the sample treated with no ethanol addition. Thus, based on these results, 20 wt.% was found to be the optimum ethanol concentration for use in the SEAA process for pretreatment of corn stover.

Keywords Lignocellulosic biomass pretreatment · Soaking in ethanol and aqueous Ammonia · Hemicellulose preservation · Hemicellulose recovery · Ethanol precipitation

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Introduction

Interest in renewable liquid fuels such as ethanol is increasing worldwide. In the United States, the goal is to produce 60 billion gallons of ethanol per year to replace 30% of the nation's gasoline consumption by 2030 [1]. Currently, all the fuel ethanol produced commercially in the USA is made from corn. Corn ethanol alone is not sufficient to meet the stated goal since the maximum production has been estimated to be only about 12 to 15 billion gallons per year [2, 3]. Thus, a renewable feedstock for ethanol production other than corn is needed and considerable attention has been given to lignocellulosic biomass. The three main sources of lignocellulosic biomass are forest products and residues, agricultural residues, and dedicated energy crops. Corn stover contributes the largest quantities among the agricultural residues in the USA. Currently, 75 million dry metric tons (MT) of corn stover can be sustainably collected and used for ethanol production. It has been estimated that by the mid-twenty-first century 232 million dry metric tons of corn stover would be available for ethanol production [4]. The potential ethanol yield of corn stover has been estimated to be 0.29 l kg^{-1} on a dry basis (76.61 gal per dry MT) [5]. Thus, by the mid-twenty-first century, 18 billion gallons of ethanol can be produced from corn stover alone.

Lignocellulosic feedstocks consist of three main components: cellulose, hemicellulose, and lignin. Bioconversion of these feedstocks via the sugar route requires enzymatic hydrolysis of cellulose and hemicellulose to fermentable sugars, which subsequently are fermented to ethanol. For efficient hydrolysis of the two carbohydrate fractions by enzymes normally a pretreatment process is needed. Typically, in a pretreatment process, some of the lignin is removed to increase accessibility of cellulose and hemicellulose to enzymes [1]. Since the objective of lignocellulosic biomass conversion is to produce ethanol from the fermentable sugars derived from cellulose and hemicellulose, it is obvious that a good pretreatment process should preserve as much of these two carbohydrate fractions as possible.

Soaking in aqueous ammonia (SAA) has been proven to be an effective pretreatment method for lignocellulosic biomass. A pretreatment method based on the use of aqueous ammonia has been investigated. In this ammonia recycle percolation method, while it also utilizes aqueous ammonia, it uses high temperature in the range of 150–210 °C. In this process, high energy input is required due to its high temperature and about half of the xylan is removed along with the lignin, which complicates xylan recovery in the downstream processing [7, 19]. The SAA process uses low temperatures to minimize degradation of fermentable sugars, formation of potentially inhibitory compounds, and solubilization of the hemicellulose. SAA is one of the few methods for pretreatment of lignocellulosic biomass in which almost 100% glucan and greater than 80% xylan are retained whereas a significant percentage of lignin is removed. Despite this high efficiency in carbohydrate preservation still about 20% xylan is solubilized together with lignin and hence, is not available for conversion to ethanol in the subsequent fermentation step [6, 7].

In this study, we investigated an improvement of the SAA method, which is designated the soaking in ethanol and aqueous ammonia method (SEAA). The key steps of the proposed SEAA process are shown in Fig. 1. In this process, the biomass is pretreated with an aqueous solution of ethanol and ammonia. After the pretreatment is a solid/liquid separation operation. The liquid enters an evaporator where ammonia is evaporated and recovered for recycle. The residual solids, which are mostly lignins, are recovered and burned as fuels in boilers. The solids from the aforementioned solid/liquid separation operation are washed with water to remove the residual ammonia. The wash water is fed to the evaporator for ammonia recovery as described previously. The washed solids are hydrolyzed with industrial cellulases and hemicellulases to produce fermentable sugars, which are fermented to ethanol in the

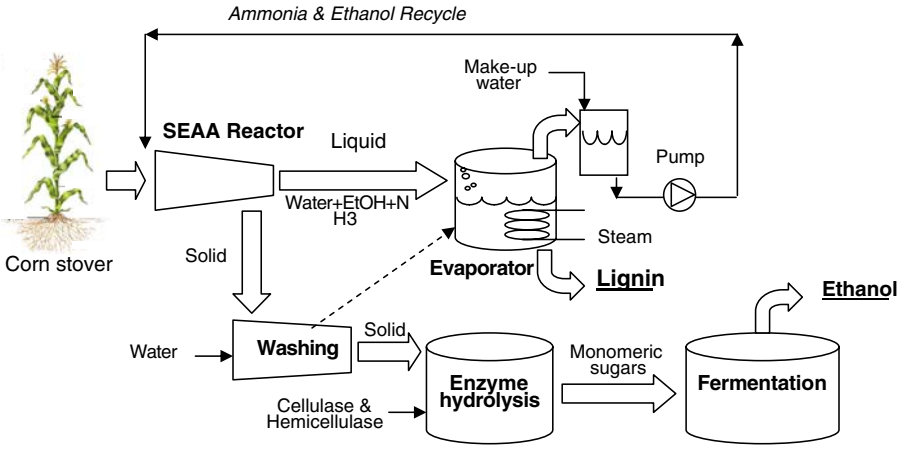

Fig. 1 Bioconversion of corn stover using the proposed SEAA process

subsequent fermentation step. The objective of the new method is to reduce the loss of xylan due to solubilization. In the SEAA method, ethanol is added to the aqueous ammonia solution. Precipitation of hemicellulose with ethanol has been used for the separation and purification of polysaccharides in lignocellulosic biomass [8–10]. We speculated that the presence of ethanol in the aqueous ammonia would cause reprecipitation of the previously solubilized xylan onto the solid matrix or reduce the extent of hemicellulose solubilization. Thus, more xylan would be available for hydrolysis to fermentable sugars and subsequently for ethanol production. It also was anticipated that the ethanol used in the pretreatment process could be recovered and recycled.

Materials and Methods

Corn Stover

Corn stover was obtained from the National Renewable Energy Laboratory (NREL) in Golden, CO, USA. It was ground and screened. The fractions having particle sizes between 10 mesh and 35 mesh (0.5 mm and 2.0 mm) were used in the experiments. The ground and screened corn stover was air dried and stored at about 25 °C, which was the temperature of

Table 1 Composition analysis of untreated corn stover.[a]

Description	Composition (wt.%±SD)[b]
Glucan	36.7±0.2
Xylan	22.1±0.2
Galactan	1.1±0.0
Arabinan	3.8±0.0
Mannan	0.3±0.0
Acid insoluble lignin	14.4±0.1
Ash	6.1±0.1

[a] Data are based on the oven-dried untreated biomass

[b] Weight percent±standard deviation (n=2)

our laboratory. At equilibrium the moisture content of ground corn stover was approximately 5% to 8%. The composition of the corn stover is shown in Table 1.

Enzymes

Cellulase enzyme, GC-220, was kindly provided by Genencor, a Division of Danisco. The activity of the enzyme was 45 FPU/mL (FPU: filter paper unit) and its protein content was 184 mg/mL. The β-glucosidase enzyme, Novozyme 188, was purchased from Sigma-Aldrich (St. Louis, MO, USA). This enzyme had an activity of 750 CBU/mL (CBU: cellobiose unit).

Pretreatment Procedures

The pretreatment of corn stover was carried out in a cylindrical reactor made of stainless steel 316. The reactor assembly is shown in Fig. 2 where its dimensions are given. The two ends of the reactor were sealed with two stainless steel 316 flanges, which were held tightly in place during each experiment by four sets of bolts and nuts located at equal distances on the circumference of the flanges. In each experiment, 5 g corn stover (dry basis) was placed in the reactor and the ammonia–ethanol solution was added. The reactor was closed and the corn stover was allowed to soak in the ammonia–ethanol solution for 5 min before the reactor was placed in a convection oven that was preset and subsequently maintained at 60 °C. In all of the experiments, the ammonia loading was kept constant at 15 wt.% of the total liquid and the solid–liquid ratio was maintained at 1:9, i.e. 5 g dry corn stover in 45 g total liquid. Four different ethanol levels were tested, which were 1, 5, 20, and 49 wt.% of the total liquid (balance was water). It should be pointed out that since 30 wt.% was the maximum concentration of the commercially available ammonium hydroxide solution our attempt to maintain constant ammonia loading and solid–liquid ratio in effect put a constraint on the maximum ethanol concentration that could be applied. This maximum ethanol concentration was 49 wt.%, which was the highest ethanol concentration used in our experiments. An experiment in which no ethanol was added also was performed. The pretreatment time in all experiments was 24 h. At the end of the experiments the entire contents of the reactor were collected and filtered. The liquid was discarded and the solids were subject to compositional analysis and enzymatic digestibility tests.

Enzymatic Digestibility Test Procedure

The enzyme digestibility tests of the pretreated corn stover were performed in duplicate according to the National Renewable Energy Laboratory Standard Method LAP-009 [11]. These tests were performed at 50 °C and pH 4.8 in 0.05 M sodium citrate buffer. Enzyme loading of 30 FPU of GC-220/g glucan supplemented with 30 CBU of β-glucosidase

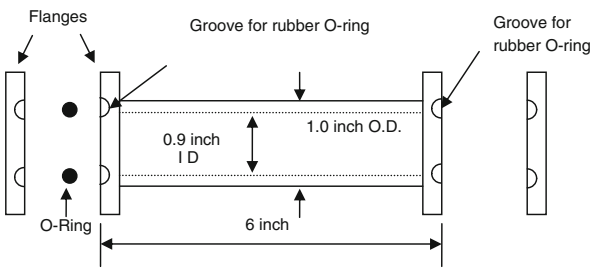

Fig. 2 The SEAA pretreatment reactor assembly

(Novozyme 188)/g glucan was used. The initial glucan concentration was 1% (*w*/*v*) based on 100 mL of total liquid and solid. All the samples used in the digestibility tests were wet samples as collected from various pretreatments. The 250-mL screw-capped Erlenmeyer flasks containing the enzyme hydrolysis preparations were placed in an incubator shaker (Lab-line, 4827F, Dubuque, Iowa) and agitated at 150 rpm. Samples were taken periodically (6, 12, 24, 48, 72, and 96 h) and analyzed for glucose, xylose, and cellobiose using high performance liquid chromatography (HPLC). Total released glucose (or xylose) after 72 h of hydrolysis was used to calculate the enzymatic digestibility. Untreated corn stover was taken through the same procedure as a reference.

Analytical Methods

The solid samples, which included treated and untreated corn stover, were subject to compositional analysis according to the NREL standard method [12]. Each sample was run in duplicate. Sugars were determined by HPLC. The system was an ISCO model 2350 (Lincoln, NE, USA) using deionized water as solvent at 0.6 mL/min combined with an Aminex® HPX-87P column (Bio-Rad Laboratories, Hercules, California) operated at 8 °C and an HP 1047A refractive index detector (Hewlett Packard, Palo Alto, California). The software used for data analysis was Chrom Perfect® Spirit version 4 build 17 (Justice Laboratory Software, Auchtermuchty, Fife, UK).

Results and Discussion

Retention of Hemicellulose by Ethanol Addition

The effects of ethanol at various concentrations on the compositions of the corn stover treated by the SEAA process are shown in Table 2. The results clearly indicate that the amounts of xylan remaining after the SEAA treatment increased with increasing ethanol concentrations. The extents by which xylan was solubilized with no ethanol and with ethanol added at 1, 5, 20, and 49 wt.% of the total liquid were calculated to be 17.2%, 16.7%, 14.5%, 10.4%, and 6.3% of the original xylan, respectively. Thus, at the highest ethanol concentration used the loss of hemicellulose in the liquid phase was reduced by 63%. It has been demonstrated that

Table 2 The effect of ethanol concentrations on the compositions of corn stover in SEAA treatment.[a]

Ethanol concentration (wt.%)	Residual solids (%)	Lignin[b] (%)	Delignification (%)	Solid	
				Glucan (%)	Xylan (%)
Untreated	100	14.4	–	36.1±1.0	22.1±0.0
0	65.1±2.5	3.8±0.5	73.6±4.9	35.3±1.0	18.3±0.6
1	65.5±1.1	4.8±0.7	65.3±6.9	35.1±0.8	18.4±0.2
5	66.6±1.5	4.2±0.3	70.8±2.9	35.4±1.4	18.9±0.3
20	68.9±2.4	4.3±0.7	70.1±6.9	35.4±0.5	19.8±0.6
49	72.5±1.8	5.3±0.4	63.2±3.9	35.6±0.6	20.7±0.5

[a] Data in the table are based on the oven-dried untreated biomass (n=2). Pretreatment conditions: 15 wt.% of ammonia concentration, 60 °C of reaction temperature, 1:9 solid–liquid ratio (by weight), and 24 h reaction time

[b] Acid insoluble lignin

hemicellulose which had been solubilized in alkali solutions could be recovered by precipitation with ethanol [13–16]. The decrease in the amounts of hemicellulose lost in the liquid phase demonstrated here probably was caused by the same mechanism, i.e. reprecipitation of the hemicellulose that had previously been solubilized by addition of ethanol.

The results in Table 2 also show that glucan was well preserved even without ethanol addition. Preservation of the cellulose fraction of corn stover during pretreatment by soaking in liquid ammonia has been demonstrated by one of the authors [6, 7]. Ethanol addition also resulted in lower removal of lignin. However, the effect of ethanol on lowering the extent of delignification was very small and not as significant as observed for hemicellulose retention.

Enzyme Digestibility Test

The results of the enzyme digestibility tests of the SEAA-treated corn stover for glucan and xylan are shown in Fig. 3. As described in the experimental procedure, the same amount of

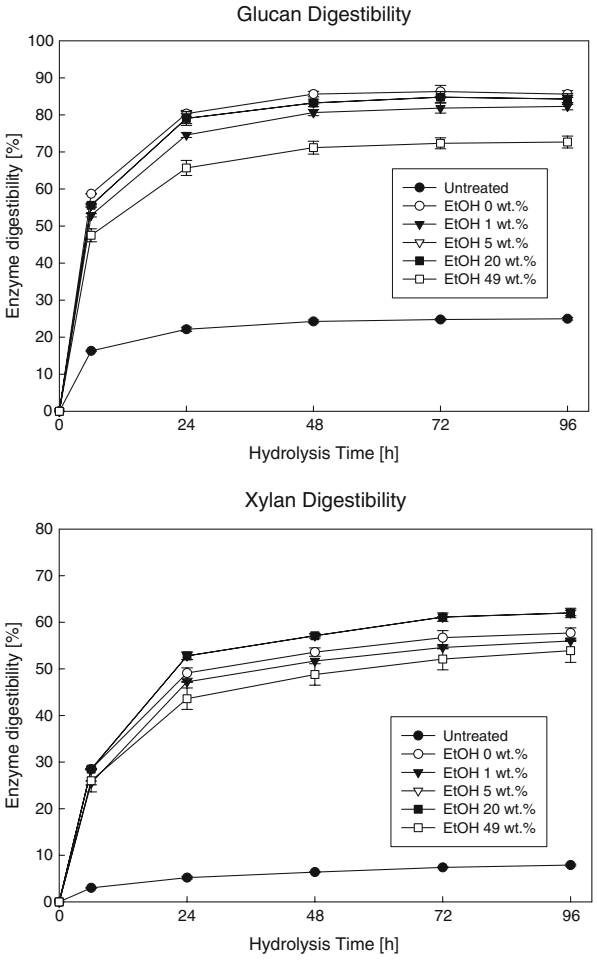

Fig. 3 Enzymatic digestibility of SEAA-treated corn stover

glucan (1 % (*w*/*v*) based on total solid plus liquid) was used in each experiment. The results in Table 2 show that the glucan contents of the corn stover samples treated by the SEAA process using different ethanol concentrations were well preserved in each experiment, but other components, i.e. xylan and lignin, varied with ethanol concentrations. Thus, different amounts of treated corn stover were used in the enzyme digestibility experiments in order to maintain 1% (*w*/*v*) glucan loadings. Consequently, the initial amounts of xylan in each experiment were different. The digestibilities were calculated based on the glucan and xylan contents of untreated corn stover.

$$\begin{aligned} \textit{Enzymatic Digestibility}(\%) = & \frac{\textit{Monomeric sugar (glucose or xylose) produced (g)} \times \textit{hydration factor}}{\textit{Initial glucan (1.0g) or Xylan(x g) in flasks}} \\ & \times (\textit{Sugar} \times \textit{retention} \times \textit{ratio}) \times 100 \end{aligned}$$

$$\text{Sugar retention ratio} = \frac{(\text{Glucan or xylan remaining after treatment})}{(\text{Total glucan or xylan content in the untreated biomass})}$$

Where, the hydration factor is 0.9 for glucose and 0.88 for xylose.

In all cases, the results plotted in Fig. 3 clearly show considerably higher digestibility of both glucan and xylan in the treated samples compared to the untreated sample. For both glucan and xylan the digestibility of the samples treated with ethanol concentrations of 1, 5, and 20 wt.% were equal to those obtained with the sample treated with ammonia solution without ethanol addition. In other words, addition of ethanol to the aqueous ammonia solution did not have a significant effect on digestibility of both of these carbohydrate fractions. In terms of total xylose produced increasing ethanol concentrations resulted in increases in production of this sugar. When ethanol was used at 49 wt.%, the digestibility of both glucan and xylan were significantly lower than those obtained with the sample treated with no ethanol addition. Ethanol at high concentrations has been reported to change physical properties of cellulose during the organosolv pulping process [17, 18]. It was probable that these changes also caused modifications of the structure of the treated corn stover in our investigation, which in turn negatively affect enzyme hydrolysis.

Xylan Retention and Selectivity

Carbohydrates, such as glucan, xylan, galactan, arabinan, and mannan, are valuable feedstocks that can be converted to fuels and chemicals by chemical and biological methods. The objective of this study was to produce a carbohydrate-rich solid cake with low lignin contents. This goal was achieved through the addition of ethanol to the ammonia solution to reduce the loss of xylan through solubilization. In the biological conversion of biomass, lignin has been known to exert a significant inhibitory effect on both enzyme and microbial activities [19–23]. Thus, a good pretreatment process is one that has high degree of lignin removal and low carbohydrate losses. To quantitatively examine these desired pretreatment characteristics, we introduced two simple concepts as follows:

$$\text{Xylan retention } [\%] = (X_R/X_T) \times 100$$

Where,

X_R Xylan remaining
X_T Total xylan content in the untreated biomass

$$\text{Selectivity}[-] = m_{\text{Lignin}}/m_{\text{Xylan}}$$

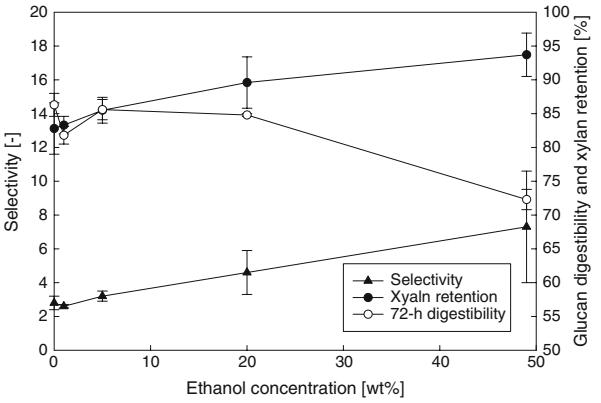

Fig. 4 Selectivity of the SEAA-treated corn stover at various ethanol concentrations. Data in the figure are based on the oven-dried untreated biomass. Pretreatment conditions: 24 h of reaction time, 1:9 (weight basis) of solid–liquid ratio, 15 wt.% ammonia concentration, 60 °C of reaction temperature. Selectivity=m_{Lignin}/m_{Xylan} (where, m_{Lignin} and m_{Xylan} are the mass loss rate of lignin and xylan in the solid, respectively)

Where, m_{Lignin} and m_{Xylan} are the mass loss rate of lignin and xylan of the corn stover during the pretreatment.

It is clear from the above equation that higher lignin removal and lower xylan loss will result in higher selectivity. Selectivity was calculated for the ethanol concentrations used in this study and plotted in Fig. 4 together with xylan retention and xylan digestibility. The results clearly show that selectivity increased as ethanol concentrations increased up to 20 wt.% in parallel with increases in xylan retention. Selectivity still increased after ethanol concentration was increased above 20 wt.% but digestibility dropped. As discussed above, very high ethanol concentrations might have affected the structure of the treated corn stover and reduced the enzyme efficiency.

Xylan retention at various ethanol concentrations are plotted in Fig. 5. The results clearly show the important role of ethanol in xylan retention. The highest ethanol concentration of 49 wt.% resulted in an 11% improvement of xylan contents of the treated corn stover over the xylan contents of the sample treated with no ethanol addition. However, the digestibility of both glucan and xylan dropped significantly when ethanol concentration higher than 20 wt.% was used. Thus, based on the experimental results obtained in this study, 20 wt.% ethanol was the optimum concentration for the SEAA pretreatment of corn stover.

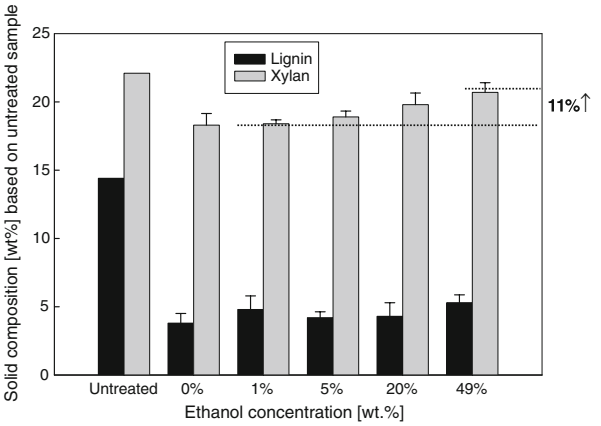

Fig. 5 Compositional change in SEAA-treated corn stover with various ethanol concentrations

Conclusion

It has been demonstrated that the addition of ethanol was effective for retention of hemicellulose during the SEAA treatment of corn stover. With optimum ethanol concentration at 20 wt.%, the carbohydrate-rich solid sample obtained from SEAA treatment contained 51.4% glucan, 26.7% xylan, and 6.2% lignin, which were almost 100% glucan retention, 89.6% xylan retention, and 70.1% lignin removal, respectively. The SEAA was proven to be an effective method to produce the carbohydrate-rich solid cake, which can be used for production of fuels and chemicals.

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