Aqua-ammonia pretreatment of corn stover for enhancing enzymatic saccharification

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Abstract: Aqua-ammonia pretreatment of corn stover was provided in a 1.2 L high pressure reactor with two ammonia:biomass ratios of 1:1 (w/w) and 2:1 (w/w); at three temperatures: 60℃, 90℃ and 120℃, and two treatment time: 5 min and 30 min. Pretreatment with water was used as control.   The pretreated samples were saccharified to fermentable sugars by commercial enzymes ACCELERASE 1500 (cellulase) and ACCELERASE XC (xylanases).   For ammonia:biomass ratio of 1:1, the yield of total fermentable sugar was 87% at 90℃ for 30 min treatment.   In case of ammonia:biomass ratio of 2:1, fermentable sugar yield increased four fold of that of control.   Maximum fermentable sugar yield of 99% was obtained for 2:1 ammonia: biomass ratio, 90℃, and 30 min treatment time.   The results of this study are very promising for improving fermentable sugar yield from corn stover using smaller amount of ammonia than the ammonia fiber explosion and other pretreatments such as strong acid or alkali pretreatments.   However, further optimization is required for reducing pretreatment time.

Keywords: aqua-ammonia pretreatment, corn stover, fermentable sugar, enzymatic saccharification, biomass

1 Introduction

Lignocellulosic biomass is one of the most abundant renewable resources for biofuel production.   Converting low-cost lignocellulosic feedstock to fuels offers outstanding benefits to the nation through: 1) a sustainable energy resource supply, 2) decreased trade deficits, 3) healthier rural economies, and 4) improved environmental quality[1].   Currently, large-scale production of ethanol from biomass involves enzymatic conversion of corn-derived starch to glucose and then fermentation of glucose to ethanol.   Starch is a polymer of glucose molecules covalently linked via α-1,4

glycosidic bonds, resulting in a loose polymeric gel structure synthesized by plants for storage of carbon and energy.   This structure allows ready access to enzymes involved in synthesizing and degrading starch as the plant grows throughout its life cycle.   Microbial amylase enzymes that hydrolyze starch to glucose followed by fermentation are currently used on a large scale by the biofuel industry to produce ethanol from corn.   However, there are concerns that production of ethanol from corn will negatively impact global food supplies and prices. Cellulose is also a polymer of glucose that has attracted much interest as an alternative to starch as a substrate for biofuel production.   However the β-1,4 glycosidic bond linking the glucose monomers results in a densely packed polymer that provides a rigid support structure allowing upright growth by plants.   This structure limits the access of cellulase enzymes and hinders conversion of cellulose to glucose[2].

There are two routes for converting lignocellulosic materials to fuels: thermochemical and biochemical.

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Compared to the high temperature and pressure requirements to achieve thermochemical biomass transformation, biochemical transformation is favored. This process comprises four major unit operations: 1). pretreatment, 2). hydrolysis, 3). fermentation and 4). product separation/purification[3]. Since the first two operations are still the hurdle for efficient use of plant feedstocks for fuels, in this study we seek to develop improved pretreatment technology for corn stover, which is an abundant crop waste in the Midwest America where average production of corn generates 3.01 to 4.27 dry tons of stover per acre (7.44 to 10.55 t/ha). Out of this, 1.77 to 3.48 dry tons per acre (4.37 to 8.60 t/ha) can be harvested as feedstocks for biorefineries[4]. Corn stover consists of non-edible parts of the corn plant: stalk, leaves and cobs left after harvest of corn grains. As one of the potential feedstocks, corn stover has been studied intensively. Hence, we chose corn stover as one of the feedstocks for this project. Corn stover has approximately 37.5% cellulose, 22.4% hemicelluloses and 17.6% lignin[3].

The purpose of pretreatment is to separate the cellulose from hemicellulose and lignin, and make the natural crystalline cellulose more amenable for degradation by converting it to an amorphous state. The proportion of cellulose, hemicelluloses and lignin varies by plant species, cultivar and growth conditions, and plant parts. Therefore the proportion of sugar monomers as well as the byproducts derived from different biomass sources affect the fermentation process to convert the sugar into biofuel.

Different approaches have been explored regarding biomass pretreatment, for instance: dilute sulfuric acid[5]; dilute alkali[6]; ammonia explosion[7,8]; lime[9]; hot water[10,11]; supercritical fluids[12,13], organosolvents[14], and ionic liquids[15]. Several of these pretreatment processes are in different stages of research and development in the upcoming cellulosic ethanol industry.

Among these, acid and alkali pretreatments have been broadly studied. However, there is still a need for an improved pretreatment process to enhance sugar release and reduce generation of toxic byproducts. Ammonia explosion and dilute ammonia pretreatment have

advantage of using relatively lower process and recycling cost[3,16]. These treatment techniques neither leave any chemical residues in the pretreated biomass nor involve any washing or conditioning steps prior to fermentation of derived sugars. Therefore, we evaluated pretreatment processes using ammonia as a pretreatment agent in addition to water so that the process cost could be lowered by reducing ammonia consumption during pretreatment. The objective of this study was to develop an aqua-ammonia pretreatment process and optimize process parameters.

2   Materials and methods

2.1   Biomass composition analysis

Freshly harvested corn stover (biomass) was obtained from the agricultural farms, Southern Illinois University, Carbondale, IL. It was sun dried to moisture content of 12% (w.b.) and ground using a Wiley laboratory cutting mill (Thomas Wiley Model 4, Arthur H. Thomas Co., Phil, PA, USA) and passed through a 2 mm screen. The ground biomass was again screened to obtain particles between 0.5 to 1.0 mm. The convection oven method was used for determination of total solids (moisture) in biomass as described in Laboratory Analytical Procedure (LAP) of National Renewable Energy Laboratory[17]. The composition of structural carbohydrates and lignin was determined by an ANKOM F57 Fiber Analyzer (ANCOM Technology, Macedon, NY) using the procedure recommended by the manufacturer and recently used by Liang et al.[18]. The samples were stored in sealed plastic bags at room temperature until use for all experiments reported in this study.

2.2   Aqua-ammonia pretreatment

A high pressure vessel (Parr 4626, Parr Instrument Co., Moline, IL) as shown in Figure 1 was used for providing aqua-ammonia pretreatment to the corn stover grind. The reactor had a maximum rupture pressure of 5000 psi and maximum tolerable temperature of 350℃. For each treatment level, 100 g biomass was charged in the reactor with 800 g deionized water. For the treatment level of 1:1 ammonia:biomass ratio, 100 g (60 mL) anhydrous ammonia was added; and for 2:1 ammonia:biomass ratio, 200 g (120 mL) anhydrous

ammonia was added in the reactor. For the control experiment, ammonia was not added. The reactor cap was closed and was heated by the temperature controlled electric heater until desired temperature was reached. The temperature was held for required treatment time (min) and then the pressure was released by opening the exhaust valve. The reactor was left in the chemical hood until it was cooled to room temperature. Three levels of pretreatment temperatures used were: 60℃, 90℃ and 120℃. Two levels of residence time used were: 5 and 30 min. The cooling time varied approximately from 30 min for 60℃ to 70 min for 120℃. The treated biomass was emptied from the reactor and the treated samples were packed in ziplock bags and stored under refrigeration until analysis of composition (as per the procedure given in 2.1) and enzymatic hydrolysis studies for saccharification to fermentable sugars.
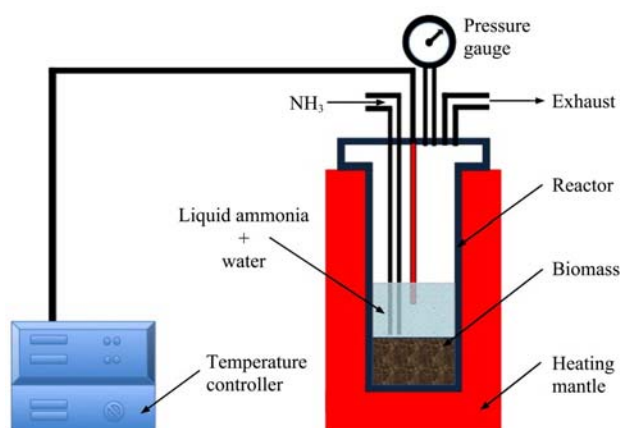

Figure 1   Experimental set-up used for aqua-ammonia pretreatment of corn stover grind (biomass).   The high pressure reactor was a 1.2 L pressure vessel (Parr 4626)

2.3   Enzymatic saccharification

Two commercial enzymes namely ACCELLERASE 1500 and ACCELLERASE XC (xylanases) supplied by Genencor, Rochester, NY, USA were used. A sample size of 0.2 g pretreated biomass sample was added to a 125 mL flask with citrate buffer (1.5 mL, 1.0 M, pH 4.8) and sodium azide (1 mL, 0.01 g/mL). The pH of the slurry was adjusted to 4.8 using acetic acid and the total volume was adjusted to 20 mL. Based on Genencor's application guidelines, 0.24 mL of ACCELLERASE 1500 and 0.25 mL of ACCELLERASE XC per gram of biomass were added to each flask.   Then the flasks were

incubated at 50℃ while shaking at   100 r/min. At different hours (0, 8, 16, 24, and 48),   1.0 mL samples were taken from each flask, centrifuged, and measured for total reducing sugars (TRS) by DNS (1,3-dinitrosalicylic acid) reagent method[19].   The percentage conversion was calculated based on the conversion of cellulose and hemicellulose portion into total reducing sugar, using the following equation:

Sugar conversion % = TRS release /(cellulose fraction *1.11+hemicellulose fraction *1.14)

where, TRS release = total reducing sugar released per gram of sample after 48 hours of enzymatic hydrolysis; cellulose fraction = cellulose content in gram per gram of biomass; hemicellulose fraction = hemicellulose content in gram per gram of biomass.

3   Results and discussion

3.1   Compositional changes

The average compositional variation of biomass with different levels of ammonia treatment is shown in Figure 2.   The water solubles fraction decreased with pretreatment with the maximum soluble removal at the treatment level 1:1 of ammonia:biomass.   The hemicellulose (C5) portion increased from 14.9% in untreated sample to 17.5% for pretreated sample at 1:1 treatment level.   Similarly, the cellulose content increased from 31.5% in untreated sample to 39.4% at the treatment level 1:1 with the maximum enrichment of cellulose at this treatment level.   Lignin content slightly increased (1%) for treatment levels 0:1 and 1:1, whereas for 2:1, the lignin content decreased (1%).   This indicates that there might be a possibility of removing lignin at higher ammonia level but it may also degrade cellulose and hemicellulose content and increase recycling problems in the downstream of pretreatment process.   Therefore, based on the variation in composition, the optimal level of ammonia biomass ratio would be 1:1.   Here we used 800 g of water per 100 g of biomass so that there was enough liquid to fill up the pore spaces of biomass in the high pressure vessel. The use of water reduced ammonia consumption in the reactor compared to ammonia fiber explosion using anhydrous ammonia[16].
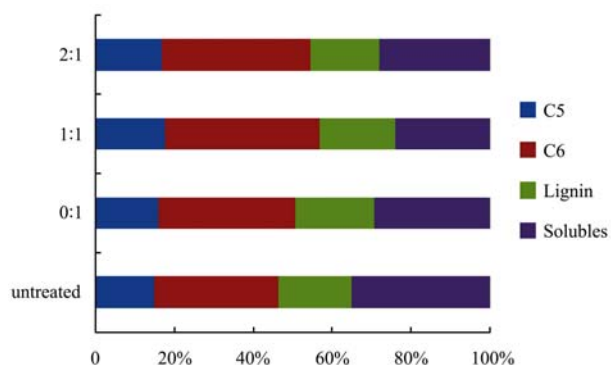
Figure 2   Compositional variation of pretreated biomass with various ammonia levels during aqua-ammonia pretreatment (average values for all treatment times and temperatures within a ammonia:biomass ratio level).   Rows indicate treatment levels, with ammonia:biomass ratio (by weight).   C5 and C6 indicate the hemicellulose and cellulose contents, respectively.

Figure 3 shows the effect of temperature on composition of biomass at the 1:1 ammonia:biomass level. The hemicellulose (C5), and lignin levels decreased with temperature, but the cellulose (C6) decreased at 90℃ but again increased at 120℃ at almost 60℃ level.   Based on changes in structural carbohydrate content, treatment at 60℃ should be beneficial as it will be less energy consuming and also have higher availability of cellulose and hemicellulose for conversion to fermentable sugars.
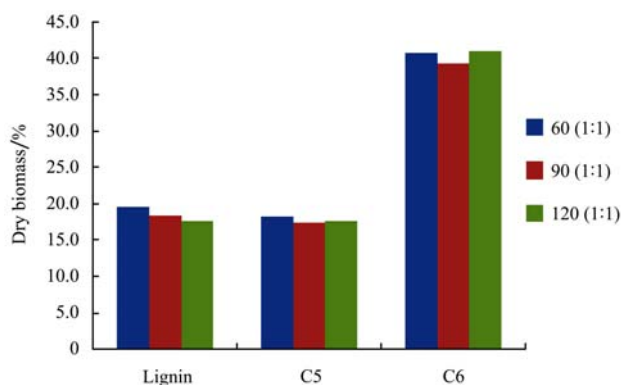

Figure 3   Compositional variation of pretreated biomass with pretreatment temperatures (60℃, 90℃ and 120℃) at the ammonia biomass ratio of 1:1.   The residence time was 30 min.

3.2   Saccharification of structural carbohydrates

Figure 4 shows the percentage conversion of structural carbohydrates into total reducing sugars by the enzymatic hydrolysis at the ammonia:biomass ratio of 1:1. The maximum conversion of 85% was obtained at 120℃ for 5 min treatment whereas 88% of structural carbohydrates were converted by pretreatment at 90℃ for

30 min.   The use of ammonia as a pretreatment agent significantly increased sugar conversion compared to the pretreatment by water alone (20%).   As seen in Figure 5, the maximum conversion of structural carbohydrates to fermentable sugars was 99% for 2:1 ammonia:biomass ratio, at 90℃ and 30 min treatment time.   When the temperature was increased to 120℃, the conversion of sugar was reduced.   This can be attributed to the degradation of cellulose and hemicellulose into charred products at 30 min treatment time.
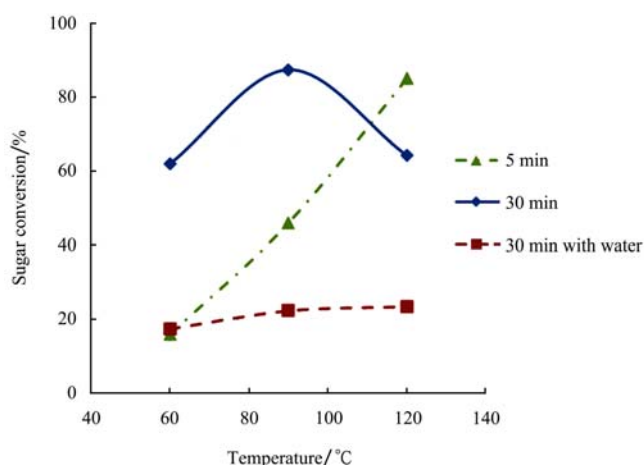

Figure 4   Percentage sugar conversion of corn stover pretreated in 1:1 ammonia:biomass ratio
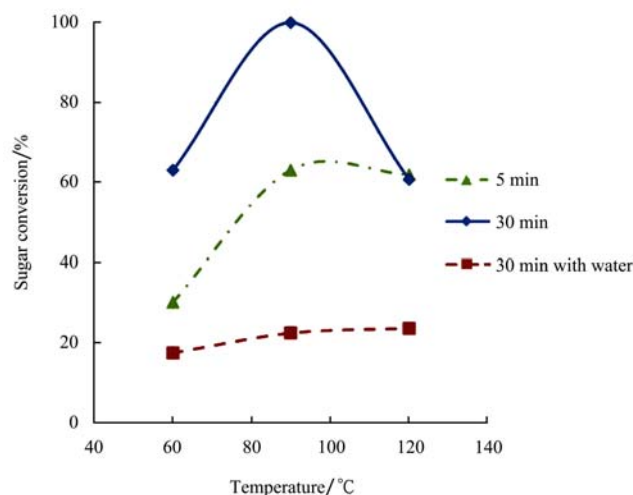

Figure 5   Percentage sugar conversion of corn stover pretreated in 2:1 ammonia:biomass ratio

The overall sugar conversion from the combined effects of pretreatment and enzymatic hydrolysis can be best represented by g TRS/100 g biomass.   The overall sugar yield was obtained by multiplying the sugar conversion percentage with the sum of cellulose and hemicellulose contents in biomass recovered after

pretreatment. As shown in Table 1, the overall sugar conversion increased with treatment time for 60℃ and 90℃, but it decreased when the temperature was 120℃. Highest overall sugar conversion was 46 g TRS/g biomass for the ammonia:biomass ratio of 1:1, treated at 90℃ for 30 min. Although higher percentage of sugar polymer conversion was observed for the 2:1 ammonia biomass pretreatment, the overall sugar yield (g TRS/100 g biomass) was almost the same as that of 1:1 ammonia:biomass ratio (Table 1). This was because the cellulose content in the 1:1 pretreated biomass was higher than that of 2:1 treated biomass. Overall, based on the final sugar yield, the best pretreatment condition should be 90℃ for the ammonia biomass ratio of 1:1, and treatment time of 30 min. However, since there is a large interval between 5 min and 30 min treatment time in this study, it is desirable to reduce pretreatment time by further study with treatment times between 5 and 30 min.

Table 1 Overall sugar conversion by combined effect of pretreatment and hydrolysis

Treatment level			Overall sugar conversion (TRS g/ 100 g biomass)
Ammonia:biomass ratio	Temperature/℃	Time/min	
1:1	60	5	8.3
		30	33.7
	90	5	22.7
		30	46.0
	120	5	44.5
		30	36.6
2:1	60	5	14.2
		30	30.0
	90	5	32.4
		30	45.8
	120	5	30.9
		30	27.2
0:1	60	30	8.3
	90	30	33.7
	120	30	22.7

4 Conclusions

Aqua-ammonia pretreatment significantly improved conversion of structural carbohydrates into fermentable sugars by commercial cellulase and xylase enzymes. Sugar conversion was maximum at 90℃. Based on overall sugar yield and lower requirement of ammonia,

the pretreatment level with ammonia:biomass ratio of 1:1 is recommended for aqua-ammonia pretreatment. Based on this study, the treatment time of 30 min is recommended. However, further optimization of treatment time may be possible, so that process time and cost can be further reduced.

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