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Dilute Sulfuric Acid Pretreatment of Corn Stover at High Solids Concentrations

Scientific Note

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INTRODUCTION

One of the more promising alternative fuels to replace gasoline is ethanol. However, in order to have a significant impact on our current oil consumption, ethanol must be both inexpensive and plentiful. Lignocellulosic biomass, from such sources as energy crops, agricultural residues, and municipal solid waste, is one resource that is plentiful and could potentially produce inexpensive ethanol (*1*).

One resource with the potential to produce large quantities of ethanol is agricultural residues. The US production of these residues is estimated at 300 million tons per year, with corn stover accounting for 150 million tons per year (*2*). This could potentially produce 15 billion gallons per year of ethanol, or approx 10% of our current gasoline consumption.

However, it is difficult to hydrolyze lignocellulosic biomass to glucose for subsequent conversion to ethanol because of the lignin-hemicellulose sheath that surrounds the cellulose and because of the crystallinity of the cellulose (*1*). Some form of pretreatment is needed to disrupt the sheath,

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increasing the susceptibility of the cellulose to enzymatic attack. Once the cellulose is converted to glucose, yeast can be used to ferment the glucose to ethanol.

One process for biomass pretreatment that has been extensively studied is the low-temperature (140–180°C) dilute sulfuric acid process (3,4). This process catalyzes the degradation of the hemicellulose fraction (primarily xylan to xylose) and the conversion of the amorphous cellulose fraction to glucose. Only a small fraction of the crystalline cellulose fraction is degraded to glucose. This process enhances the enzymatic digestibility (ability to convert the cellulose to glucose enzymatically) of the remaining substrate. However, degradation products of both glucose (5-hydroxymethylfurfural) and xylose (furfural) are produced that are toxic to yeast (2), and may inhibit enzyme and yeast activity. Both of these products are contained in the liquid hydrolyzate produced during pretreatment. However, little work has been done to investigate the feasibility of converting pretreated solids with hydrolyzate to ethanol.

Clausen and Gaddy (5) report the conversion of corn stover hydrolyzate to ethanol after a mild acid pretreatment followed by a concentrated acid hydrolysis to convert the cellulose to glucose. Performance equal to a synthetic glucose media was obtained; however, overall yields were not reported. Knappert et al. (6) pretreated corn stover with acid concentrations from 0 to 1.2% for 0.22 min at 180–220°C, using a cellulase loading of 40 IU/g substrate at a solids concentration of 2.5%. Good conversions (70–100%) were obtained after 2 d of enzymatic saccharification. Wayman et al. (7) pretreated corn stover with SO_2 and 160°C steam for 30 min at a solids concentration of 33%. After 2 d of enzymatic saccharification, the sugar yield was 86.5% of theoretical. Sugar yields of 38% after 1 d at 50°C and an enzyme loading of 15 g/substrate was obtained from a steam explosion pretreatment of corn stover at a pressure of 560 psig (3960 kPa, 250°C) for 30 min (*8*).

In this study, we investigated the effectiveness of a dilute sulfuric acid, high-solids (20–30%) pretreatment at different conditions on the enzymatic digestibility of corn stover. Corn stover (stalks and cobs) is defined as the residue remaining in the field after the corn kernels are harvested from the cob. In these experiments, the acid concentration was held constant, and the residence time and temperature were varied using a three-level full factorial design.

Although the dilute acid pretreatment of corn stover at some of the conditions used in this study have been investigated by others (3,4,6), the work was at lower solids concentrations (< 10%). This study examined the performance of a pretreatment system for steaming acid-impregnated corn stover at high solid concentrations (> 20%). Steaming of acid-impregnated wood chips has been investigated by the Forest Products Laboratory (9), but not on agricultural residues. Furthermore, this system more accurately reflects the type of system being considered for the conversion of biomass to ethanol (4). As such, our system was designed to pretreat large quantities of material and to assess the performance of the pretreated material in terms of enzymatic digestibility. It was not possible or desirable to obtain accurate yields or temperature profiles of the solids from these experiments for the generation of kinetic data.

MATERIALS AND METHODS

Feedstock

Corn stover was obtained as a rolled bale from a recently harvested corn field in eastern Colorado. The material was field dried and had a moisture content of 10–20%. The entire bale (approx 300 kg) was knife milled (Allsteel Knife Mill, 30 hp [22.4 kW]) through a 1/8-in (3.2-mm) rejection screen.

Pretreatment

The feedstock was prepared for pretreatment by soaking the particles in a 1.0 wt% solution of sulfuric acid at a 10 wt% solids concentration for 1 d. Excess liquid was removed by vacuum filtration, leaving particles of 20–30% solids concentration. The solids were loaded onto open trays and placed inside a 1.0 m long by 0.15 m in diameter cylindrical reactor. The reactor was jacketed for preheating of the solids to 120°C, direct steam injection then rapidly brought the solids up to reaction temperature. Temperature is measured by a K-type thermocouple inserted into the interior of the vessel. Timing begins when the indicated temperature reaches the targeted reaction temperature. However, the solids temperature probably lags the indicated temperature to some extent. At the end of the reaction time, the interior of the reactor is vented to flash cool the solids. The temperatures and residence times examined in these experiments are listed in Table 1.

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed in a 100-mL shake flask in a shaking incubator at 45°C. Triplicates were performed for each of the experiments listed in Table 1. The solids concentration of washed pretreated solids was 2.0 wt%, and the enzyme was Genencor Laminex cellulase (So. San Francisco, CA) at a loading of 40 IU/g solids. Citrate buffer (0.05*M*) was used to maintain pH at 4.8. Tetracycline at 40 g/mL and cycloheximide at 30 g/mL were used to control bacterial and fungal infections. At the end of 5 d, total sugars, defined as glucose and cellobiose after hydrolysis to glucose, were measured, and the digestibility was calculated as total sugars divided by potential glucose.

Table 1
Experimental Conditions and Corn Stover Composition Before and After Pretreatment

Experiment number	Temperature, °C	Residence time, min	Glucan, %	Xylan, %
Corn stover	–	–	38.6	20.4
1[a]	140	5.0	–	–
2	140	10.0	45.6	11.7
3	140	20.0	53.8	12.5
4	160	5.0	57.4	12.4
5	160	10.0	55.3	7.4
6	160	20.0	57.4	5.5
7	180	5.0	52.2	1.4
8	180	10.0	49.8	2.4
9	180	20.0	53.9	3.5

[a] Composition data not available.

Sugar Measurement

Total sugars produced during enzymatic hydrolysis were determined by incubating a sample with an equal vol of 2.0 mg/mL Novo 188 (Danbury, CT) β-glucosidase at 37°C for 2 h, which converts any remaining cellobiose to glucose. The glucose concentration was measured with a Yellow Springs Instrument analyzer (Yellow Springs, OH).

Corn Stover Composition

The procedure for analyzing the composition of these samples was based on the protocol developed by Olof Theander of the University of Agricultural Sciences in Uppsala, Sweden, for the analysis of lignocellulosic materials (*10*). This is a unique procedure that unifies the methods of analysis for wood and herbaceous materials.

Initially, air-dried samples were ground to pass a 1-mm mesh. Samples of this material were then oven dried (105°C) and analyzed for moisture and ash content. Soxhlet extraction was carried out with 95% ethanol for 6 h to determine the content of extractives of the samples and to prepare the materials for further analysis. The extractives-free samples were then digested with 72% sulfuric acid (3 mL) for 1 h at 30°C. After dilution with 84 mL of distilled water, the samples were digested again at 125°C for 1 h in an autoclave. Acid digestion of the extractives-free corn stover hydrolyzes the carbohydrates to their constituent sugars. The concentrations of the sugars in the filtrates from the acid digestions were determined by gas chromatography after reduction and acetylation of the sugars to their alditol acetates. Reduction was performed using potassium borohydride;

acetylation was then accomplished using acetic anhydride and 1-methylimidazole (*10*). The alditol acetates were quantitatively determined by gas chromatography using myo-inositol as an internal standard. The polysaccharide compositions of the original samples were calculated from the concentrations of the alditol acetates after applying loss factors, which correct for the small amount of degradation of the sugars that occurred during the acid digestion.

RESULTS AND DISCUSSION

Corn Stover Composition

The results of a preliminary analysis of the composition of corn stover and the pretreated material are also shown in Table 1. The composition of corn stover is similar to the composition reported by Sitton et al. (*11*) of 35% hexosans and 15% pentosans. The glucan content of all pretreated material is higher than the raw material, which is expected because little of the glucan is dissolved during pretreatment, whereas a much larger fraction of the hemicellulose is dissolved. This increases the relative amount of glucans.

In general, the more severe pretreatment conditions lead to more dissolution of the xylan. At a temperature of 140°C (experiments 2, 3) nearly half the xylan is converted to other products. As expected, at 160°C (experiments 4–6), there is a decrease in xylan content with increasing residence time. At 180°C (experiments 7–9), there is little xylan left after pretreatment. These data are somewhat inconsistent in that we expected that the xylan content would decrease with increasing residence time. However, this slight variability may be within the experimental error of the measurement.

Enzymatic Digestibility

The results of the enzymatic digestibility studies are presented in Fig. 1. The numbers given are the average yields for the three determinations, and error bars are 1 SD. The low yields at 140°C indicate that this temperature is not effective for pretreatment of corn stover. At 160°C, the yields increase from 56% at 5 min to 65% and finally 78% at the longer residence times. The longer time removes more of the xylan (as shown by the composition analysis). This increases the accessibility of the cellulose to the enzyme. Thus, the yields increase with increasing residence times. As expected, the highest yields were obtained at 180°C (96% at 10 min). The highest yield would be expected for experiment 9 (20-min residence time); however, the 77% yield is much lower than expected.

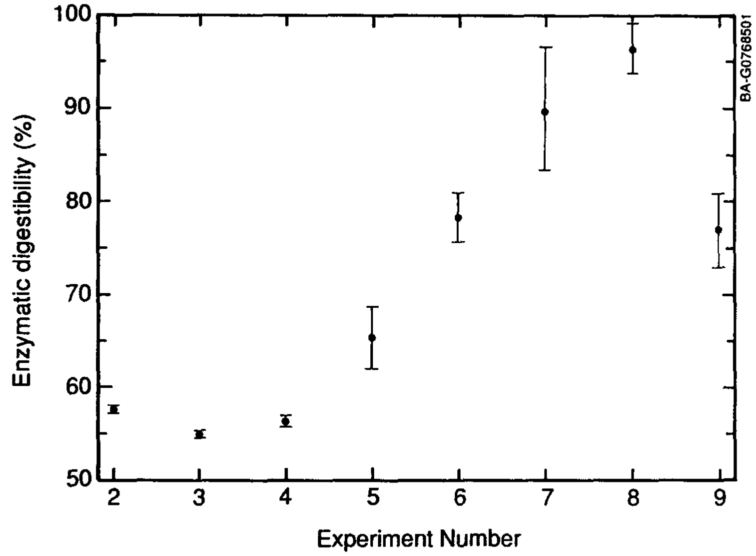

Fig. 1. Five-day enzymatic digestibility (40 IU/g solids) for each pretreatment experiment.

CONCLUSIONS

The enzymatic digestibility of a pretreated substrate is enhanced by increasing the severity of the pretreatment conditions, apparently because a greater fraction of the hemicellulose sheath surrounding the cellulose is dissolved. This allows the enzyme greater access to the cellulose. However, optimizing enzymatic digestibility will not optimize the production of ethanol. Although increasing the severity of the pretreatment conditions will decrease the remaining xylan, it will also result in the conversion of xylose to furfural. This is a loss of a potential carbon source for conversion to ethanol. The more severe conditions also result in the conversion of more of the cellulose to glucose and subsequent conversion to 5-hydroxymethylfurfural (HMF). Although the digestibility of the pretreated material may be enhanced by the more severe conditions, the conversion of this material to ethanol by yeast may be poor because of the higher levels of furfural and HMF, which are toxic to yeast. The best pretreatment conditions are those that produce the highest ethanol yields, which is influenced by the enzymatic digestibility of the pretreated substrate, the xylose yield, and the production of toxic byproducts.

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