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Optimization of Dilute-Acid Pretreatment of Corn Stover Using a High-Solids Percolation Reactor

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Abstract

We have previously demonstrated that pretreatment of corn stover with dilute sulfuric acid can achieve high digestibility and efficient recovery of hemicellulose sugars with high yield and concentration. Further improvement of this process was sought in this work. A modification was made in the operation of the percolation reactor that the reactor is preheated under atmospheric pressure to remove moisture that causes autohydrolysis. This eliminated sugar decomposition during the preheating stage and led to a considerable improvement in overall sugar yield. In addition, liquid throughput was minimized to the extent that only one reactor void volume of liquid was collected. This was done to attain a high xylose concentration in the hydrolyzate. The optimum reaction and operating conditions were identified wherein near quantitative enzymatic digestibilities are obtained with enzyme loading of 15 FPU/g glucan. With a reduced enzyme loading of 5 FPU/g glucan, the enzymatic digestibility was decreased, but still reached a level of 92%. Decomposition of carbohydrates was extremely low as indicated by the measured glucan and xylan mass closures (recovered sugar plus unreacted) which were 98% and 94%, respectively. The data obtained in this work indicate that the digestibility is related to the extent of xylan removal.

Index Entries: Corn stover; dilute-acid hydrolysis; percolation process; pretreatment; high sugar recovery; low enzyme loading.

Introduction

In the biomass-to-ethanol process, the feedstock and enzyme are two primary cost factors *(1)*. For a pretreatment to be economically feasible, efficient sugar recovery and high enzymatic digestibility (especially at low enzyme loadings) must be attained. It is particularly important to minimize sugar decomposition in the case of dilute acid pretreatment. It

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not only improves the sugar yield but also gives a beneficial effect on the subsequent bioconversion process by reduction of toxic components. It is well known that sugar decomposition generates various toxins including hydroxymethlyfurfural (HMF), furfural (*2*), and furans. Our previous work (*3*) have indicated that acid pretreatment of corn stover using a percolation reactor could provide high enzymatic digestibility as well as efficient recovery of hemicellulose sugars. The primary objective of this study is to seek further improvement of this process. Improvements were sought from two different angles. One was to give further insight into the process over a broader range of reaction conditions. The other was to adjust the reactor operating conditions. In either case, the focus of the improvement was on minimizing sugar decomposition, yet with an important constraint that a high glucan digestibility and an acceptable level of sugar concentration and yield in the pretreatment liquor are ensured.

Experimental Methods

Materials

Washed and air-dried corn stover was supplied by BioMass AgriProducts (Harlan, IA) and was knife-milled at NREL (National Renewable Energy Laboratory). It was screened and the fraction between 2 mm and 20 mesh was used as the feedstock of this work. It was stored at refrigeration temperature wherein the moisture content varied from 9 to 14 wt%. The chemical composition (dry basis) of the corn stover was 36.8% glucan, 21.7% xylan, 2.6% arabinan, 0.68% galactan, 0.3% mannan, and 17.2% lignin. Alpha-cellulose was purchased from Sigma (cat. no. C-8200, lot no. 11K0246). The cellulase enzyme (Spezyme CP, lot no. 301-00348-257) was provided by Genencor, Inc. through NREL. The average activity was 31.2 filter paper units (FPU)/mL. The β-glucosidase (Novozyme 188, lot no. 11K1088) was purchased from Sigma at an activity of 750 cellobiase units (CBU)/mL.

Analysis

The liquid samples were analyzed for sugars by HPLC equipped with Bio-Rad Aminex HPX-87P column and a refractive index detector (Shodex, Model-71). The total sugars (monomers+oligomers) in the hydrolyzate and the sugar contents in solid were measured following the procedures of NREL CAT-LAP- No. 014 and 002 (*4*), respectively. The hydrolyzate liquor contained significant amounts of oligomers and was subjected to secondary hydrolysis (4% H_2SO_4, 121°C, 1 h) to determine the oligomer contents. The Klason lignin content in the solid was measured by the procedure of NREL LAP-No. 003 (*4*).

Experimental Setup and Operation

The percolation reactor system was described elsewhere (*3*). The operation of the reactor was initiated with the reactor preheating. After initial series of experiments, it became apparent that the moisture retained in the biomass promotes autohydrolysis causing considerable sugar degradation. Therefore, in the present experiments, the reactor was preheated under atmospheric pressure with the vent valve of the collection tank open. After 25 min of preheating, the reactor system reached the desired temperature (160–180°C) and the biomass feedstock became totally moisture-free. The reactor was then immediately pressurized to 260 psi, at which point acid was pumped into the reactor (1 in. ID × 10 in. L) at a flow rate of 10 mL/min. The pretreatment was carried out until one reactor void volume (27 mL) of liquid came out of the reactor. The total time of reactor operation was 5.7 min and the mean reaction time for the entire solids was estimated to be 4.4 min. Upon completion of pretreatment reaction, the pump was stopped and the remaining reactor liquid was flushed out with nitrogen with concurrent cooling of the oven. Nitrogen was passed through the reactor by release of the reactor outlet pressure, which enables quick quenching of reactor to occur. Liquid samples were collected at three different points: (1) the reactor effluent—contained most of the hemicellulose sugars; (2) nitrogen-flush-out liquid (NFOL)—filled the reactor void volume, less concentrated than the effluent but contained considerable amount of sugars; and (3) liquid trapped in the solids (LTS)—stayed in the pores of the corn stover, not flushed out by nitrogen. The sugars in LTS were extracted with water for determination of its constituents.

Enzymatic Hydrolysis

The enzymatic digestibility of the treated biomass was measured by the procedure of NREL CAT-LAP-No. 009 (*4*). The enzymatic digestibility test was conducted at 50ºC in a laboratory-shaking incubator (150 rpm) with working volume of 50 mL in 250-mL Erlenmeyer flasks. The washed solid containing 0.5 g of glucan was added to the flask to attain 1% (w/v) of glucan. Spezyme CP (cellulase) was supplemented with 30 CBU (cellobiase units)/g glucan of Novozyme 188 (cellobiase) for the digestibility test. Alpha-cellulose was used as the reference substrate.

Results and discussion

Recovery of Hemicellulose Sugars

A percolation (flow-through) reactor works well for the recovery of sugar because the sugar products in the reactor effluent are removed from the reactor as soon as they are produced, thereby minimizing decomposition (*5*). The sugar recovery yields and mass closures are shown in Table 1.

Table 1
Sugar Distribution in Pretreatments and Mass Balance Closures[a]

Pretreatment conditions[b]		Yield in effluent		Yield in NFOL[c]		Yield in LTS[d]		Remained in solids		Mass closure	
Acid concentration (wt%)	Temperature (°C)	Glucose	Xylose	Glucose	Xylose	Glucose	Xylose	Glucan	Xylan	Glucan	Xylan
0.2	160	2.7%	20.6%	2.0%	27.3%	0.4%	7.5%	96.1%	40.4%	101.2%	95.8%
	170	3.3%	29.8%	2.1%	29.4%	0.4%	6.8%	93.2%	29.1%	99.0%	95.0%
	180	3.9%	37.4%	1.6%	24.8%	0.4%	5.3%	93.8%	26.5%	99.7%	94.1%
0.5	160	4.5%	49.3%	2.0%	25.8%	0.4%	4.3%	92.2%	14.8%	99.2%	94.1%
	170	5.8%	62.5%	1.9%	18.1%	0.8%	4.2%	89.1%	8.5%	97.5%	93.3%
	180	5.1%	54.9%	1.8%	19.9%	0.6%	3.7%	90.7%	11.3%	98.4%	89.8%
1.0	160	6.0%	69.1%	1.8%	16.0%	0.6%	2.3%	92.3%	8.6%	100.7%	96.0%
	170	6.3%	73.3%	1.2%	10.1%	0.5%	2.2%	90.7%	8.4%	98.7%	94.0%
	180	6.5%	66.9%	1.8%	10.9%	1.4%	1.9%	88.4%	5.6%	98.0%	85.2%

[a]Yields are expressed as the total of sugar monomers and oligomers.
[b]Acid flow rate = 10ml/min. Reaction time = 4.4 min.
[c]NFOL = nitrogen flush out liquid.
[d]LTS = liquid trapped in solid.

Taking the run with 1 wt% acid and 170ºC as an example, 8.4% of xylan retained in solids after pretreatment, and hence 91.6% of xylan was removed, of which 85.6% was collected as xylose and xylo-oligomers, and only 6% was lost by decomposition. Xylan mass closures from these runs were all above 93% except the two with 0.5 wt% and 1 wt% acids at 180ºC, in which xylan mass closures were 89.8% and 85.2%, respectively. The glucan mass closure was consistently high throughout with no more than 2% of glucose decomposition over the entire range of reaction conditions. Because only one reactor void volume of liquid was collected from the reactor, the sugars present in effluents were obtained in concentrated form. In the runs made with 1 wt% acid, 69–75% of the total xylan was obtained in the effluents, which retained 6 wt% of xylose concentration. If all the liquids (effluent plus NFOL and LTS) were recovered, the overall xylose yield would increase to 86%, but the concentration would decrease to 3 wt%.

Enzymatic Digestibility

Pretreatment of corn stover by dilute acid has been investigated by a number of different researchers. Knappert et al. (*6*) carried out dilute-acid pretreatment of corn stover in a continuous-flow reactor with solid concentration of 5% applying a fixed residence time of 0.22 min. Quantitative cellulose digestibility was achieved after 48 h for the substrate treated with 0.9 wt% acid and 216ºC using an enzyme loading of 26 FPU/g glucan (calculated from the table data). Um et al. (*7*) pretreated corn stover in a batch reactor operated with 2% (w/v) sulfuric acid at 121ºC for 120 min. They reported 80% digestibility with 40 FPU/g glucan enzyme loading. But the most intensified research along this line was conducted at the National Renewable Energy Laboratory. At the early stage, a continuous bench-scale cylindrical reactor operated at high solid concentration (20–30 wt%) was used for corn stover pretreatment (*8*). With an enzyme loading of 40 IU/g solids (or 80.3 IU/g glucan), the 5-d enzymatic digestibility was reported to be 96% for the substrate treated with 1 wt% sulfuric acid at180ºC for 10 min. Later, a pilot-scale process was developed with a reactor capacity of 1 t corn stover/d operated at 20 wt% solids concentration (*9*). Over a range of conditions (165–195°C, 0.5–1.4 wt% acid, and 3–12 min), the highest cellulose conversion yields (digestibility) were 80–87%. Recently, Schell et al. (*10*) reported that cellulose digestibility of 96% was also achievable for the corn stover pretreated with the same reactor system under optimized reaction conditions.

In the present work, the enzymatic digestion of corn stover was carried out at a comparatively low enzyme level (no higher than 15 FPU/g glucan). The enzymatic digestibilities of the glucan in the untreated and treated corn stover are listed in Table 2. For corn stover pretreated with 1% acid, 160–180ºC, the 96-h digestibilities were in the range of 92–98% using an enzyme loading of 15 FPU/g glucan.

Table 2
Summary of Sugar Recovery and 96-h Enzymatic Digestibility of Treated Corn Stover[a]

Pretreatment conditions[b]		Accountibility		96-h Enzymatic digestibility of Glucan
Acid concentration (wt%)	Temperature (°C)	Glucan	Xylan	
0.2	160	101.2%	95.8%	64.6%
	170	99.0%	95.0%	61.8%
	180	99.7%	94.1%	78.9%
0.5	160	99.2%	94.1%	85.3%
	170	97.5%	93.3%	90.6%
	180	98.4%	89.8%	93.4%
1.0	160	100.7%	96.0%	93.4%
	170	98.7%	94.0%	99.1 ± 0.2%
	180	98.0%	85.2%	98.8 ± 0.1%

[a]Enzymatic hydrolysis conditions: 1% (w/v) glucan concentration; 15 FPU/g glucan cellulase; pH 4.8; 50°C.

[b]Acid flow rate was hold at 10 mL/min and reaction time at 4.4 min.

The enzyme loading was further reduced to 10 and 5 FPU/g glucan for the substrate treated with 1 wt% acid and 170°C. As shown in Fig. 1, reduction of the enzyme loading resulted in significant decrease in the initial enzymatic hydrolysis rate and slight decrease in the digestibility. For the extremely low enzyme loading of 5 FPU/g glucan, the 96-h glucan digestibility was 92%. The digestibilities observed in this work are higher than or close to those reported by other groups (*6, 7*) taking the enzyme loading into consideration, and are positively comparable to those reported by Schell et al. (*9*), who obtained 87% of cellulose conversion (digestibility) for the pretreated corn stover employing 15 FPU/g cellulose in SSF (simultaneous saccharification and fermentation) test.

The digestibility of glucan appears to be proportional to the degree of xylan removal (Fig. 2). This agrees with an observation by Um et al. (*7*) that the removal of xylan improved the exposure of the cellulose to enzyme attack and, consequently, the digestibility. Although lignin was considered as an important factor hindering the access of enzyme to cellulose (*11–15*), we have observed high digestibilities despite high lignin content in some of our pretreated corn stover samples (*3*). This implies that xylan and lignin are parallel factors affecting the enzymatic digestion. The removal of either xylan or lignin somehow makes the biomass amenable for the enzymatic digestion.

Acid treatment also caused structural changes to corn stover, as shown by the SEM (scanning electron microscopy) pictures in Fig. 3. It is evident that dilute-acid treatment increases the surface area and the

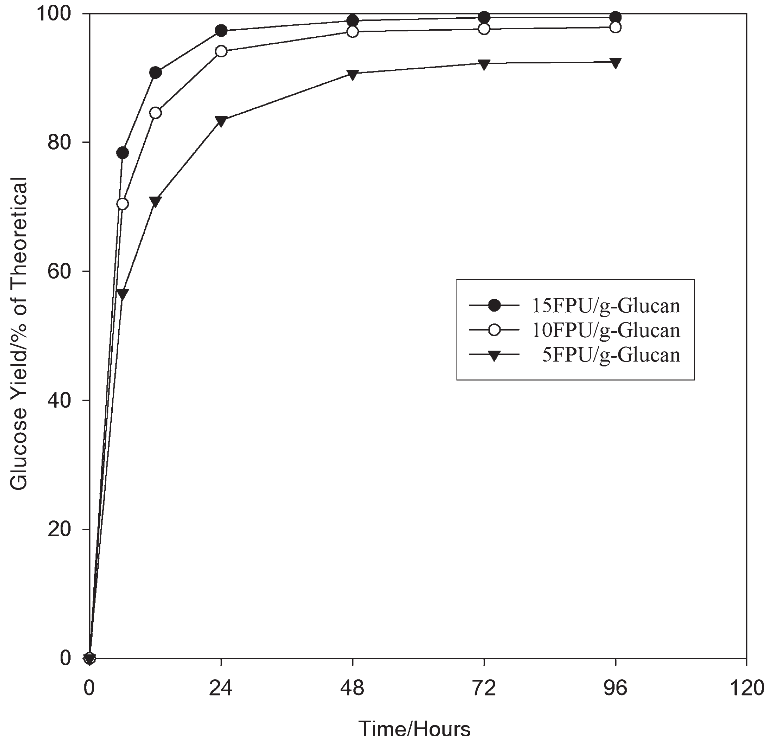


Fig. 1. Enzymatic hydrolysis of pretreated corn stover with various enzyme loadings. Pretreatment conditions: 1% acid, 10 mL/min flow rate, 170ºC, 4.4 min.

porosity of corn stover, consequently, the enzyme accessibility to cellulose. This observation agrees with earlier finding by Grethlein (*16*).

Evaluation of the Pretreatment

Table 2 summarizes the digestibility of treated corn stover and sugar recovery under various pretreatment conditions. The data indicate that pretreatment with 1 wt% acid and 170ºC is a near optimum condition. Under this particular pretreatment, 94% of xylan and 98.7% of glucan were accounted for, and quantitative enzymatic digestibility was obtained. Pretreatment at 180ºC with 1 wt% acid level also achieved a quantitative enzymatic digestibility. However, 15% xylose decomposition occurred in this run indicating that the reaction condition was too severe. In the low severity runs (0.2 wt% acid), high glucose and xylose accountabilities were maintained, but the digestibilities were significantly lower.

There are two important factors to be considered in our pretreatments in regard to the fact that the biomass in the reactor was not uniformly treated. First, because only small amount of liquid (approximately one reactor void volume) passed through the reactor, the pretreatment time for the

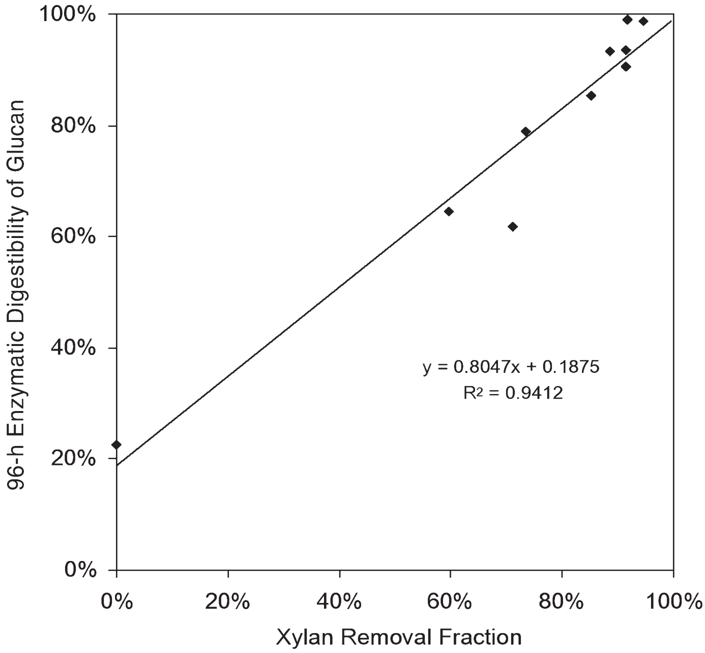
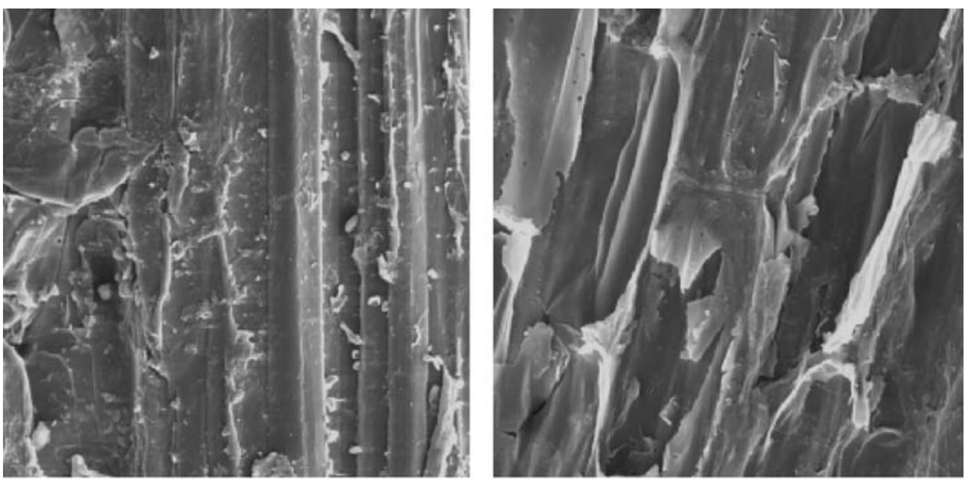


Fig. 2. Correlation between digestibility and xylan removal.



Untreated Corn Stover — Percolation Process, 1% Acid, 170°C, 4.4 min

Fig. 3. Scanning electron micrographs of treated and untreated corn stover (500×).

solids varied considerably depending on the axial position of the reactor, the reaction time of the biomass at the reactor inlet being nearly double that of the biomass at the outlet. Second, the acid was continually consumed as the liquid moved along the reactor because of the buffering components in corn stover. The pH value thus varied significantly from the reactor inlet (pH < 1.0) to the outlet (pH 2–3). Therefore, the corn stover solids at different axial positions were treated with different severities. This may lead to a non-uniform reactive situation in the reactor and considerably different composition among the solids remaining in the reactor. The composition data of pretreated solids presented in this work were the average. To overcome these potential problems, different strategies may be applied in reactor design and operation. A two-staged reverse-flow simulated counter-current reactor is one example (*5*).

There is also a concern as to whether acid impregnation into biomass was a factor influencing the rate of the overall pretreatment process. In our work, corn stover was moisture-free prior to acid introduction. The acid then penetrated into the particles through the pores for the reaction to occur. Because the corn stover was treated only for a short period of time and the reaction and impregnation occur simultaneously, it is uncertain whether the biomass attained uniformly wet condition by the end of the pretreatment. Nonetheless, the pretreatment process appeared to progress very well as shown by near complete removal of xylan and high digestibilities. We therefore conclude that the acid penetration is not a factor significantly affecting the pretreatment.

Conclusion

Dilute-acid pretreatment of corn stover with a percolation reactor was investigated over a broad range of reaction conditions. The optimum conditions were identified as 1 wt% acid, 170°C, and 4.4 min (the average reaction time for the reactor particles). The optimum pretreatment run resulted in 73% of xylose yield and 6 wt% xylose concentration. With recovery of trapped liquid, the xylose yield increased to 86%, but the xylose concentration decreased to 3 wt%. The treated and washed corn stover attained near quantitative digestibility with enzyme loading of 15 FPU/g glucan at 1 wt% solids concentration. With reduced enzyme loading of 5 FPU/g glucan, the digestibility was 92%. Decomposition of sugars was less than 6% as 98% of glucan and 94% of xylan were accounted for. The high yield of sugar was attributed to the unique characteristics of the reactor (flowthrough type) and adjustment of reactor operation (preheating under atmospheric pressure and quick quenching with nitrogen flush). Acid impregnation was not a factor significantly affecting the pretreatment process for the particle size used in this work. The digestibility of corn stover correlates directly with the extent of xylan removal.

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