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

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Abstract

Pretreatment of corn stover by dilute sulfuric acid was investigated using a laboratory percolation (flowthrough) reactor operated under high-solids conditions. The effects of reaction conditions and operating parameters on the performance of the percolation reactor were investigated seeking the optimal range in which acceptable levels of yield and sugar concentration could be attained. It was demonstrated that 70–75% recovery of xylose and 6 to 7% (w/w) xylose concentration were attainable. The high sugar concentration was obtained as a result of dense packing of dry corn stover and the low liquid throughput. Xylose was mostly unreacted, rather than decomposed. The cellulose and the unreacted xylan of treated corn stover were both effectively hydrolyzed by a "cellulase" enzyme preparation that also exhibits some activity on xylan. The xylose yield was affected significantly by the flow rate under the same reaction time and conditions. This behavior appears to be related to sugar decomposition, mass transfer resistance, and the fact that acid is neutralized by the buffering components of the biomass.

Index Entries: Corn stover; dilute-acid hydrolysis; hemicellulose; percolation process; pretreatment; xylan.

Introduction

Treatment of biomass by hot dilute acid is a well-known pretreatment method. In this process, most of the hemicellulose and some of the lignin is removed. Since hemicellulose and lignin provide a shield to the cellulose fibers, removal of these substances increases accessibility of cellulose to

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cellulase enzymes and, consequently, the rate and extent of enzymatic hydrolysis. Hemicellulose is a heteropolymer composed of a variety of sugar residues such as glucose, xylose, galactose, arabinose, and mannose. Since hemicellulose occupies approx 20% of the total biomass, nearly half as much as the cellulose contribution, it is important to recover and utilize this portion of sugars.

A percolation reactor (packed-bed flowthrough type) was proven to work well for biomass pretreatment *(1–4)*. The unique feature of this reactor is that it permits concurrent discharge of sugar products during the reaction process, thereby suppressing sugar decomposition and improving sugar yield. The percolation process also removes greater amounts of lignin than a batch process and can result in enhanced enzymatic digestibility of cellulose. In addition to the yield and digestibility of cellulose, there is another important performance measure in pretreatment: the concentration of sugars produced in the process. It must attain a certain level for a pretreatment process to be economically feasible. The percolation reactor performs reasonably well in this regard because it is a packed-bed reactor, which allows a high solid/liquid ratio.

In this present study, we were mainly interested in recovering hemicellulose sugar in high concentration while minimizing sugar decomposition. We were not particularly concerned with the xylose yields obtainable at the pretreatment stage because the unreacted hemicellulose can potentially be hydrolyzed enzymatically in the next step. Enzymatic hydrolysis of xylan is feasible because many cellulase preparations exhibit xylanase activity. We explored the optimal reaction and operating conditions of the percolation reactor with these constraints in mind. We were also interested evaluating the treated corn stover for enzymatic digestibility of the glucan and the remaining xylan.

Materials and Methods

Corn Stover and Chemicals

Washed and dried corn stover was supplied by BioMass AgriProducts (Harlan, IA) and was knife-milled at National Renewable Energy Laboratory (NREL). It was screened and the fraction between 20 and 60 mesh was used as the feedstock of this work. The corn stover was stored at a temperature wherein the average moisture content was 6.6% (w/w). The chemical composition ([w/w], 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. The cellulase enzyme used for the digestibility was Spezyme CP (lot no. 301-00348-257; Genencor, Palo Alto, CA). It was supplemented with β-glucosidase (Novozyme 188) to break down cellobiose to glucose.

Analytical Methods

The liquid samples were analyzed for sugars by high-performance liquid chromatography (HPLC) operated with a Bio-Rad Aminex HPX-87P

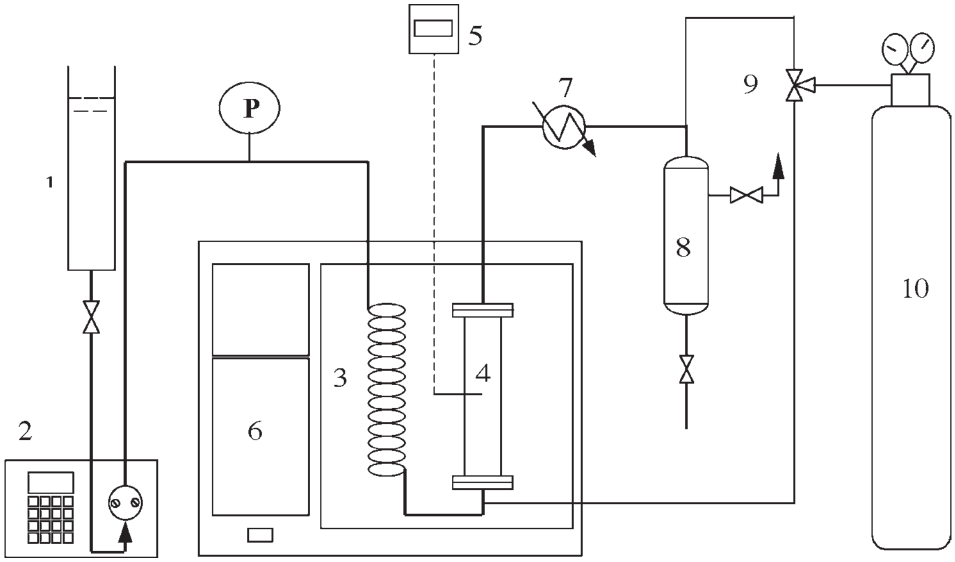

Fig. 1. Laboratory setup for percolation reactor system: 1, acid tank; 2, HPLC pump; 3, preheating coil; 4, percolation reactor; 5, thermometer; 6, gas chromatography oven; 7, heat exchanger; 8, product collecting tank; 9, three-way valve; 10, nitrogen gas.

column and a refractive index detector (Shodex RI-71, JM Science Inc., Grand Island, NY). The amounts of total sugars (monomers + oligomers) in the hydrolysate and the sugar contents in the solid were measured by NREL standard analytical procedure no. 014 and 002, respectively *(5)*. The sugars in the hydrolysate contained a high amount of oligomers. The hydrolysates were put through secondary hydrolysis (4% H_2SO_4, 121°C, 1 h) to determine the oligomer content. The enzymatic digestibility of the treated biomass was measured by NREL standard analytical procedure no. 009 *(5)*. The enzymatic digestibility test was conducted at 50°C in a laboratory shaking incubator (150 rpm) with a working volume of 50 mL in a 250-mL Erlenmeyer flask. The washed solid containing 0.5 g of glucan was added to the flask to attain 1% (w/v) glucan. Two levels of enzyme dosage were applied: 15 and 60 filter paper units (FPU)/(g of glucan) of Spezyme CP supplemented with 30 cellobiase units of Novozyme 188/(g of glucan).

Experimental Setup and Operation

The percolation reactor system is illustrated in Fig. 1. The reactor was made of Monel tubing (2.29 cm id × 9 cm). Hastelloy C-276 tubing (1/8 in.) was used for connections and for preheating coil. The reactor system had a provision to apply back pressure to 300 psig with N_2 gas. In the percolation reactor operation, 10.7 g of biomass (containing 10 g of dry solids) was packed into the tubular reactor with an internal volume of 37 cm^3. Under typical operating conditions, the reactor void fraction was 75% (v/v); thus, the reactor was operated with 25% (v/v) solids concentration. The reactor

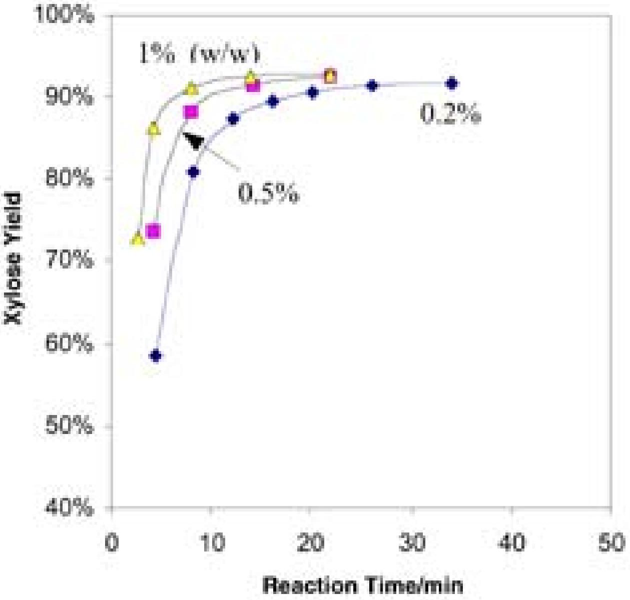

Fig. 2. Time courses of xylose production at different acid concentrations: 180°C, acid flow rate of 10 mL/min.

and preheating coil were placed in a forced convection oven for temperature control. The reactor system was preheated to a desired temperature, at which point the dilute acid was pumped into the reactor through a preheating coil by a piston pump (Acuflow-Series III, LabAlliance, PA). The reaction temperature was monitored by a thermocouple inserted into the center of the reactor bed and at the end outlet section of the reactor. The reactor effluent (hydrolysate) was quenched by a cooling heat exchanger connected immediately after the reactor.

Results and Discussion

Behavior of Percolation Reactor

Figure 2 shows the time courses of xylose production from the percolation reactor with variation of acid level. The acid flow rate was 10 mL/min, corresponding to an input liquid:solid ratio of 1 g of liquid/(g of dry feedstock·min of reaction time). Time zero was defined as the point when the first drop of liquid exited the reactor. Since the reactor contained dry biomass, the reaction time represents that of the particles at the reactor exit point. The particles at the entrance of the reactor would have a longer reaction time. The xylose production curves are similar in shape for all acid concentrations, the xylose yield rapidly increasing early in the reaction and then leveling off thereafter. The effluent contained sugars in both monomeric and oligomeric forms. The xylose oligomers accounted for 40–80% of

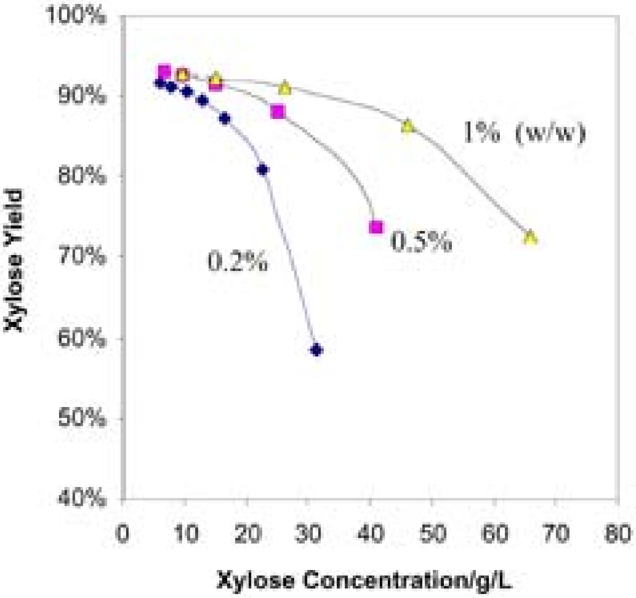

Fig. 3. Relationship between xylose yield and xylose concentration at different acid levels: 180°C, acid flow rate of 10 mL/min.

the total monomeric and oligomeric xylose. The amount of xylose expressed in Fig. 2 is therefore interpreted as xylose plus oligomers. For all acid levels, the maximum xylose yields surpassed 90%. At the 4-min point, the yield of xylose increased from below 55 to above 86% as the acid level increased from 0.2 to 1%. We found that the pH of the liquid exiting the reactor was in the range of 2.0–3.5 for a reaction time of <2.5 min. This indicates that a large fraction of the acid was neutralized in the reactor owing to the buffering components existing in the corn stover. This unusual situation is related to the high-solids condition that we applied.

In theory, the first drop exiting the reactor should have the highest concentration *(2)*. It decreases gradually as the reaction progresses. With longer reaction time, the overall cumulative sugar yield increased whereas the sugar product was diluted. The yield and sugar concentration therefore had an inverse relationship in the percolation reactor, as indicated in Fig. 3.

Figures 4 and 5 present the effects of temperature on xylose production. Temperature showed a distinct positive effect on yield and concentration within the range of 160–180°C. The effects of temperature and acid concentration were consistent with those of previous kinetic models in which the hydrolysis rate constant followed an Arrhenius relationship for temperature and increased proportionally with acid concentration raised to a certain exponent *(6–8)*.

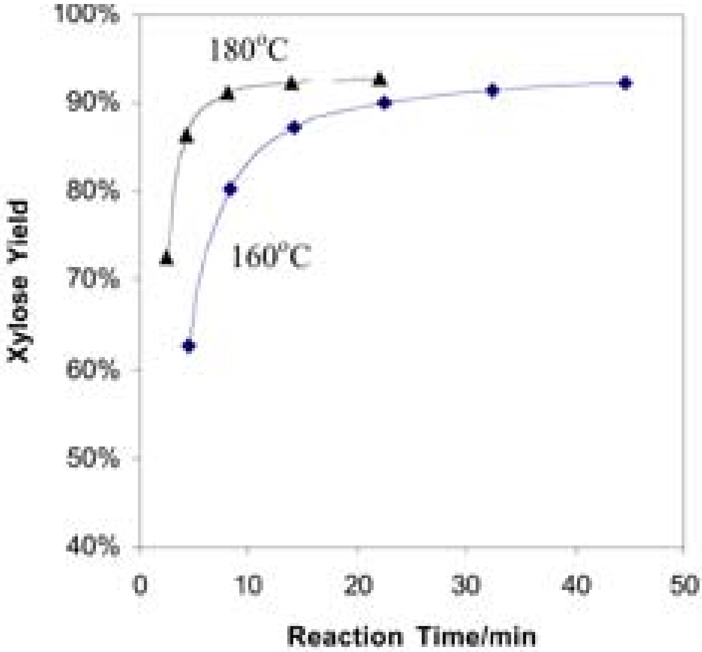

Fig. 4. Time courses of xylose production at 160 and 180°C: 1 wt% acid concentration, acid flow rate of 10 mL/min.

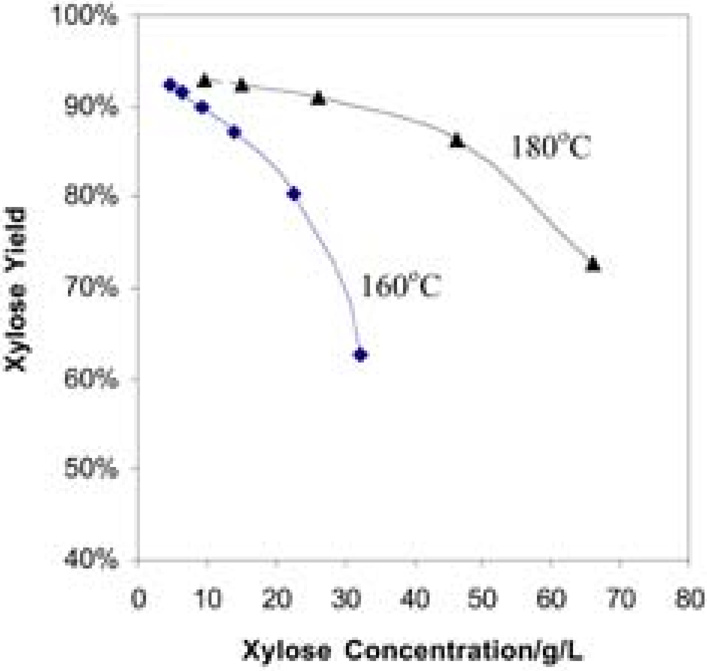

Fig. 5. Relationship between xylose yield and xylose concentration at 160 and 180°C: 1% (w/w) acid, acid flow rate of 10 mL/min.

Mass Balance of Xylan

Interesting in Fig. 3 is the run in which the yield of xylose is 73% and the concentration is 66 g/L (or 6.6 wt%). This occurred under the conditions of 1% (w/w) acid, 180°C, and 2.5 min of reaction time (recovery of 25 mL of total fluid). Of the 27% unrecovered xylan, 9% was left unreacted, and 9% was left in the liquid trapped inside the reactor. The effective yield was then actually 82%, since the sugars trapped in the retained liquid would be available for conversion in subsequent process steps. Only 7% of xylan was unaccounted for, most likely owing to decomposition. We have found that most of the xylan decomposition occurs during the preheating stage, which was done under 300 psi of nitrogen backpressure. It appears that the moisture content in the biomass under high temperature and pressure caused autohydrolysis and subsequent decomposition. The measured decomposition of xylan during preheating (180°C, 25 min) was 4.8%, which accounts for the majority of the decomposition in the entire process. We recently found that decomposition during the preheating period could be reduced to a nearly zero level when done under atmospheric pressure. From this observation, we contend that when properly operated dilute-acid percolation pretreatment can be carried out with negligible xylan decomposition.

One may consider further reducing the liquid input to obtain an even higher sugar concentration, which will then result in less xylose recovery. Assessing the data we have obtained to this point, we think it is possible to attain a 50% yield of xylose with a 10% sugar concentration. This is nearly at the level where it can be used as a fermentation substrate without further concentration. The low yield of sugar during the pretreatment stage is not a detriment in this case because the balance of xylan mostly stays intact unreacted, rather than being decomposed. The unreacted xylan can then be hydrolyzed enzymatically by xylanase normally embodied in "cellulase" *(9,10)*. This may bring the overall xylose yield for the combined pretreatment and enzymatic process to a level economically acceptable. It is also noteworthy that the enzymatically produced sugar is nontoxic to microorganisms. The xylose trapped in the reactor after the run can be recycled into the subsequent run to retain a high sugar concentration. In some process scenarios, the entrained xylose-rich liquid can remain in a slurry with the pretreated solids and be used in a simultaneous saccharification and cofermentation, allowing fermentation of both xylose and enzymatically released glucose using a single cofermenting microorganism. It is also technically feasible to operate the percolation reactor such that no residual liquid is left in the reactor at the end of the run. This can be done by introducing the desired amount of liquid by pumping, and pushing it through the reactor with hot air, which would further reduce liquid input and increase sugar concentration. This is similar to a process concept recently adopted and being tested at NREL: high-solids pretreatment by Pneuma-Press® filtering operation *(11)*.

Sugars other than xylose (glucose, arabinose, galactose, and mannose) were also generated during the acid hydrolysis of corn stover hemicellu-

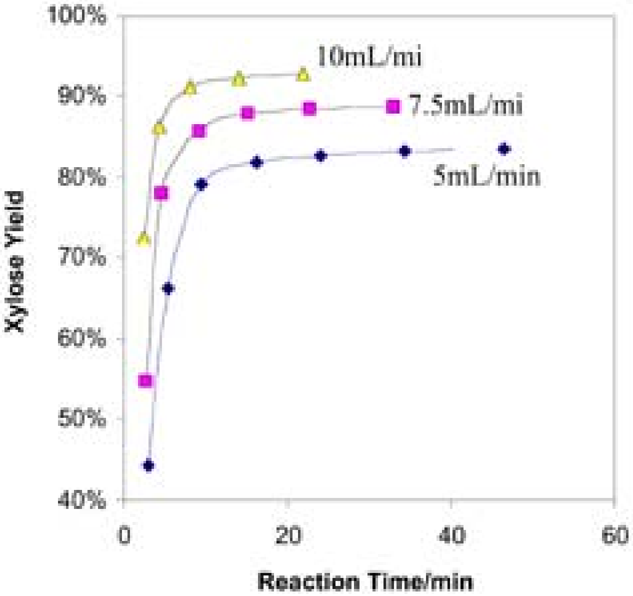

Fig. 6. Time courses of xylose production with various acid flow rates: 180°C, 1% (w/w) acid concentration.

lose. The glucose yield was much lower than xylose. As a reference point, in the run where 73% of xylose was recovered with a concentration of 6.6% (w/w), the glucose yield was only 4.5%. The glucose produced in the pretreatment is believed to have originated from hemicellulose and amorphous cellulose.

Effect of Flow Rate on Performance of Percolation Reactor

The effects of acid flow rate on xylose yield and concentration in the percolation reactor are shown in Figs. 6 and 7. The temperature and acid concentration were respectively fixed at 180°C and 1% (w/w) for these runs. The acid flow rate showed significant positive effects on both concentration and yield over the range of 5–10 mL/min. The maximum xylose yields observed were 83.5 and 92.9% for acid flows of 5 and 10 mL/min, respectively. The increase in yield was insignificant beyond the flow rate of 10 mL/min. The hydrolysis rate was also improved by an increase in acid flow rate, as indicated by the different slopes of the curves in Fig. 6. At the early phase of the reaction, taking the 3-min point as an example, a xylose yield of 44% was attained with a 5 mL/min flow rate. It rose rapidly to 65% with a 10 mL/min flow rate. No significant increase in hydrolysis rate was observed beyond the flow rate of 10 mL/min.

Figure 7 presents the relationship between xylose yield and concentration at various acid flow rates. Again, the inverse relationship between the yield and the sugar concentration was reaffirmed. The experimental run discussed earlier was also included: the highest xylose concentration (6.6%) was obtained at a flow rate of 10 mL/min with a xylose yield of 73%.

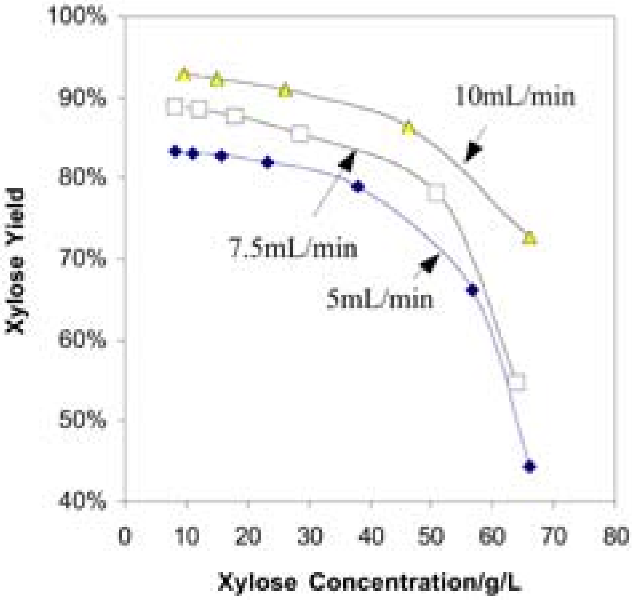

Fig. 7. Relationship between xylose yield and xylose concentration with various acid flow rates: 180°C, 1% (w/w) acid concentration.

The rate of the hydrolysis reaction is dependent only on temperature and acid concentration. The extent of reaction is also dependent on reaction time. From this viewpoint, the xylose yield profiles vs reaction time should be constant for all flow rates, but obviously they are not (*see* Fig. 6). One of the primary reasons for this behavior is the decomposition of sugars. The hydrolysis reaction occurs only in the solid phase, whereas the decomposition reaction occurs only in the liquid phase. Therefore, the hydrolysis is controlled by clock time, but the decomposition is controlled by residence time. Lower flow rate allows longer residence time, thus inducing higher decomposition and lower xylose yield.

There are other factors that may play a role in how the flow rate is affecting reactor performance. Liu and Wyman *(12)* found that higher flow rates induce faster xylose (and lignin) removal in a percolation reactor, which is not explainable by traditional kinetics. They postulated that there may be a mass transfer effect in the reactor. This may partly explain our experimental results because of the relatively low flow rates applied in our work. The presence of buffering components in corn stover may also interfere with reactor performance. It has been reported that corn stover has the neutralizing capacity of 43.7 mg of H_2SO_4/g of dry substrate *(7)*. As indicated earlier, we observed that the pH of the reactor effluents indeed rose to 2.0–3.5 for all the samples taken before 2.5 min, a drastic change in pH from the input pH of near 1.0. This would add to the effect of flow rate on reactor performance since the overall pH in the reactor is influenced by the flow rate: a low flow rate induces high pH because a higher percentage of

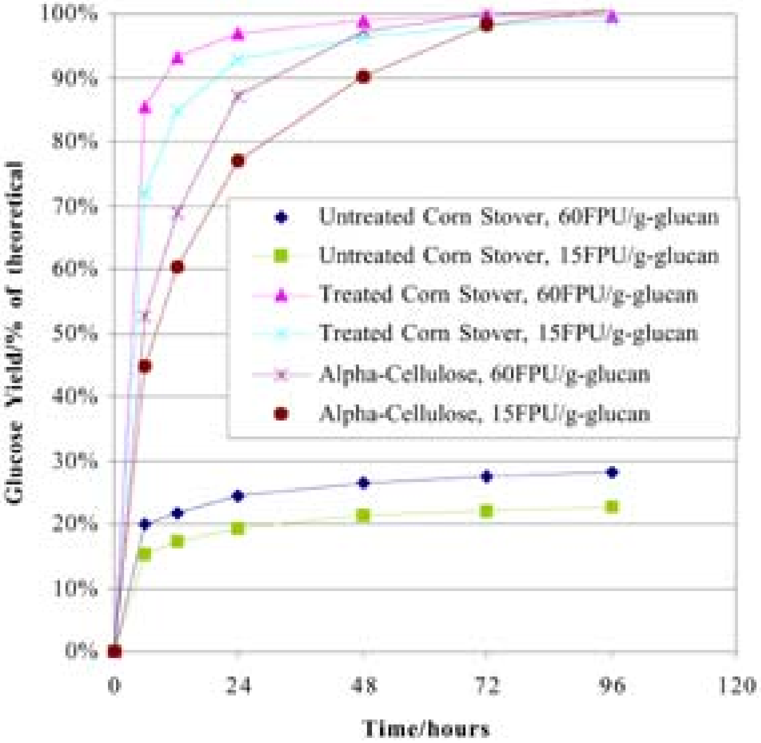

Fig. 8. Enzymatic digestibility of corn stover pretreated with 1% (w/w) acid at 180°C and a flow rate of 10 mL/min for 3 min followed by nitrogen-gas-through quenching.

acid is consumed. We believe that the strong influence of flow rate on the yield and concentration of the percolation reactor as observed in our work is owing to the combined effects of neutralization of acid by the buffering components, sugar decomposition, and mass transfer.

Enzymatic Digestibility of Treated Corn Stover

To assess the efficiency of acid pretreatment with the percolation process, a standard enzymatic hydrolysis test was conducted for pretreated corn stover. The sample was taken from the run under the conditions of 1% (w/w) acid, 180°C, 10 mL/min flow rate, and 3 min. The reaction was stopped by nitrogen gas throughquenching. The pretreated solids had a composition ([w/w], dry basis) of 63.4% glucan, 2.4% xylan, and 25.0% Klason lignin.

Figure 8 shows the enzymatic digestibility test profiles of the glucose for the pretreated corn stover. Untreated corn stover and α-cellulose were used as the control and reference substrate, respectively. The 96-h glucan conversion of untreated corn stover was only 28 and 23% with respective cellulase loadings of 60 and 15 FPU/(g of glucan). With the pretreated corn stover, much higher glucan conversions were observed after 96 h at both levels of enzyme loading. It is quite evident that the high-solids dilute-acid percolation process is a very effective pretreatment method. The efficacy of the pretreatment can be further enlightened by comparison of the profiles of treated corn stover with those of α-cellulose (lot no. 11K0246; Sigma, St. Louis, MO). Although quantitative glucan digestibility was also found with α-cellulose after 96 h, initial glucan hydrolysis of α-cellulose was substantially slower that that of pretreated corn stover. Interestingly, the pretreated substrate contains a high level of lignin (25% Klason lignin). Although lignin is regarded as one of the main factors controlling the enzymatic digestion, it appears that low lignin in the substrate is not a necessary condition to achieve high-level digestibility in the case of dilute-acid pretreatment.

Figure 8 also shows that most of the glucose from the pretreated corn stover was formed before 12 h. Higher enzyme loading resulted in substantially faster hydrolysis in the early phase, as indicated by the significantly different glucose yields at 6 h. The difference gradually diminished with time, having little effect on the terminal glucan digestibility. It is also notable that the residual xylan, although only 2.4% after pretreatment, was hydrolyzed by the cellulase enzyme (data not shown). At 96 h, 77 and 83% of xylan were hydrolyzed with enzyme loadings of 15 and 60 FPU/(g of glucan), respectively.

Conclusion

Pretreatment of corn stover with dilute acid was investigated using a laboratory-scale percolation reactor. The performance of the reactor was assessed regarding recovery of hemicellulose sugars over a broad range of reaction and operating conditions. It was demonstrated that the percolation reactor could produce xylose with a 73% yield and 6.6% (w/w) concentration. Less than 10% of the total xylan was decomposed during this process. Retention of high sugar concentration was possible because the reactor was operated with a low amount of liquid throughput, about one reactor void volume. The high solid-to-liquid ratio caused significant changes in the pH in the reactor owing to neutralization by the buffering components of corn stover. This also amplified the effect of flow rate on reactor performance. An enzymatic digestibility test was performed for the treated corn stover. Quantitative conversion of glucan was observed with enzyme loadings of 60 and 15 FPU/(g of glucan). The same test showed concurrent hydrolysis of the remaining xylan with the resulting digestibility in the vicinity of 80%.

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