0273-2289/04/115/0951–0963/$25.00

Ammonia Fiber Explosion Treatment of Corn Stover

Farzaneh Teymouri, Lizbeth Laureano-Pérez, Hasan Alizadeh, and Bruce E. Dale*

Department of Chemical Engineering and Materials Science, 2527 Engineering Building, Michigan State University, East Lansing MI 48824, E-mail: bdale@egr.msu.edu

Abstract

Optimizing process conditions and parameters such as ammonia loading, moisture content of biomass, temperature, and residence time is necessary for maximum effectiveness of the ammonia fiber explosion process. Approximate optimal pretreatment conditions for corn stover were found to be temperature of 90°C, ammonia:dry corn stover mass ratio of 1:1, moisture content of corn stover of 60% (dry weight basis), and residence time (holding at target temperature), of 5 min. Approximately 98% of the theoretical glucose yield was obtained during enzymatic hydrolysis of the optimal treated corn stover using 60 filter paper units (FPU) of cellulase enzyme/g of glucan (equal to 22 FPU/g of dry corn stover). The ethanol yield from this sample was increased up to 2.2 times over that of untreated sample. Lowering enzyme loading to 15 and 7.5 FPU/g of glucan did not significantly affect the glucose yield compared with 60 FPU, and any differences between effects at different enzyme levels decreased as the treatment temperature increased.

Index Entries: Ammonia fiber explosion; corn stover; enzymatic hydrolysis; simultaneous saccharification and fermentation; moisture content; residence time.

Introduction

Utilizing solar energy and extracting energy from the carbon fixed by photosynthesis in plants offers a potentially attractive solution to establish clean and sustainable resources for both energy demands and raw material needs. It has been estimated that 10^{11}–10^{12} tons of carbon is fixed annually around the world by photosynthesis of higher plants. Potentially, this renewable resource can provide approx 10 times our current energy demand from all sources *(1)*.

*Author to whom all correspondence and reprint requests should be addressed.

The potential for using lignocellulosic biomass material to produce energy carriers such as electricity, gases, and transportation fuels is well recognized. Biomass energy produced in an efficient and sustainable manner can offer numerous economic, environmental, and social benefits compared to fossil fuels. One liquid fuel that has the potential to match the convenient attributes of petroleum fuels is ethanol produced from lignocellulosic biomass. In ethanol production, enzymatic hydrolysis of cellulose to glucose is a very attractive route, because nearly theoretical yields of glucose are possible *(2,3)*.

A major problem in the commercialization of this potential is the inherent resistance of lignocellulosic materials toward conversion to fermentable sugars *(4)*. To improve the efficiency of enzymatic hydrolysis, a pretreatment step is necessary to make the cellulose fraction accessible to cellulase enzymes. Delignification, removal of hemicellulose, and decreasing the crystallinity of cellulose produce more accessible surface area for cellulase enzymes to react with cellulose *(5)*.

The ammonia fiber explosion (AFEX) process treats lignocellulosic biomass with liquid ammonia under pressure followed by explosive pressure release to enhance conversion of structural carbohydrates (cellulose and hemicellulose) to fermentable sugars. Instantly releasing the pressure in the AFEX process disrupts the fibrous structure of biomass and increases the accessible surface area, which improves the digestibility of the biomass. However, a previous study *(6)* has shown that increasing the blowdown pressure from 1.4 to 2.3 and 3 atm did not affect the reactivity of the treated biomass and the results were similar, but at higher pressure, 4 atm, less reactivity was observed. AFEX treatment also increases the digestibility of the biomass by decrystallizing cellulose, prehydrolyzing hemicellulose, and reducing lignin content of the treated material.

A major obstacle to the commercialization of enzymatic hydrolysis of biomass is the high cost of the enzymes. One way to reduce this cost is to use much less enzyme per unit of biomass hydrolyzed. Previous work has shown that the effective enzymatic hydrolysis of AFEX-treated biomass at enzyme loadings as low as 5 filter paper units (FPU)/g of dry biomass was achieved by adjusting the pretreatment parameters *(6)*. The objectives of the present study, were to determine the best AFEX conditions for pretreatment of corn stover, to employ different enzyme loading levels (60, 15, and 7.5, FPU/g of glucan), and to compare the enzymatic hydrolysis results and ethanol yield.

Materials and Methods

Substrate

The biomass material studied was corn stover (includes all aboveground portions of the corn plant except the grain and cob). Milled (passed through a 6-mm screen) and dried (about <10% moisture content) corn stover was provided by National Renewable Energy Laboratory (NREL)

Table 1
Composition of Corn Stover

Component	Mass percentage (dwb)
Glucan	36.1
Xylan	21.4
Arabinan	3.5
Mannan	1.8
Galactan	2.5
Lignin	17.2
Protein	4.0
Acetyl	3.2
Ash	7.1
Uronic acid (estimated)	3.6
Nonstructural sugars	1.2
Total	101.6

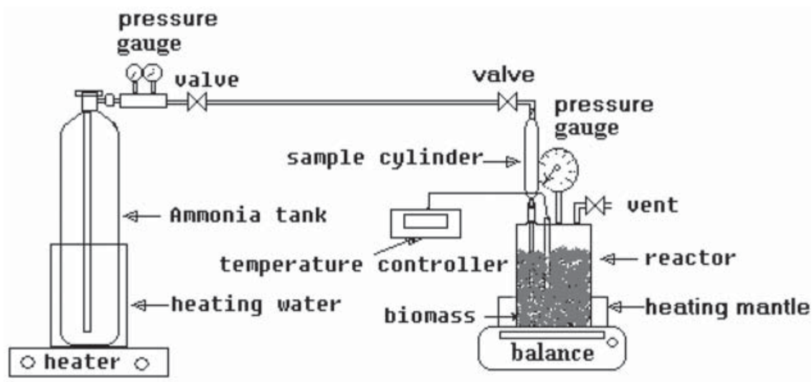

Fig. 1. Schematic diagram of laboratory AFEX apparatus.

(Golden, CO), as was its composition (Table 1). Liquid anhydrous ammonia was from AGA (Lansing, MI).

AFEX Treatment

The reactor consisted of a 300-mL stainless steel pressure vessel (Parr, Moline, IL) (Fig. 1). The vessel was loaded with prewetted corn stover (at desired moisture content). The vessel was topped up with stainless steel spheres (approx 1 mm in diameter) to occupy the void space and thus minimize transformation of the ammonia from liquid to gas during loading.

The lid was then bolted shut. Using the precalibrated ammonia sample cylinders, the predetermined amount of liquid ammonia was delivered to the vessel. The vessel was heated by a 400-W Parr heating mantle to the desired temperature. After holding the vessel at the target temperature for the selected residence time, the exhaust valve was rapidly opened to relieve the pressure and accomplish the explosion. Both pressure and temperature drop very rapidly. The treated samples were removed and allowed to stand overnight in a fume hood to evaporate the residual ammonia.

Treatment of the corn stover with ammonia resulted in a darkening of corn stover compared with untreated sample but did not otherwise change the macroscopic appearance of the substrate. The treated samples were kept in plastic bags in a refrigerator for further analysis.

Enzymatic Hydrolysis

We essentially followed the NREL standard biomass enzymatic hydrolysis protocol (LAP-009). The NREL standard protocols can be obtained from the following website: www.ott.doe.gov/biofuels/analytical_methods.html.

All the samples were hydrolyzed in a pH 4.8 citrate buffer with the desired cellulase enzyme (Celluclast 1.5 L [Novozyme] provided by NREL, CAS 9012-548; activity: 28 FPU/mL) loading (60, 15, and 7.5 FPU/g of glucan), and β-glucosidase from Sigma (St. Louis, MO) at 40 IU/g of glucan. All the samples were hydrolyzed at 50°C with gentle rotation (75 rpm) for a period of 168 h. At predetermined time intervals (0, 3, 6, 24, 48, 72, and 168 h), 1 mL of hydrolysate was taken for sugar analysis. Sugar analysis was performed using a Bio-Rad (Richmond, CA) high-performance liquid chromatography (HPLC) unit equipped with an Aminex HPX87P carbohydrate analysis column and a Bio-Rad Deashing Cartridge guard column. The mobile phase used was degassed HPLC water at a flow rate of 0.6 mL/min at 85°C. The injection volume used was 20 µL with a run time of 20 min.

Simultaneous Saccharification and Fermentation

Simultaneous saccharification and fermentation (SSF) experiments were conducted according to NREL standard protocol (LAP-008). Each SSF flask was loaded with 3% (w/w) glucan, 1% (w/v) yeast extract, 2% (w/v) peptone, 0.05 M citrate buffer (pH 4.8), the appropriate amount of cellulase enzyme to achieve 15 FPU/g of glucan, and the appropriate amount of *Saccharomyces cerevisiae* D_5A (provided by NREL) inoculum (starting optical density of 0.5). The SSF flasks were equipped with water traps to maintain anaerobic conditions and were incubated at 37°C with gentle rotation (130 rpm) for a period of 168 h.

At time intervals of 0, 3, 6, 24, 48, 72, 96, and 16 8h, a 2-mL aliquot was removed aseptically from each flask. The samples were centrifuged, and the supernatants were filtered for sugar analysis on HPLC and ethanol analysis by gas chromatography (GC). At the last time point, a sample from each SSF flask was streaked on a YPD (yeast extract, peptone, dextrose)

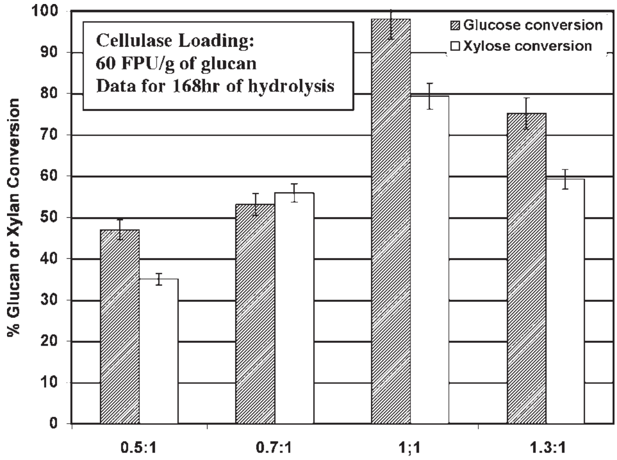

Fig. 2. Effects of ammonia loading (g of NH3:g of dry biomass) on enzymatic conversion of glucan and xylan for AFEX treatment of corn stover at 90°C and 60% moisture content (dwb). All the runs were kept at the set temperature for 5 min.

plate to check for any contamination. No contamination was observed in this series of SSF experiments.

The samples taken from fermentation at different time intervals were analyzed for ethanol yield by a GC 17 Shimadzu (Columbia, MD) unit. The injection temperature was 240°C and the detector temperature was 255°C. The column was first maintained at 80°C up to 3 min, followed by a temperature program at 15°C/min up to 125°C for 6 min.

Results and Discussion

Effects of Ammonia-to-Biomass Ratio on Enzymatic Hydrolysis of AFEX-Treated Corn Stover

Figure 2 shows the effect of ammonia-to-biomass ratio (0.5:1, 0.7:1, 1:1, and 1.3:1 g of anhydrous ammonia:g of dry biomass) on the subsequent enzymatic hydrolysis of AFEX-treated corn stover. Enzymatic hydrolysis was carried out for 168 h. Glucan conversion increased with increasing ammonia loading and attained a maximum value at a mass ratio of 1:1. Xylan conversion also showed the same trend as glucan conversion in response to ammonia loading. Ammonia at this loading provided maximum overall enhancement of reactivity during pretreatment. It is known that ammonia can react with lignocellulosic materials by ammonolysis of the ester crosslinks of some uronic acids with the xylan units and by cleaving the bonds linking hemicellulose and lignin *(7)*. However, it is evident from Fig. 2 that further increases in ammonia loading decreased glucan conversion. It is possible that extra liquid ammonia plasticizes *(8)* the cellulose and thereby reduces the disruptive effect of sudden pressure release.

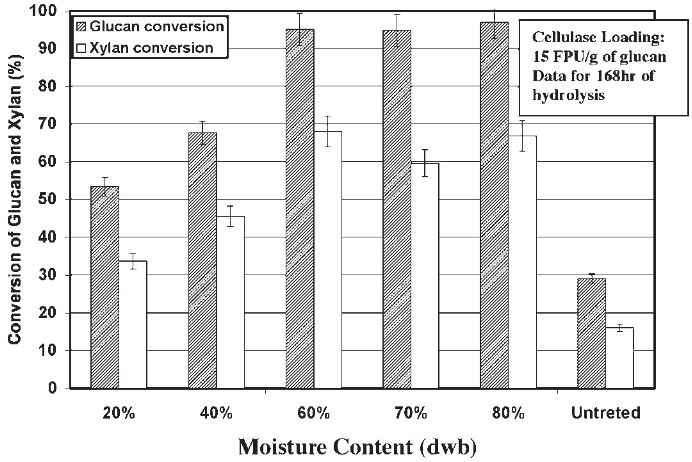

Fig. 3. Effects of moisture content on glucan and xylan conversion of AFEX-treated corn stover at 90°C and 1:1 ammonia loading. All runs were kept at the set temperature for 5 min.

Based on these observations, the ammonia ratio of 1:1 is considered to be the optimum ammonia loading ratio for AFEX treatment of corn stover. Previous work also recognized the same ammonia ratio as the optimum ratio for AFEX treatment of corn fiber *(9)*.

Effects of Moisture Content on the Enzymatic Hydrolysis of AFEX-Treated Corn Stover

Effects of different moisture contents (20, 40, 60, 70, and 80% dry weight bsis [dwb]; for example, to make 20% moisture content [dwb] 20 g of water was added to 100 g of dry corn stover) on the subsequent enzymatic hydrolysis are presented in Fig. 3. Glucan conversion increased with increasing moisture content and attained a maximum value at 60% moisture content. Xylan conversion also showed the same trend as glucan conversion in response to moisture content. Even though at higher moisture content, ammonia is more diluted, apparently the affinity of ammonia for biomass components (e.g., cellulose, hemicellulose) is still sufficiently strong so that the ammonia reacts adequately with the structural polymers (cellulose and hemicellulose). Previous studies have postulated that the moisture in the biomass allows formation of ammonium hydroxide, which hydrolyzes hemicellulose and thereby enhances the overall effect of AFEX treatment *(10)*. As Fig. 3 shows, further increases in moisture content beyond 60% (70 and 80% dwb), did not improve either glucan or xylan conversion. Dilution of ammonia at these moisture contents may reduce the affinity of ammonia for biomass. Based on these data, we have selected 60% moisture content (dwb) as the optimum moisture content for AFEX treatment of corn stover.

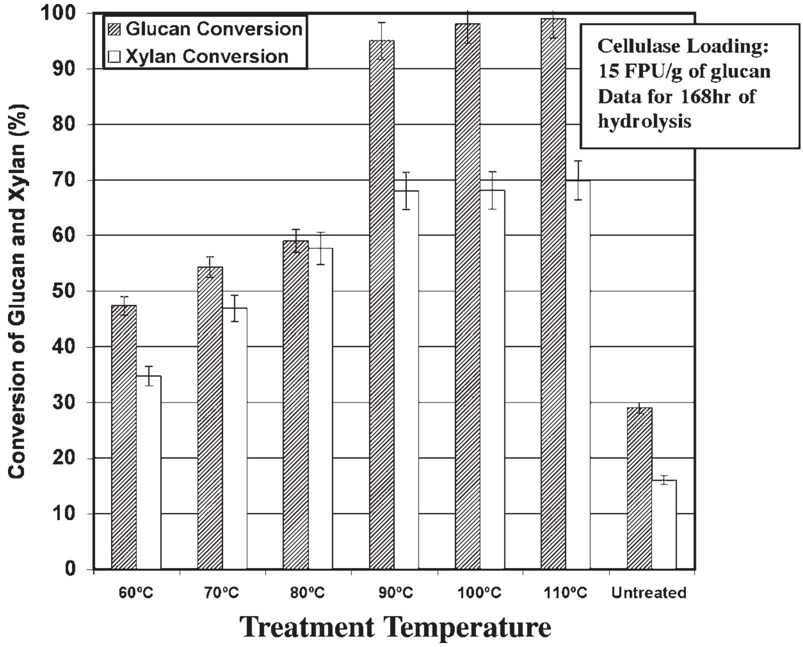

Fig. 4. Effects of treatment temperature on glucan and xylan conversion of AFEX-treated corn stover at 60% moisture content (dwb) and 1:1 ammonia loading. All the runs were kept at the set temperature for 5 min.

Effects of Temperature on the Enzymatic Hydrolysis of AFEX Treated Corn Stover

The data in Fig. 4 illustrate that increasing temperature dramatically enhances conversion of glucan to glucose. Pretreatment temperature is a very important variable, because it determines the amount of ammonia vaporized during the explosive flash and influences system pressure. At higher temperatures, more ammonia vapors flash, and, therefore, greater disruption of biomass fiber structure probably occurs. In addition, higher temperature accelerates the chemical reactions such as alkaline hydrolysis of hemicellulose. As Fig. 4 shows, increasing temperature from 90 to 100 and 110°C improved the conversion of glucan to glucose by only about 1%. Apparently further increases in treatment temperature beyond 90°C do not have much additional beneficial effect.

The ultimate goal of the AFEX treatment is to increase the yields of fermentation products such as ethanol by increasing the digestibility of the biomass. Therefore, selected runs that showed higher glucan and xylan conversion were chosen for further SSF analysis. The best treatment temperature is to be selected based on the fermentation results, not just on enzymatic hydrolysis; the fermentation results are presented later in this article.

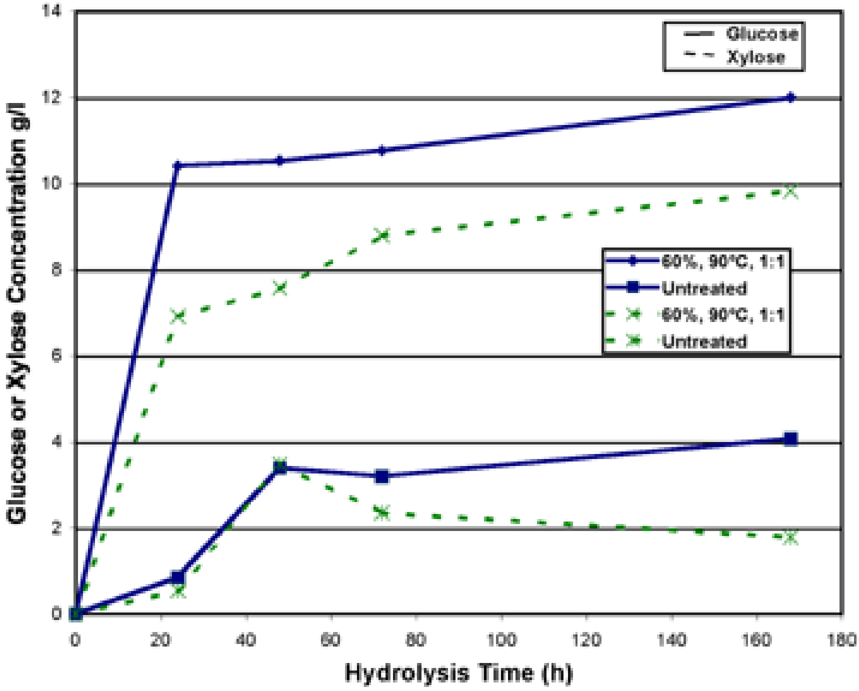

Fig. 5. Glucose and xylose concentration vs hydrolysis time at enzyme loading of 60 FPU/g of glucan.

Enzymatic Hydrolysis Time Profile

Figure 5 shows glucose and xylose production profiles of AFEX-treated and untreated corn stover samples at enzyme loading levels of 60 FPU/g of glucan. In all cases, the curves that describe sugar production vs time have a similar shape. The AFEX-treated sample showed a higher degree of digestibility than the untreated sample. Figure 5 also demonstrates that the initial rate of digestion of treated material was higher than of untreated corn stover. The 24-h yields of glucose and xylose from the AFEX-treated sample are more than 10 times the yields from untreated stover. As this data shows, AFEX treatment approximately doubled the yield of glucose and quadrupled the yield of xylose vs untreated corn stover.

Figure 6 illustrates glucose production vs time for our best AFEX run (60% moisture, 90°C, and 1:1 ammonia ratio), with the data normalized to the 3-d yield. The 3-d glucose yield is a convenient measure of enzymatic susceptibility of the AFEX-treated biomass. The normalized curve allows determination of glucose yield at times other than 3 d. Based on Fig. 6, about 96% of the 3-d glucose was released in 24 h and about 97% of glucose was released in 48 h. These data demonstrate the high digestibility of the AFEX-treated corn stover.

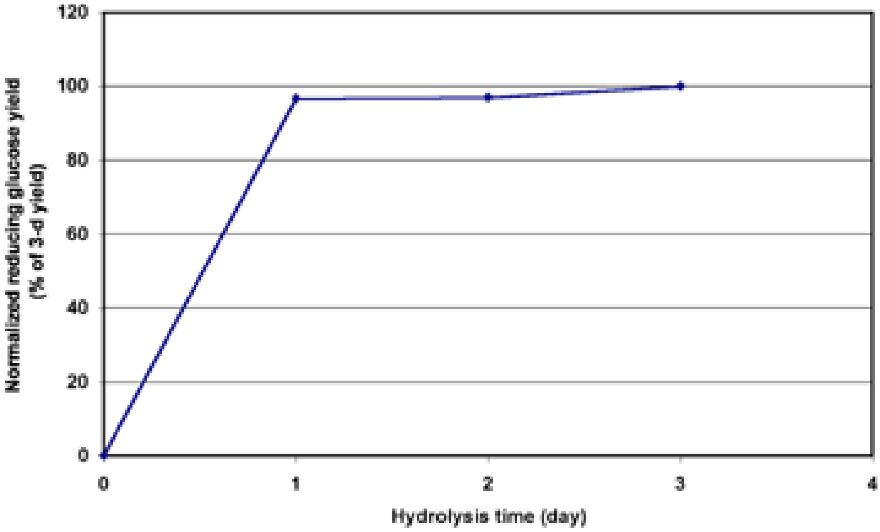

Fig. 6. Normalized hydrolysis profile for corn stover treated at 60% moisture content (dwb), 90°C, and 1:1 ammonia loading ratio, with enzyme loading of 60 FPU/g of glucan.

Simultaneous Saccharification and Fermentation

As Fig. 7 shows, throughout the SSF process, glucose produced by the cellulase enzyme was almost completely consumed by the yeast and converted to ethanol. The rate of ethanol production was quite rapid during the first 6 h of the fermentation. The AFEX-treated sample attained the maximum amount of ethanol after 96 h of the SSF process. As seen in Fig. 7, the AFEX-treated sample produced more than twice as much as ethanol compared with an untreated sample. Even though this sample showed slightly lower glucose yield in enzymatic hydrolysis compared with the samples treated under the same conditions except with higher temperature (100°C), as Fig. 8 shows, it still produced a higher ethanol yield. The higher temperatures may produce some inhibitory material, perhaps lignin fragments, that affects the performance of the yeast and reduces the SSF productivity.

On the other hand, the concentration of xylose produced consistently increased throughout the SSF process. Our yeast (*S. cerevisiae* D_5A) does not have the ability to utilize xylose and to convert it to ethanol. The data in Fig. 7 are also similar to those we observed in our enzymatic hydrolysis.

Effects of Longer AFEX Residence Time

In an effort to explore the effects of longer treatment time on the hydrolysis results, a series of AFEX experiments (temperature of 90–110°C, moisture content of 60–70% dwb, all at 1:1 ammonia loading ratio) were conducted; the reactor was maintained at the target temperature

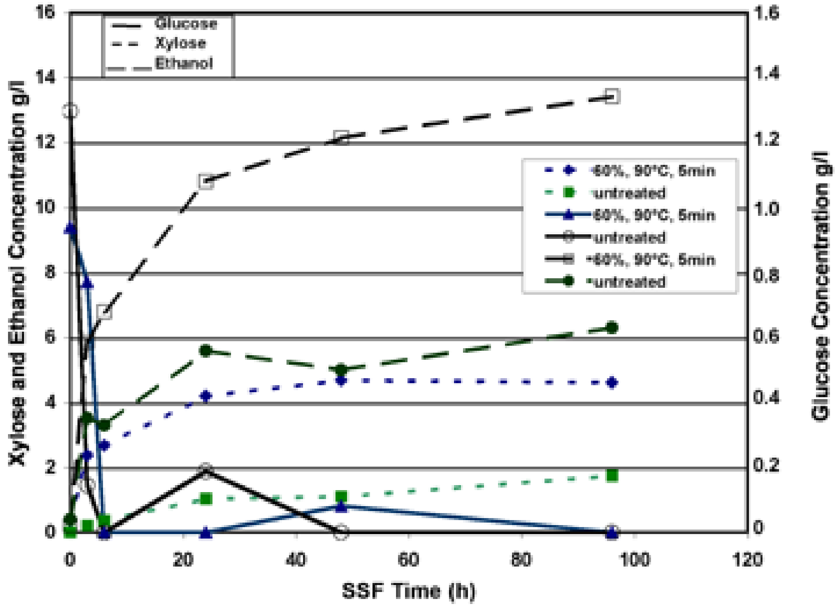

Fig. 7. SSF time profile for glucose, xylose and ethanol concentration.

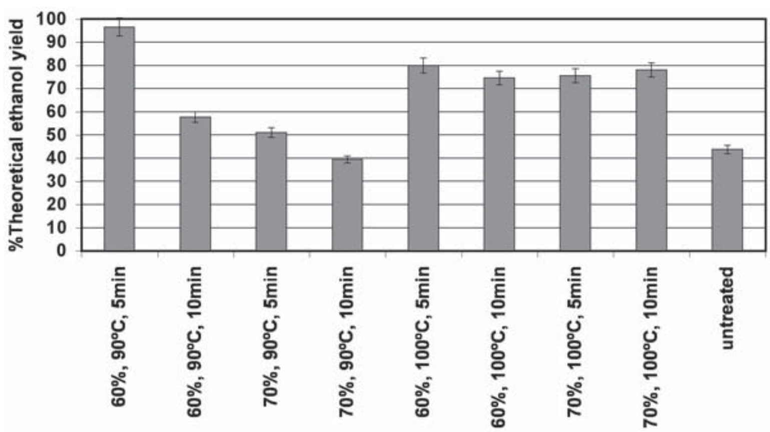

Fig. 8. SSF results of some AFEX runs with longer residence times.

for 5, 10, or 15 min. Increasing the treatment time in some cases slightly increased glucan and xylan conversion and in some cases decreased these conversions (data not shown). Since these effects were different in each set of AFEX runs, we were not able to find any correlation among the treatment time and other conditions of the AFEX runs. Therefore, to be able to choose the best treatment time, we selected some of the runs that showed greater glucan and xylan conversion with increasing time for further SSF experiments. The results of these SSF experiments are shown in Fig. 8.

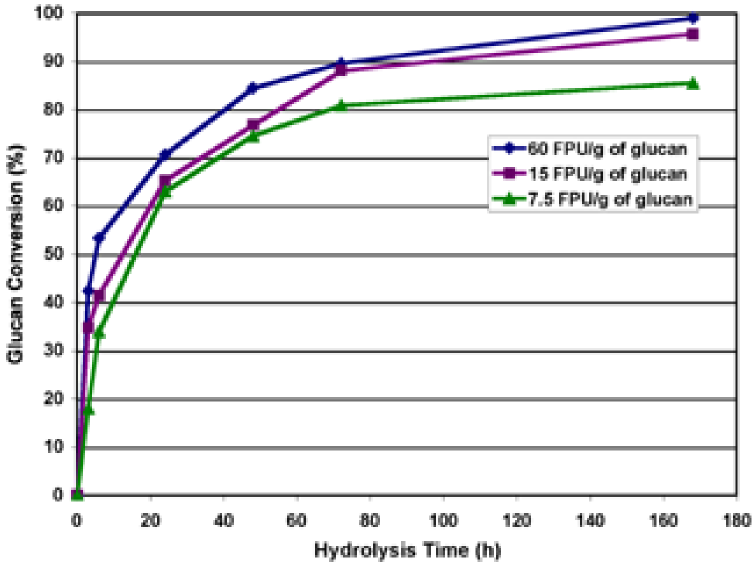

Fig. 9. Glucan conversion profile of AFEX-treated corn stover (60% moisture content [dwb], 1:1 ammonia loading, and 90°C) at different enzyme loadings.

Note that all the runs (with one exception) with longer treatment time showed lower ethanol yield. However, the hydrolysis results of these runs all showed greater glucan and xylan conversion. As mentioned previously, perhaps during longer treatment times some inhibitory materials were produced that in turn reduced the yield of the fermentation. Based on these findings, 5 min is currently considered the best residence time for the AFEX treatment of corn stover.

Effects of Different Cellulase Enzyme Loadings

The cost of enzyme used for saccharification of cellulosic materials strongly influences the overall economics of the biomass conversion process. One way to decrease enzyme cost is to use less enzyme per kilogram of biomass. Therefore, we performed enzymatic hydrolysis on AFEX-treated corn stover sample with three different cellulase loadings (7.5, 15, and 60 FPU/g of glucan). As Figs. 9 and 10 demonstrate, increasing cellulase loading from 15 to 60 FPU/g of glucan did not make an appreciable difference in glucan or xylan conversion of AFEX-treated samples. These data demonstrate the high digestibility of the AFEX-treated corn stover.

Conclusion

Optimum AFEX treatment conditions are quite broad. However, the highest glucan and xylan conversions and ethanol yields from AFEX-treated corn stover were achieved at about 1:1 kg of ammonia/kg of dry biomass, 60% (dwb) moisture content, 90°C, and 5-min residence time in a

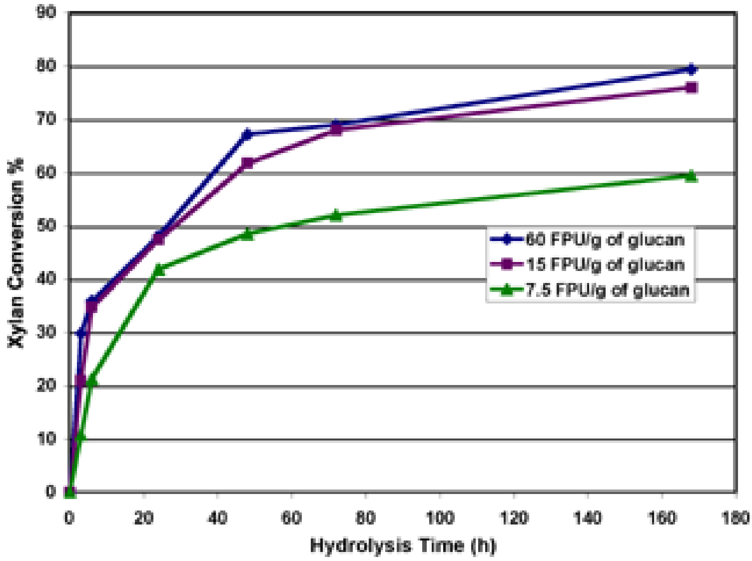

Fig. 10. Xylan conversion profile of AFEX-treated corn stover (60% moisture content [dwb], 1:1 ammonia loading, and 90°C) at different enzyme loadings.

batch AFEX reactor. It appears possible, at least within some limits, to achieve similar hydrolysis results by increasing temperature while reducing ammonia levels; that is, we can"trade off" these two parameters.

In addition, increasing temperature and moisture content enhanced the AFEX treatment, at least up to some limit. Above about 90°C, higher sugar yields by enzymatic hydrolysis were not accompanied by higher ethanol yields following SSF. Presumably, some fermentation inhibitors are formed, perhaps lignin fragments, at higher AFEX temperatures.

Furthermore, enzymatic hydrolysis of the corn stover treated under optimal AFEX conditions showed almost 98% glucan conversion and 80% xylan conversion vs 29 and 16% for untreated corn stover, respectively (at an enzyme loading of 60 FPU/g of glucan). Unlike acidic pretreatments, AFEX does not generate sugar monomers. The cellulase mixture in our study was developed for hydrolysis of acid-pretreated materials and has about 1% by weight xylanase activity (J. Cherry, personal communication, Nov. 2002). Enzyme cocktails with enhanced xylanase activity would presumably completely hydrolyze AFEX-treated xylans.

Moreover, lowering the cellulase loading from 60 FPU/g of glucan to 7.5 or 15 FPU/g of glucan did not make appreciable differences in glucan or xylan conversion of AFEX-treated corn stover. These results are very important in terms of process economics. We are doing additional studies to determine how low the cellulase loading can be made and still obtain high glucose and xylose yields.

Finally, AFEX-treated corn stover more than doubled the amount of ethanol production compared with untreated corn stover in an SSF process.

Acknowledgments

We thank the NREL for providing corn stover, cellulase enzyme, and yeast. We also thank Dr. Mariam Sticklen for the use of her laboratory and equipment. This work was supported by The USDA Initiative for Future Agricultural and Food Systems Program through contract no. 00-52104-9663.

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