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Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover

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Abstract

Biological processing of cellulosic biomass to fuels and chemicals would open up major new agricultural markets and provide powerful societal benefits, but pretreatment operations essential to economically viable yields have a major impact on costs and performance of the entire system. However, little comparative data is available on promising pretreatments. To aid in selecting appropriate systems, leading pretreatments based on ammonia explosion, aqueous ammonia recycle, controlled pH, dilute acid, flowthrough, and lime were evaluated in a coordinated laboratory program using a single source of corn stover, the same cellulase enzyme, shared analytical methods, and common data interpretation approaches to make meaningful comparisons possible for the first time. Each pretreatment made it possible to subsequently achieve high yields of glucose from cellulose by cellulase enzymes, and the cellulase formulations used were effective in solubilizing residual xylan left in the solids after each pretreatment. Thus, overall sugar yields from hemicellulose and cellulose in the coupled pretreatment and enzymatic hydrolysis operations were high for all of the pretreatments with corn stover. In addition, high-pH methods were found to offer promise in reducing cellulase use provided hemicellulase activity can be enhanced. However, the substantial differences in sugar release patterns in the pretreatment and enzymatic hydrolysis operations have important implications for the choice of process, enzymes, and fermentative organisms.

Keywords: Corn stover; Pretreatment; Hydrolysis; Sugars; Enzymatic digestion

1. Introduction

As mentioned in the introductory paper for this special issue, this project was a multi-institutional effort funded by the US Department of Agriculture Initiative for Future Agriculture and Food Systems Program to develop comparative technical and economic information on pretreatment of cellulosic biomass by leading options using a single source of corn stover, a shared supply of cellulase enzyme, identical analytical methods, and identical approaches to data analysis. Comparative data of this nature are sorely needed to aid in selection of pretreatment options for commercial uses but are unfortunately lacking. The pretreatments investigated and corresponding lead investigators and institutions for this study were (1) aqueous ammonia recycle pretreatment by Y.Y. Lee at Auburn University, (2) water only and dilute acid hydrolysis by co-current and flowthrough systems by Charles Wyman at Dartmouth

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College, (3) ammonia fiber explosion (AFEX) by Bruce Dale at Michigan State University, (4) controlled-pH pretreatment by Michael Ladisch of Purdue University, and (5) lime pretreatment by Mark Holtzapple at Texas A&M University. In addition, feedstock and enzyme supply, other logistical support, and economic analyses were provided through Richard Elander of the National Renewable Energy Laboratory (NREL) made possible by funding from the Office of the Biomass Program of the US Department of Energy. These participants are all members of a Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) that has the mission of developing a fundamental understanding of biomass hydrolysis that will facilitate commercialization, accelerating the development of next generation technologies that dramatically reduce the cost of sugars from cellulosic biomass, and training future engineers, scientists, and managers.

This paper presents a brief overview of the project and the approach applied to define sugar yields from hemicellulose and cellulose. Then, key xylose and glucose yield results are presented for each technology for the operations of pretreatment and subsequent enzymatic hydrolysis. Overall, the goal is to provide a single source of comparative information that will assist readers in understanding the unique features and performances of leading options for releasing sugars from cellulosic biomass. References are given to the source of the data summarized for the reader to obtain more detailed information on each system, if desired.

2. Background

The tasks undertaken in this project were to apply leading pretreatment technologies to (1) prepare corn stover for conversion to products, (2) characterize resulting fluid and solid streams, (3) close material and energy balances for each pretreatment process, (4) determine cellulose digestibility and liquid fraction fermentability/toxicity, and (5) compare performance of pretreatment technologies on a consistent basis. The project period was from late 2000 to late 2003, and the emphasis of the project was on research quality to be sure the data would be useful to potential practitioners. A single source of corn stover provided by NREL through BioMass AgriProducts from a source in Harlan IA was washed and dried in small commercial operation and knife milled to pass through a 1/4 in. round screen prior to distribution to the participants. The composition of this feedstock, as measured by NREL, was 36.1% glucan, 21.4% xylan, 3.5% arabinan, 2.5% galactan, 1.8% mannan, 17.2% lignin, 7.1% ash, 4.0% protein, 3.6% uronic acid, 3.2% acetyl, and 1.2% non-structural sugars on a dry weight basis.

2.1. Calculation of sugar yields

Material balances were closed for each pretreatment system by measuring the composition and total mass of each liquid and solid stream leaving pretreatment and converting this data to amounts of sugars produced, as illustrated by an example for AFEX in Fig. 1. The procedures applied were as described in the Introduction paper to this special volume. Yields were then calculated based on the xylose and glucose available in the corn stover fed to the systems. Thus, based on the material composition used for this study and the appropriate increase in mass with hydrolysis, at most about 40.1 mass units of glucose and 24.3 mass units of xylose could be produced from 100 mass units of corn stover feed giving a total sugar potential of 64.4 units per 100 units of bone dry feed. It is important to note that although accurate analysis and measurements are quite simple in concept, they are challenging for biomass, and extensive time and care are essential to obtain meaningful information.

The consideration of sugar yields must account for the fact that the overall system consists of two stages for each pretreatment system evaluated and that not all of the sugar is released as monomers. In Stage 1, biomass is pretreated to open up the structure of the residual solids and facilitate access by enzymes to obtain high yields. Enzymes are then added to the pretreated solids in Stage 2. For many operations, some amounts of glucose and xylose are released in Stage 1 and recovered in the liquid stream. Furthermore, although cellulase enzyme is added to Stage 2, it has enough xylanase activity to hydrolyze a substantial portion of the xylan, and both xylose and glucose are typically found in the liquid streams from Stage 2. Thus, yields of each sugar are

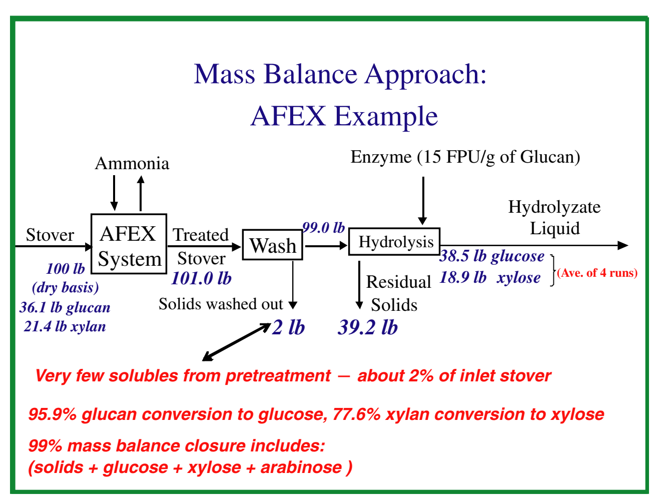

Fig. 1. Example of the material balance approach when applied to the ammonia fiber explosion (AFEX) process. As shown, yields of about 59.8% for glucose, 29.3% for xylose, and 89.1% for total glucose plus xylose were obtained based on the maximum potential production of 64.4 lb of total sugars per 100 lb of dry corn stover.

reported for each stage as appropriate. Because some of the pretreatments produce sugar oligomers as well as monomers in Stage 1, the yields of each sugar from each stage are further differentiated to reflect this information.

Comparison of the amount of each sugar monomer or oligomer produced to the maximum potential amount for that sugar would give the percent yield of each. However, it is important to recognize that corn stover, along with most other forms of cellulosic biomass, are richer in glucose than xylose, and as a result, yields of glucose have a greater impact than those of xylose. Thus, sugar yields were defined in this project by dividing the amount of xylose or glucose or the sum of the two by the maximum potential amount of both sugars to better reflect the relative contribution to overall sugar production. On this basis, the maximum xylose yield is calculated as 24.3/64.4 or 37.7%, the maximum glucose yield is 40.1/64.4 or 62.3%, and the maximum amount of total xylose and glucose is 100%.

In the case of fermentation to ethanol, the definition of yields applied here is appropriate in principle because the theoretical yields are the same from all sugars and the yields of sugars reflect reasonably closely the amount of ethanol that could be obtained. Thus, consideration of total sugars released will closely approximate the total amount of ethanol that could be produced. However, some organisms suffer from diauxic effects that reduce or prevent fermentation of low concentrations of xylose or other sugars that exist in solutions containing greater concentrations of glucose. Furthermore, although some organisms can ferment sugar oligomers, others do not, making conversion of the oligomers released during pretreatment more challenging or perhaps impossible at reasonable cost in such cases. For these reasons, the utility of each sugar type can vary with the process chosen, and total sugar yields may not be equally relevant for all applications. However, the breakdown of data by stage and sugar type should allow the user to assess the yield of sugars appropriate for the intended application. In addition, caution is urged in use of this data in that it may be possible to adjust pretreatment and hydrolysis conditions somewhat to achieve higher yields of selected sugars from those reported here for maximum total yields.

3. Comparative results

Details for each of the pretreatment systems applied in this project are provided in other papers in this volume, and this section will focus on summarizing data on a common basis to facilitate comparisons. However, it is important to realize that the use of somewhat different enzymes and other process specifics could alter the results for some of the systems from those reported.

3.1. Distinguishing features

Several observations are important to note in terms of the different effects of pretreatment on the characteristics of the pretreated biomass. First, pretreatments at lower pH by dilute acid, controlled pH, and flowthrough all gave a liquid fraction containing most of the sugars from hemicellulose and a solid residue that contained most of the cellulose from the original corn stover. Furthermore, little lignin was removed by dilute acid or controlled-pH pretreatments, whereas the flowthrough or partial flow operations removed as much as 75% of the lignin in addition to hemicellulose. On the other hand, the high-pH technologies based on lime and liquid ammonia recycle removed lignin and left a solid residue containing most of the cellulose and hemicellulose. Although AFEX is also a high-pH process, it did not generate free liquid and did not separate the lignin or hemicellulose from the cellulose. Rather, a solid material was left that looked very much like the original substrate with the cellulose and hemicellulose well preserved and essentially 100% of the feedstock recovered as dry matter.

3.2. Xylose sugar yields

Tables 1 and 2 document the yields of xylose from hemicellulose for enzyme loadings of 60 and 15 FPU/g glucose in the original corn stover, respectively. The pretreatment technologies are listed in order of increasing pH, and reasonably high xylose yields were achieved for all systems. Most of the xylose was released in pretreatment, Stage 1, for dilute acid, flowthrough, partial flow, and controlled-pH pretreatment. Furthermore, most of the xylose was released as monomers for just the dilute acid system. On the other hand, the high-pH pretreatments by ARP and lime released a large percentage of xylose sugars in the second stage, with about half being solubilized in the second stage for ARP and two thirds for lime. Essentially all of the xylose was released in the second stage for AFEX.

3.3. Glucose sugar yields

Tables 1 and 2 summarize glucose yields for cellulase loadings of 60 and 15 FPU/g glucose in the original corn stover, respectively. All of the pretreatments considered resulted in a small fraction of the total glucose being released in Stage 1 with most solubilized in Stage 2. Nonetheless, total glucose yields were close to the maximum possible of 62.3% for all pretreatments at the higher enzyme loading and dropped only slightly when cellulase use was cut by 75%. Thus, all pretreatments were effective in making cellulose accessible to enzymes. The glucose released was predominately as monomers, demonstrating virtually complete hydrolysis by cellulase.

Table 1
Yields of xylose and glucose for each pretreatment system studied followed by enzymatic hydrolysis with a loading of 60 FPU/g glucan in the original corn stover

Pretreatment system	Xylose yields			Glucose yields			Total sugars		
	Stage 1	Stage 2	Total xylose	Stage 1	Stage 2	Total glucose	Stage 1	Stage 2	Combined total
Dilute acid (Lloyd and Wyman, 2005)	32.1/31.2	3.3	35.3/34.5	3.9	53.3	57.2	36.0/35.1	56.6	92.5/91.7
Flowthrough (Liu and Wyman, 2005)	36.3/1.7	0.8/0.7	37.1/2.4	4.5/4.4	57.0	61.5/61.4	40.8/6.1	57.8/57.7	98.6/63.8
Partial flow pretreatment (Liu and Wyman, 2005)	31.5/2.8	–	–	4.3/4.2	–	–	–	–	–
Controlled pH (Mosier et al., 2005)	21.8/0.9	8.9	30.7	3.5/0.2	54.7	58.2	25.3/1.1	63.6	88.9
AFEX (Teymouri et al., 2004)		ND/30.2	ND/30.2		61.8	61.8		ND/92.0	ND/92.0
ARP (Kim et al., 2005; Kim and Lee, 2003)	17.8/0	17.0	34.8/17.0	0	59.4	59.4	17.8/0	76.4	94.2/76.4
Lime (Kim and Holtzapple, 2005)	9.2/0.3	20.2	29.4/20.5	1.0/0.3	59.5	60.5/59.8	10.2/0.6	79.7	89.9/80.3

Stage 1 refers to pretreatment and Stage 2 refers to the enzymatic digestion of the solids produced in pretreatment. The first value reported in each column is for total sugars released into solution, and the second is for just the monomers released. A single value indicates release of only monomers. Yields are defined based on the maximum potential sugars released from the corn stover used of 64.4 g per 100 g of dry solids with the maximum potential xylose being 37.7% and the maximum potential yield of glucose being 62.3% on this basis. ND = not determined.

Table 2
Yields of xylose and glucose for each pretreatment system studied followed by enzymatic hydrolysis with a loading of 15 FPU/g glucan in the original corn stover

Pretreatment system	Xylose yields			Glucose yields			Total sugars		
	Stage 1	Stage 2	Total xylose	Stage 1	Stage 2	Total glucose	Stage 1	Stage 2	Combined total
Dilute acid (Lloyd and Wyman, 2005)	32.1/31.2	3.2	35.3/34.4	3.9	53.2	57.1	36.0/35.1	56.4	92.4/91.5
Flowthrough (Liu and Wyman, 2005)	36.3/1.7	0.6/0.5	36.9/2.2	4.5/4.4	55.2	59.7/59.6	40.8/6.1	55.8/55.7	96.6/61.8
Partial flow pretreatment (Liu and Wyman, 2005)	31.5/2.8	2.6/2.4	34.1/5.2	4.3/4.2	51.2	55.5/55.4	35.8/7.0	53.8/53.6	89.6/60.6
Controlled pH (Mosier et al., 2005)	21.8/0.9	9.0	30.8/9.9	3.5/0.2	52.9	56.4/53.1	25.3/1.1	61.9	87.2/63.0
AFEX (Teymouri et al., 2004)		34.6/29.3	34.6/29.3		59.8	59.8		94.4/89.1	94.4/89.1
ARP (Kim et al., 2005; Kim and Lee, 2003)	17.8/0	15.5	33.3/15.5	0	56.1	56.1	17.8/0	71.6	89.4/71.6
Lime (Kim and Holtzapple, 2005)	9.2/0.3	19.6	28.8/19.9	1.0/0.3	57.0	58.0/57.3	10.2/0.6	76.6	86.8/77.2

Stage 1 refers to pretreatment and Stage 2 refers to the enzymatic digestion of the solids produced in pretreatment. The first value reported in each column is for total sugars released into solution and the second is for just the monomers released. A single value indicates release of only monomers. Yields are defined based on the maximum potential sugars released from the corn stover used of 64.4 g per 100 g of dry solids with the maximum potential xylose being 37.7% and the maximum potential yield of glucose being 62.3% on this basis.

The data show slightly higher yields for lime, ARP, and flowthrough technologies that remove substantial amounts of lignin. However, the fact that AFEX achieved slightly higher yields even though no lignin was removed suggests that lignin removal is not essential to enhance the digestibility of corn stover cellulose. Understanding the causes of these differences could suggest new approaches to enhanced cellulose digestion.

3.4. Total sugar yields

The total sugar yields are also shown in Tables 1 and 2. All of the pretreatments realized overall sugar yields of around 90% at high enzyme loadings, with some advantage being apparent for systems using flowing water either alone (flowthrough) or with ammonia (ARP), apparently due to lignin removal and greater recovery of hemicellulose. However, yields for the other technologies were not substantially lower. Furthermore, when cellulase loadings were lowered substantially to 15 FPU/g, all of the yields were still similar. Thus, all pretreatments achieved high total sugar yields.

3.5. Conditions for maximum yields

Table 3 summarizes the key conditions employed to maximize the yields to those reported in Tables 1 and 2. Most of the temperatures were in the range of 160–200 °C. However, AFEX used only 90 °C whereas lime applied 55 °C. Thus, high temperatures are not essential to achieving good sugar recovery. Except for the four-week period applied for lime, all pretreatments were run for between 5 and 24 min, making it possible to conduct most of them in relatively small vessels. On the other hand, a novel approach such as pretreatment in a pile would be needed to handle the large volumes of biomass for lime pretreatment. Chemical demands are as listed.

4. Conclusions

This project took a major step toward addressing a compelling need for comparative data on the performance of leading pretreatment technologies, and the information gathered through this first coordinated evaluation of biomass pretreatment and subsequent enzymatic hydrolysis supports several important conclusions. As expected, dilute acid, neutral pH, and water-only pretreatments solubilized mostly hemicellulose whereas addition of lime or percolation with ammonia removed mostly lignin. On the other hand, when ammonia was released at the end of the pretreatment process via AFEX, neither lignin nor hemicellulose was physically removed from cellulose and other components. When water was pushed through biomass in a flowthrough mode, virtually all of the hemicellulose and up to about

Table 3
Reaction conditions employed to obtain maximum total sugar yields from corn stover for each pretreatment system

Pretreatment system	Temperature, °C	Reaction time, min	Chemical agent used	Chemical loading	Other notes
Dilute acid (Lloyd and Wyman, 2005)	160	20	Sulfuric acid	0.49% in water	25% solids concentration during run in batch tubes
Flowthrough (Liu and Wyman, 2005)	200	24	None	0	Continuously flow just hot water at 10 mL/min for 24 min
Partial flow pretreatment (Liu and Wyman, 2005)	200	24	None	0	Flow hot water at 10 mL/min from 4–8 min, batch otherwise
Controlled pH (Mosier et al., 2005)	190	15	None	0	16% corn residue slurry in water
AFEX (Teymouri et al., 2004)	90	5	Anhydrous ammonia	1.0 g/g dry biomass	62.5% solids in reactor (60% moisture dry weight basis)
ARP (Kim et al., 2005; Kim and Lee, 2003)	170	10	Ammonia	15	Flow aqueous ammonia at 5 mL/min without presoaking
Lime (Kim and Holtzapple, 2005)	55	4 weeks	Lime	0.08 g CaO/g biomass	Purged with air

75% of the lignin were removed with or without addition of very dilute sulfuric acid. Thus, different pretreatments can affect biomass in very different ways.

Although removal of hemicellulose and lignin differed, high yields of glucose were achieved by enzymatically hydrolyzing the remaining solids for all of these pretreatments. The digestibility was somewhat better for higher pH and flowthrough methods, likely due to the removal of lignin that can interfere with the accessibility of cellulase to cellulose and non-productively adsorb lignin. However, the AFEX process achieved excellent enzymatic digestion at low enzyme loadings even though essentially no lignin or hemicellulose was removed. This difference suggests that ammonia affects lignin and possibly hemicellulose differently than other additives, reducing the ability of lignin to adsorb enzyme and/or to impede its access to cellulose.

An interesting observation was that the enzyme formulation used contained enough xylanase activity to solubilize a large portion of the xylan remaining for the high-pH pretreatments. Thus, although AFEX, lime, and ammonia percolation approaches produced moderate to low yields of xylose during the pretreatment step itself, the enzymes hydrolyzed much of the residual xylan in the pretreated solids, and a substantial fraction of the xylan could be recovered from the residual solids over the two stages for the high-pH methods. Much less xylan remained in the pretreated solids at optimum conditions that prevented excessive xylose degradation for pretreatments at lower pH, but some of that left in the solids was still broken down to xylose by enzymes, noticeably enhancing yields. Thus, increasing hemicellulase activity promises improved yields for all methods and could have particularly important benefits for neutral or alkaline pretreatment.

A related factor is the release of oligomers during pretreatment and their implications for process design. At one extreme, dilute sulfuric acid pretreatment released almost all of the xylose as monomers, and these sugars are readily fermented by many organisms. On the other hand, pretreatments by methods such as flowthrough or controlled-pH technologies gave high relative amounts of oligomers, which are not as easily utilized and may require additional steps such as post hydrolysis with acids or enzymes to break the oligomers into monomeric species. Consequently, these differences in the pattern of oligomer release must be considered in selecting a pretreatment system.

Overall, the results show interesting tradeoffs among the pretreatments evaluated. The enzymatic digestibility is similar for all methods, but those at high (alkaline) pH offer somewhat lower enzyme loadings for a given yield due to lignin removal. However, AFEX seems to particularly benefit in this regard even though little lignin is removed. Furthermore, although some pretreatments release primarily xylose during pretreatment and primarily glucose in subsequent enzymatic hydrolysis, other pretreatments release mixtures containing appreciable concentrations of both xylose and glucose sugars during enzymatic hydrolysis. Because most organisms preferentially use glucose instead of xylose or other sugars, fermenting this sugar mixture in the enzymatic hydrolyzate can present important challenges. Thus, the choice of pretreatment technology is not simple and must consider the sugar release patterns and solids concentrations for each pretreatment as well as their compatibility with the process, enzymes, and fermentative organisms.

It is also important to keep in mind that the data reported here pertain specifically to corn stover. Similar performance may be expected for other agricultural residues such as wheat straw or rice straw and possibly for herbaceous crops such as switchgrass. However, such interpretations are extrapolations and cannot be substantiated without data. Furthermore, it would be unrealistic to imply that similar results would be achieved with hardwoods such as poplar, and much different results would almost certainly be observed for softwoods. In fact, experiments of the type reported here for corn stover are needed with each of these feedstocks before any meaningful conclusions can be drawn on the relative merits of these different pretreatment technologies for application to multiple crops. On that basis, another project built around a team augmented from that reporting here is in progress to develop comparative data on how each pretreatment performs with poplar wood. In addition, this project will examine in more depth how leading pretreatment technologies impact hydrolyzate conditioning needs for effective hydrolyzate fermentation by recombinant organisms, cellulase effectiveness, the effect of hemicellulase addition on performance, the relationship of enzyme formulation to performance, and substrate features and their effect on digestibility.

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References

Kim, S., Holtzapple, M.T., 2005. Lime pretreatment and enzymatic hydrolysis of corn stover. Bioresource Technology, this volume, doi:10.1016/j.biortech.2005.01.014.

Kim, T.H., Lee, Y.Y., 2003. Pretreatment of corn stover by soaking in aqueous ammonia. AIChE Annual Meeting, November, San Francisco, CA.

Kim, T.H., Lee, Y.Y., Kim, J.S., Sunwoo, C.S., 2005. Pretreatment of corn stover by low-liquid ammonia percolation process. Biotechnology and Bioprocess Engineering, in press.

Liu, C., Wyman, C.E., 2005. Partial flow of compressed-hot water through corn stover to enhance hemicellulose sugar recovery and enzymatic digestibility of cellulose. Bioresource Technology, this volume, doi:10.1016/j.biortech.2005.01.012.

Lloyd, T.A., Wyman, C.E., 2005. Total sugar yields for pretreatment by hemicellulose hydrolysis coupled with enzymatic hydrolysis of the remaining solids. Bioresource Technology, this volume, doi:10.1016/j.biortech.2005.01.011.

Mosier, N., Hendrickson, R., Ho, N., Sedlak, M., Ladisch, M.R., 2005. Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioresource Technology, this volume, doi:10.1016/j.biortech.2005.01.013.

Teymouri, F., Laureano-Perez, L., Alizadeh, H., Dale, B.E., 2004. Ammonia fiber explosion treatment of corn stover. Applied Biochemistry and Biotechnology 113–116, 951–963.