

Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids

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Abstract

A number of previous studies determined dilute acid pretreatment conditions that maximize xylose yields from pretreatment or glucose yields from subsequent digestion of the pretreated cellulose, but our emphasis was on identifying conditions to realize the highest yields of both sugars from both stages. Thus, individual xylose and glucose yields are reported as a percentage of the total potential yield of both sugars over a range of sulfuric acid concentrations of 0.22%, 0.49% and 0.98% w/w at 140, 160, 180 and 200 °C. Up to 15% of the total potential sugar in the substrate could be released as glucose during pretreatment and between 15% and 90+% of the xylose remaining in the solid residue could be recovered in subsequent enzymatic hydrolysis, depending on the enzyme loading. Glucose yields increased from as high as 56% of total maximum potential glucose plus xylose for just enzymatic digestion to 60% when glucose released in pretreatment was included. Xylose yields similarly increased from as high as 34% of total potential sugars for pretreatment alone to between 35% and 37% when credit was taken for xylose released in digestion. Yields were shown to be much lower if no acid was used. Conditions that maximized individual sugar yields were often not the same as those that maximized total sugar yields, demonstrating the importance of clearly defining pretreatment goals when optimizing the process. Overall, up to about 92.5% of the total sugars originally available in the corn stover used could be recovered for coupled dilute acid pretreatment and enzymatic hydrolysis. These results also suggest that enhanced hemicellulase activity could further improve xylose yields, particularly for low cellulase loadings.

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1. Introduction

Pretreatment by hemicellulose hydrolysis has been applied to many substrates (Hsu, 1996), with Table 1 summarizing some studies for pretreatment of corn residues, the substrate of interest in this investigation. For water only pretreatment of corn stalks, maximum yields of about 53% of potential xylose were achieved with no more than 8% glucose yield in pretreatment at a log severity of 3.5 (Rubio et al., 1998; Schultz et al., 1984;

Tortosa et al., 1995). For corn stover, the highest xylose yields varied from 55% to over 70% (Carrion et al., 1989; Lamptey et al., 1985; Lloyd and Wyman, 2003), and for corn cobs, the highest xylose yield reported was 72% (Garrote et al., 2001). Glucose yields for enzymatic digestion of the cellulose in the solid residues from pretreatment with only water added ranged from 25% (Schultz et al., 1984) to about 95% of potential glucose, with the latter achieved by attrition milling of the residue during enzymatic hydrolysis (Lamptey et al., 1985).

Adding small amounts of a sulfuric acid significantly enhanced xylose yields from hemicellulose (Bhandari et al., 1984). The maximum xylose yields reported were

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Table 1
Results reported in the literature for pretreatment of corn residues

Substrate	Acid, %	Temperature, °C	Time, min	Xylose yield, %	Glucose yield, %	References
Corn stalk	0	^a	^a	53.0	<8.0	Rubio et al. (1998)
Corn cob	0	202	39 ^b	72.2	N/A	Garrote et al. (2001)
Corn stover	0	200	15	53.0	N/A	Stuhler (2002)
Corn stover	0.49	200	32 ^c	62.5	8.4	Bhandari et al. (1984)
Corn stover	0.92	182	36 ^c	78.1	14.5	Bhandari et al. (1984)
Corn stover	1.47	155	31 ^c	78.7	18.7	Bhandari et al. (1984)
Corn stover	0.92	160	5–10	93	25	Torget et al. (1991)
Corn stover	1.2	180	0.5–1	84	N/A	Esteghlalian et al. (1997)
CC/CS ^d	1.22	140	40	90	N/A	Lee et al. (1994)
Corn stover	1.0	190	1.5	95	N/A	Tucker et al. (2003)
Corn stover	1.35	191	0.75–1	77	N/A	Schell et al. (2003)

The basis for all yield values is the original glucose or xylose individually and not the combined potential glucose and xylose as applied in this paper.

^a $\text{Log } R_0 = \text{Log}[t(\text{min}) * \exp((T(C) - 100)/14.75)] = 3.5$.

^b Target temperature. Non-isothermal heat-up from 60 °C to target over time period.

^c Target temperature. Non-isothermal heat-up from room temperature to target over time period.

^d Corn cob/corn stover mixture.

93% for corn stover (Torget et al., 1991) and 95% (Tucker et al., 2003) and 90% (Lee et al., 1994) for a combination of corn cobs and corn stover. For those reporting enzymatic digestion of the pretreated solids, glucose yields were over 90% (Torget et al., 1991). However, although substantially better xylose yields were observed when acid was added for pretreatment, capital and operating costs would be reduced and other processing operations simplified if less acid could be used while maintaining good yields (Lynd et al., 1996; Woolley et al., 1999).

In this paper, data is reported on the effect of sulfuric acid catalyzed and water only autocatalyzed pretreatment on sugar yields from corn stover for comparison to performance data developed for other leading pretreatment technologies using an identical feedstock. We record recovery of glucose and xylose sugars in both pretreatment and subsequent enzymatic digestion and show the value of considering cumulative yields of these sugars from both stages instead of focusing on only xylose in the first stage or glucose in the second, as typically reported. We also report on the effect of acid on performance with the goal of identifying possible paths to reduce its use.

2. Methods

2.1. Experimental reaction systems

Three different batch reaction systems were used to study the effects of added acid on pretreatment: the steaming apparatus located in the Alternative Fuels Users Facility (AFUF) of the National Renewable Energy Laboratory (NREL), small diameter tubes, and a 1 l stirred pressure vessel. A description of the NREL steaming apparatus and steel tube reactors and the pro-

cedures for their use were published previously (Lloyd and Wyman, 2003). The NREL system was used for all experiments without acid, while the tube and Parr reactors were used for the dilute acid pretreatments. For all tube tests, pairs of tubes were treated identically, and the contents of one tube were used to determine pretreatment performance while the contents of the other tube were used to determine cellulose digestibility.

Pretreatment was also performed with a 1-l Parr reactor to prepare larger quantities of cellulose enriched solids for enzymatic hydrolysis. For these tests, a 1-l reactor made of Hastelloy C (Parr Instruments, Moline, IL) was fitted with a 3.5 in. diameter helical impeller on a two-piece shaft. The impeller was driven by a variable speed DC motor assembly (A1750HC, Parr Instruments, Moline, IL). Temperature was monitored with a K-type thermocouple (Extech Instruments, 421501) and a 1/8-in. stainless steel thermocouple probe (Omega Engineering Co., Stamford, CT) inserted through an outlet in the vessel and shaped to prevent interference with the rotating impeller.

To treat a batch of corn stover in the Parr reactor, a sample was presoaked at room temperature in the appropriate concentration of dilute sulfuric acid solution at 5% solids (w/w) for at least 4 h. The presoaked slurry was transferred to the reactor which was sealed and attached to the impeller drive motor suspended by a chain hoist mounted on a wall crane. The impeller speed was set to about 100 RPM, and the vessel was lowered into a sand bath set at 320 °C to the bottom of the reactor head flange. This procedure allowed for a rapid heat-up of the agitated contents to 160 °C in about 2 min. As the target temperature was being approached, the reactor was raised out of the sand bath to where the bottom was between 1 and 2 cm above the sand surface. Small adjustments with the hoist, either up or down, were made manually to maintain

the temperature within ± 2 °C of the target temperature. At the end of the reaction period, the vessel was removed from the sand bath and quenched in a 19 l poly bucket filled with room temperature water. Once cooled, the reactor was opened, and the contents filtered using a 12.5 cm diameter Whatman no. 1 filter paper in a Buchner funnel. Clean deionized water was washed through the filter cake until the filtrate pH was at least 6 (typically about 1 l).

2.2. Analytical procedures

All chemical analysis procedures applied in this work were the same as those used by all investigators and were based on the Laboratory Analytical Procedures (LAPs) documented by NREL and described earlier in this journal issue. Analytical protocols used in this work were:

Moisture determination (based on LAP-001).

Analysis of sugars in treated and untreated solids (based on LAP-002).

Analysis of monomer sugars in hydrolyzates (based on LAP-013).

Oligomer determination (based on LAP-014).

2.3. Enzymatic hydrolysis

The procedure used by other investigators for enzymatic hydrolysis of pretreated residues (based on LAP 009) was modified slightly to accommodate the smaller solid residues from the tubes. After filtering and washing the contents from a tube, the wet solid residue (about 1 dry gram) was transferred to a 100 ml shake flask, and 30 ml of 0.05 M acetate buffer was added. The flask was put in a shaking water bath (Lab-Line Instruments model 3840, Melrose Park, IL) set at 50 °C and 150 RPM and allowed to equilibrate for several hours. The appropriate amounts of cellulase (Genencor, Spezyme CP) and cellobiase (Novozyme, Novozym 188) enzyme were then added. At 24, 48, and 72 h, 0.5 ml aliquots were removed and filtered, and the filtrates were analyzed for glucose and xylose.

2.4. Sugar material balances and accounting

Material balances were calculated based on HPLC analysis of all five biomass sugars. In general, glucose balances were $100 \pm 5\%$. Xylose balances were $100 \pm 5\%$ at initial reaction times but steadily declined with reaction times due to degradation. The balances for galactose, arabinose, and mannose were not consistent due to their low concentrations coupled with interference among their HPLC peaks. Thus, data on the fate of these three sugars were not considered reliable. However, it would be useful to overcome this analytical lim-

itation as these sugars could contribute up to an additional 12% to total yields.

2.5. Determining acid neutralization capacity of corn stover

The neutralizing capacity of the corn stover substrate was measured by a dry ashing method described by Springer and Harris (1985). Approximately 10 g of dry corn stover was added to a glass filtering crucible (30 ml, 30-M; Kimax) and placed into a 525 °C muffle furnace (Barnstead/Thermolyne, model 48000; Dubuque, IA) for 8 h. The resulting ash was weighed and placed into a 250 ml beaker to which 50 ml of DI water was added before being set on a stir plate (Cole Parmer, Magnetic 6 × 6; Chicago, IL) to be mixed by a 1 in. × 1/4 in. Teflon coated stir bar. A pH probe (Fisher Scientific; Pittsburgh, PA) attached to a pH meter (VWR Scientific Products Corporation, VWR 8000; Willard, OH) was suspended in the mixed slurry, and the ash slurry was titrated with reagent grade 0.01 N H₂SO₄ dispensed from a 50 ml burette to a constant pH of 7. The volume of titrant was recorded, and the neutralizing capacity calculated. On this basis, the neutralizing capacity of the corn stover substrate was determined to be 17.3 mg H₂SO₄/g dry corn stover.

2.6. Adjustments to acid concentration

It is generally agreed that the active hemicellulose hydrolysis accelerant is the hydronium ion whose availability or activity is indicated by a solution's pH (Abatzoglou et al., 1990; Torget et al., 1991). Because the hydronium ion activity of sulfuric acid solutions is a function of both temperature and sulfate salt concentration and pH cannot be measured reliably at temperatures above about 100 °C, the hydronium ion activity must be estimated (Lloyd and Wyman, 2004; Springer and Harris, 1985). Thus, pH values at reaction conditions were calculated based on the relationship developed by Lloyd and Wyman (2004) to take into account neutralization and temperature and are included in Tables 3–5.

2.7. Severity parameter calculation

The combined severity parameter facilitates comparison of a broad range of yield data by coupling the reaction conditions of time, temperature, and acid concentration into a single variable. The severity factor was defined as (Overend and Chornet, 1987):

$$R_0 = t \cdot \exp[(T_H - T_R)/14.75], \quad (1)$$

where t is reaction time in minutes, T_H is the hydrolysis temperature in °C, and T_R is a reference temperature, most often 100 °C. To include the effect of dilute acid,

the combined severity factor has been used (Chum et al., 1990):

$$\log CS = \log R_0 - \text{pH}. \quad (2)$$

3. Results and discussion

For clarity in reporting these results, the terms Stage 1 and Stage 2 refer to pretreatment of the corn stover and enzymatic digestion of the pretreated solids, respectively, in this paper. Glucose and xylose yields were reported for the two hydrolysis stages separately and as the total for both stages. Also, the term ‘combined yields’ is used to refer to the sum of the yields of glucose and xylose in an individual stage, and the term ‘total yield’ to refer to the sum of the glucose and xylose yields in both stages. Time, temperature, and acid concentration were varied for dilute acid pretreatment (Stage 1) in batch tubes. For steam gun pretreatment without acid addition, the variables were time and temperature. Following pretreatment the washed solid residue was enzymatically digested at selected enzyme loadings and times in Stage 2.

All yields reported were normalized to the total potential glucose and xylose in the original untreated feedstock to provide a perspective on the relative contribution of each sugar to total sugar recovery. Based on the original substrate composition of 36.1% glucan and 21.4% xylan and the appropriate change in mass due to hydrolysis, the maximum potential recovery of glucose and xylose was 0.401 g and 0.243 g, respectively, per 1.0 g of the dry weight of corn stover giving a combined total of 0.644 g sugars/g of dry feed. Thus, glucose could contribute 62.3% and xylose 37.7% of the total of these sugars if both of their relative maximum possible contributions were realized. Yield was

then defined as the amount of sugar monomers and oligomers recovered in the mother liquor and wash water from either Stage 1 or Stage 2 divided by the maximum total glucose and xylose sugars that could be recovered from the quantity of stover treated. This approach reflects the greater potential contribution of glucose to the total yield and demonstrates the importance of recovering a large fraction of the potential glucan.

3.1. Water-only autocatalyzed hydrolysis

Results for water-only steam gun experiments using the NREL apparatus are summarized in Table 2. The highest Stage 1 xylose yield of 17.4% was at a pretreatment temperature of 190 °C and a time of 22 min. The highest Stage 2 glucose yield of 35.0% was for a Stage 1 temperature and time of 210 °C and 6 min, respectively. A pretreatment temperature of 210 °C and time of 6 min gave the highest total glucose and xylose yield of 56.8%. Thus, we can see that conditions for the highest xylose yield in Stage 1 did not give the best glucose yields in Stage 2 or total sugar yields from both stages.

Table 2 also presents just xylose oligomer data for each water-only steam gun test. Solubilized xylose was almost entirely as oligomers at short reaction times underscoring the depolymerization mechanism of xylan solubilization. Furthermore, because xylose degrades to non-carbohydrate products, the maximum xylose yields were limited and could be quantified by comparing the rates of solubilization and degradation. As a point of reference, consider the data for 210 °C. The rate constant for solubilization assuming a first order reaction was about 0.45 min⁻¹ and the rate constant for xylose degradation was about 0.15 min⁻¹ (data not shown), giving a ratio of the former to the latter of 3:1. In this case, the xylose yield was a maximum at 6 min and rapidly declines as the reaction continues.

Table 2
Summary of performance for water only steam gun tests

Temperature, °C	Time, min	Log R_0	Stage 1			Stage 2 ^a		Total yield, %
			Glucose yield, %	Xylose yield, %	Xylan-oligomer yield, %	Glucose yield, %	Xylose yield, %	
150	107	3.5	1.3	5.7	5.5	13.2	5.2	25.4
170	27	3.5	1.4	7.3	7.2	15.1	7.4	31.2
170	87	4.0	2.8	16.1	NA ^b	27.4	1.3	47.6
190	7	3.5	1.7	9.4	9.2	17.7	12.5	41.3
190	14	3.8	2.2	16.1	15.1	26.8	5.4	50.5
190	22	4.0	2.8	17.4	14.1	31.4	1.2	52.8
190	71	4.5	1.2	0.6	0.3	15.7	1.7	19.2
210	2	3.5	2.2	13.8	13.4	22.8	8.4	47.2
210	6	4.0	2.9	17.4	14.5	35.0	1.5	56.8
210	18	4.5	1.3	0.8	0.4	16.5	0	18.6

The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38.

^a Digestion tests and results provided by Bin Yang.

^b NA = not available.

3.2. Dilute acid pretreatment

All timed series tests were performed in 1/2 in. diameter Hastelloy C tubes except as noted, and results for the highest combined Stage 1 xylose and glucose yields are summarized in Table 3 for each acid/temperature combination. Results from these tests showed the highest pretreatment xylose yield occurred at a temperature of 140 °C, a sulfuric acid concentration of 0.98%, and a time of 40 min. The highest combined glucose and xylose yield in Stage 1 was measured at a pretreatment temperature of 160 °C, a sulfuric acid concentration of 0.98%, and a time of 5 min.

Table 3 also shows xylan oligomer content at the point of the highest total yields when corn stover was pretreated with dilute sulfuric acid. The amount of xylan oligomers as a fraction of the total solubilized xylose was greatest at short pretreatment times, coinciding with the breakdown of the fast xylan fraction (Esteghlalian

et al., 1997). In general, the relative amount of xylan oligomers in the hydrolyzate decreased as acid concentration increased at conditions corresponding to the highest xylose yields.

Fig. 1 illustrates the partitioning of glucose and xylose yields over time in Stage 1 for a 0.98% H₂SO₄ concentration. At 180 °C, the maximum yield for just xylose in Stage 1 occurred at 2 min reaction time while the maximum combined glucose and xylose yield in Stage 1 occurred at 5 min. It is interesting to note that the reaction time for the highest xylose and highest total glucose and xylose yields did not coincide at 160 and 180 °C. This variation resulted from an increase in maximum xylose solubilization with decreasing temperature and an initial increase followed by a decrease in xylose yield due to degradation, with time. On the other hand, glucose yields continually increased with time in Stage 1.

Fig. 2 shows Stage 1 monomeric and oligomeric xylose yield data versus time for dilute acid tests performed

Table 3
Summary of tests with the best xylose yield results for Stage 1 from each acid/temperature time series

Time, min	Acid, pH ^a	Temperature, °C	LogCS	Xylose as monomer, %	Xylose as oligomer, %	Total xylose yield, %	Glucose yield, %	Combined yield, %
40	2.36	160	1.01	10.5	16.5	27.0	3.9	30.9
10	2.43	180	0.93	15.0	14.3	29.3	4.0	33.3
5	2.48	200	1.16	17.4	8.4	25.8	3.6	29.4
80	1.60	140	1.48	28.4	0	28.4	3.1	31.5
20	1.62	160	1.45	31.2	0.9	32.1	3.9	36.0
5	1.64	180	1.42	29.8	2.4	32.2	4.1	36.3
40	1.23	140	1.55	29.4	3.0	32.4	4.3	36.7
5	1.24	160	1.22	25.6	9.1	34.5	3.9	38.3
2	1.25	180	1.40	29.6	1.3	30.9	3.8	34.7

The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38.

^a Adjusted for neutralization and temperature.

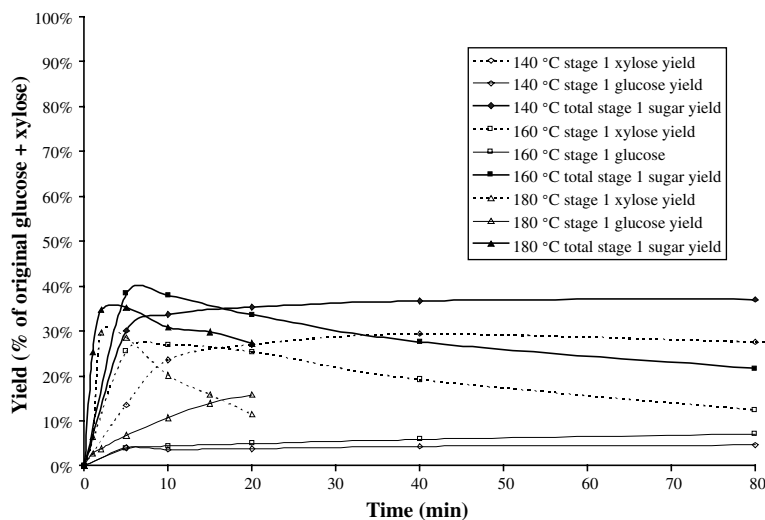


Fig. 1. Stage 1 sugar yields for corn stover pretreated with 0.98% H₂SO₄ for three different temperature/time series. The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38.

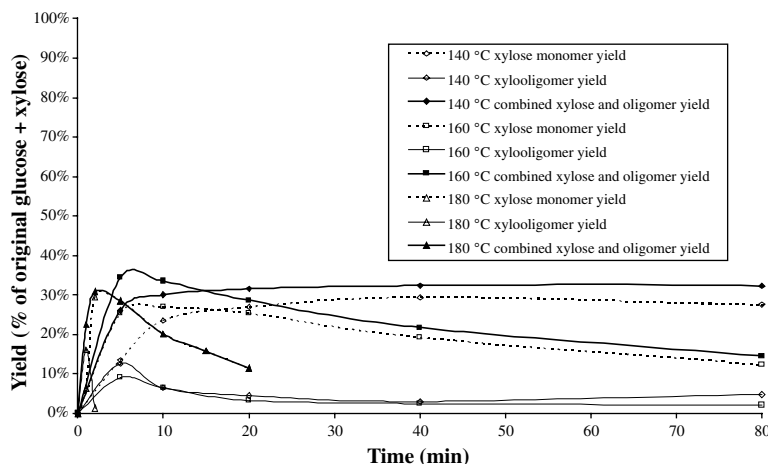


Fig. 2. Stage 1 xylose yields for corn stover pretreated with 0.98% H_2SO_4 and three different temperatures showing the distribution of monomers and oligomers. The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38.

at a 0.98% acid concentration. As for water-only yield results, significant amounts of xylan oligomers were present at short reaction times, however, unlike water-only experiments, xylan oligomer yields reached a maximum early and then dropped rapidly, presumably as a result of forming xylose. Again, this is consistent with a depolymerization mechanism. The rate constant for solubilization assuming a first order reaction was about 0.9 min^{-1} and the rate constant for xylose degradation was about 0.06 min^{-1} (calculation not shown). Thus, the ratio of these two values of 15:1 with acid was much greater than the 3:1 ratio found for water-only experiments at $210 \text{ }^\circ\text{C}$ and showed that acid addition enhanced yields by increasing xylan solubilization much faster than xylose degradation.

Glucooligomers were measured for both Stage 1 water-only steam gun and dilute acid experiments. Because only small amounts of glucose were released in Stage 1 and the post-hydrolysis procedure to quantify oligomers was indirect and hence imprecise, glucooligomer values were not consistent, and for these reasons, glucooligomer yields are not reported. However, both the fraction of glucooligomers and their time course history were roughly similar to that of xylan oligomers, that is, 50% or more glucose present in the Stage 1 hydrolyzate were as oligomers at short reaction times and the amount of glucooligomers declined with increasing reaction time. The presence of glucooligomers in Stage 1 hydrolyzates was consistent with the depolymerization of the amorphous glucan contained in the structure of cellulose microfibrils (Rondeau-Mouro et al., 2003; Weil et al., 1994).

3.3. Enzymatic digestion of dilute acid pretreated residue

Residual solids were initially treated at a high enzyme loading of 60 FPU/g glucan in the feedstock per the

NREL procedure to obtain the maximum possible yields of sugars through digestion, and this Stage 2 yield data for filtered and washed Stage 1 solid residues are presented in Table 4. Note that Stage 2 yields were about 16% as glucose and 9% as xylose for corn stover prior to pretreatment. The highest Stage 2 glucose yield occurred for a 5 min pretreatment time at $200 \text{ }^\circ\text{C}$ and a sulfuric acid concentration of 0.22%. The highest Stage 2 xylose yield occurred for a pretreatment time of 40 min at $160 \text{ }^\circ\text{C}$ and a sulfuric acid concentration of 0.22%. And finally, the highest combined glucose and xylose yield from Stage 2 occurred for a 5 min pretreatment time at $200 \text{ }^\circ\text{C}$ with a sulfuric acid concentration of 0.22%.

Fig. 3 illustrates the partitioning of glucose and xylose released in Stage 2 for Stage 1 pretreatment with an acid concentration of 0.98% at three different temperatures and various times. It is interesting to note that pretreatment conditions for the highest glucose yield alone and highest combined glucose and xylose yield in Stage 2 did not coincide at $180 \text{ }^\circ\text{C}$. This mismatch can be explained by the fact that at higher pretreatment temperatures, Stage 2 glucose yields actually declined for longer pretreatment times, and residual xylose was highest for short pretreatment times. Thus, if only Stage 2 glucose yields are considered, a longer residence time might be selected than desirable to maximize the yield of combined sugars from the two stages.

3.4. Total Stage 1 and Stage 2 yields for dilute acid pretreatment

Total Stage 1 and Stage 2 results are summarized in Table 5 for pretreatment conditions that maximized the total yield for each acid/temperature combination. The highest total glucose and xylose yields were for pretreatment at $140 \text{ }^\circ\text{C}$ with a sulfuric acid concentration of

Table 4

Summary of tests with the best glucose yield results from Stage 2 digestion for each acid/temperature time series

Time, min	Acid, pH ^a	Temperature, °C	Log CS	Glucose yield, %	Xylose yield, %	Combined yield, %
120	2.36	160	1.49	46.9	4.7	51.5
40	2.43	180	1.53	56.9	0.0	56.9
5	2.48	200	1.16	57.7	2.9	60.6
180	1.60	140	1.83	44.5	3.6	48.1
120	1.62	160	2.23	56.2	2.4	58.6
10	1.64	180	1.72	53.6	3.0	56.6
120	1.23	140	2.03	53.0	2.0	55.0
20	1.24	160	1.83	51.1	0.9	52.0
5	1.25	180	1.80	55.3	1.3	56.6

The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38.

^a Adjusted for neutralization and temperature.

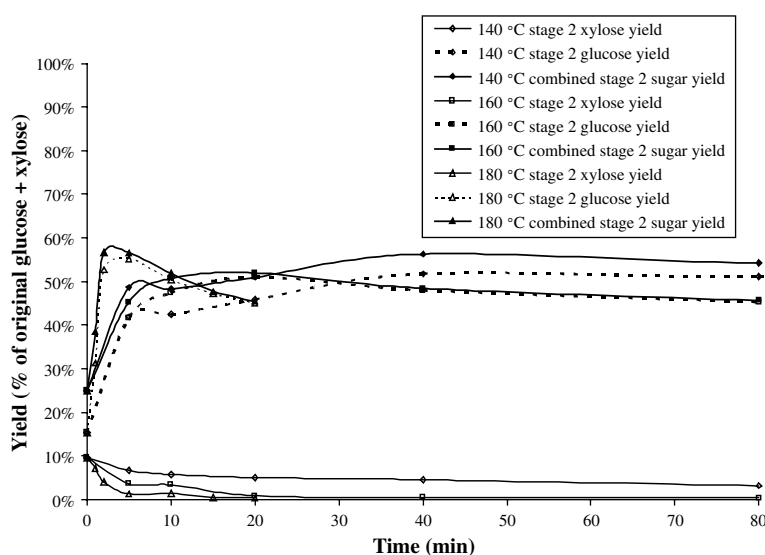


Fig. 3. Stage 2 yields for corn stover pretreated with 0.98% H₂SO₄ and at three different temperatures with an enzyme loading of 60 FPU/g original glucan. The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38.

Table 5

Summary of total Stages 1 and 2 results for best overall combined yields for each acid/temperature time series

Temperature, °C	pH ^a	Time, min	Log CS	Stage 1					Stage 2			Total yield, %
				Xylose as monomer, %	Xylose as oligomer, %	Xylose yield, %	Glucose yield, %	Combined yield, %	Glucose yield, %	Xylose yield, %	Combined yield, %	
160	2.36	60	1.18	18.1	8.1	26.2	3.9	30.1	43.8	5.0	48.8	78.9
180	2.43	10	0.93	15.0	14.3	29.3	3.6	33.0	50.1	3.4	53.5	86.5
200	2.48	5	1.16	17.4	8.4	25.8	3.6	29.4	57.7	2.9	60.6	90.0
140	1.60	80	1.48	28.4	0	28.4	3.1	31.5	43.3	4.3	47.6	79.1
160	1.62	20	1.45	31.2	0.9	32.1	3.9	36.0	53.2	3.8	57.0	91.0
180	1.64	5	1.42	29.8	2.4	32.2	4.1	36.3	52.8	3.4	56.2	92.5
140	1.23	40	1.55	29.4	3.0	32.4	4.3	36.7	51.8	4.5	56.3	93.0
160	1.24	10	1.52	27.0	6.6	33.5	4.4	37.9	47.4	3.4	50.7	88.7
180	1.25	5	1.80	28.5	0.0	28.5	6.8	35.3	55.3	1.3	56.6	91.8

The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38.

^a Adjusted for neutralization and temperature.

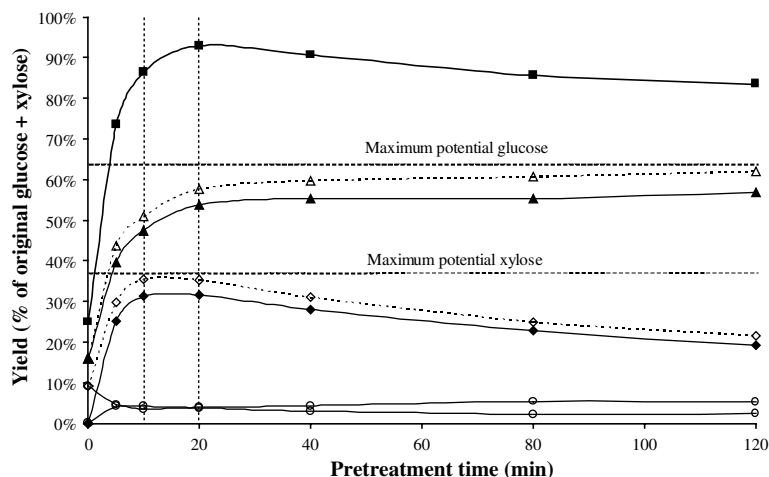


Fig. 4. Sugar partitioning for Stage 1 time series tests performed at 160 °C and 0.49% H₂SO₄ and a Stage 2 run for 72 h with an enzyme loading of 60 FPU/(g of original glucan before pretreatment). The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38. Note: the dashed vertical lines at 10 and 20 min represent the reaction time at which maximum xylose yield and maximum overall sugar yield occur, respectively. Symbol key: (■) total combined Stage 1 and Stage 2 xylose and glucose; (△) combined Stage 1 and Stage 2 glucose; (▲) Stage 2 glucose; (◇) combined Stages 1 and 2 xylose; (◆) Stage 1 xylose; (◊) Stage 1 glucose; (○) Stage 2 xylose.

0.98% and 40 min reaction time or at 160 °C with a sulfuric acid concentration of 0.49% and a reaction time of 20 min or at 180 °C with a sulfuric acid concentration of 0.49% and a reaction time of 5 min. Fig. 4 illustrates the partitioning of glucose and xylose yields for pretreatment at 160 °C and 0.49% H₂SO₄. As with a 0.98% acid concentration, it can be seen that the highest Stage 1 yield did not coincide with the highest total yield. That is, the highest Stage 1 yield of xylose occurred at a reaction time of 10 min. By contrast, the maximum combined yield of glucose and xylose at this acid concentration occurred at a reaction time of 20 min, resulting in a 2% increase over the shorter residence time yields. For Stage 2, the 20 min Stage 1 reaction time led to combined glucose and xylose yields that were about 12% higher than the total sugar yield for a pretreatment of 10 min. Overall, we found that the maximum total sugar yields of several time/temperature series tests occurred at Stage 1 reaction times longer than those for maximum Stage 1 xylose yields.

3.5. Effect of enzyme loading

All Stage 1 solid residues were initially treated in Stage 2 with 60 FPU of Spezyme CP and 120 CBU Novozym 188 per gram of original glucan to establish a ceiling on digestion yields. However, because these loadings are too costly to be competitive, the effect of lower enzyme loadings on Stage 2 performance was determined for solids pretreated at conditions selected for their high overall yields and relatively mild pretreatment conditions: 40 min at 140 °C with 0.98% H₂SO₄ and 20 min at 160 °C with 0.49% H₂SO₄. The larger quantities of solids required for these tests were prepared in the 1 l

stirred Parr apparatus according to the method described. Stage 1 yields from the larger stirred batch were very close to those with the steel tubes. However, Stage 2 yields for these enzyme loading comparison tests were slightly less than the batch tube yields but well within experimental error. For consistency among data for different enzyme loadings, the results for the Stage 2 and overall total yields were based on the solids from the Parr reactor and not the higher values from the tube runs.

Table 6 presents 6 day digestion data for solids from the two pretreatment conditions at enzyme loadings of 3, 7, 15, and 60 FPU of Spezyme CP/g original glucan with Novozym 188 added at a ratio of 2:1 CBU:FPU. Yields from both pretreatment conditions were very similar, with slightly higher yields observed at the lower Stage 1 temperature. Total sugar yields for both samples dropped slightly when the enzyme loading was reduced from 60 to 15 FPU/g original glucan and about 3% when the enzyme loading was reduced from 60 to 7 FPU/g original glucan.

3.6. Severity parameter analysis

Fig. 5 shows a plot of the data from all dilute acid tests performed using the alloy steel tube apparatus for Stage 1 pretreatment followed by digestion of the cellulose in the residual solids at an enzyme loading of 60 FPU/g original glucose versus the log of the combined severity parameter (Eq. (2)) based on adjusted pH values. This information shows that much of the data for each sugar in each stage follows a trend but that some conditions result in substantial scatter in the data that departs from the overall trend. The highest total

Table 6
Effect of enzyme loading on Stage 2 digestion performance

Pretreatment conditions	Enzyme loading ^a	Glucose yield, %	Xylose yield, %	Combined stage 2 yield, %	Total yield, %
A	60	50.7	3.7	54.5	91.1
A	15	50.6	3.6	54.2	90.9
A	7	47.8	3.2	51.0	87.7
A	3	40.3	2.6	42.9	79.6
B	60	53.3	3.3	56.6	92.6
B	15	53.2	3.2	56.4	92.4
B	7	50.5	2.9	53.5	89.5
B	3	43.1	2.2	45.3	81.3

Pretreatment was performed in the Parr reactor for conditions A: 140 °C, 0.98% H₂SO₄ for 40 min; and B: 160 °C, 0.49% H₂SO₄ for 20 min. The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38.

^a FPU/g of original glucan.

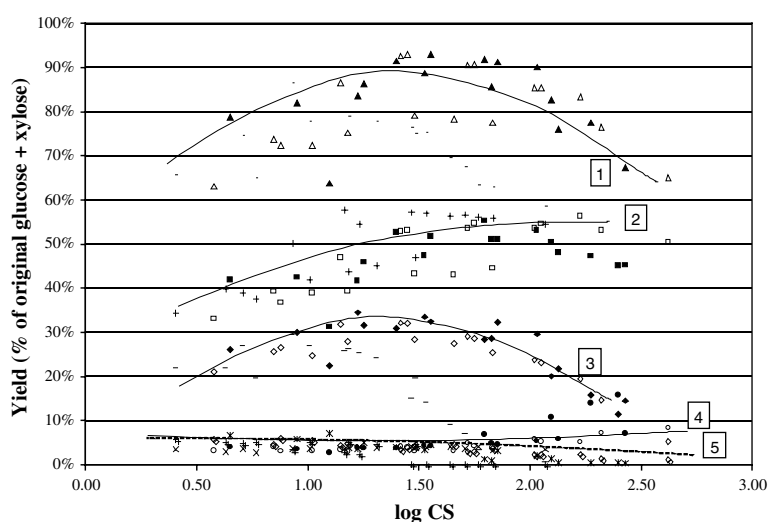


Fig. 5. Component sugar yields vs. log combined severity parameter for all temperature/acid time series tests with lines drawn to reflect approximate data trends. Trend lines 1–5 are total Stage 1 and Stage 2 sugar, Stage 2 glucose, Stage 1 xylose, Stage 1 glucose, and Stage 2 xylose, respectively. The basis for all yield values is total potential glucose plus xylose in the corn stover used. To determine the percent glucose yield based on just original glucose, divide the glucose values in the table by 0.62. For xylose only as a percent, divide the xylose values by 0.38. Symbol key: (▲) 0.98 H₂SO₄ combined total; (△) 0.49 H₂SO₄ combined total; (–) 0.22 H₂SO₄ combined total; (■) 0.98 H₂SO₄ digestion glucose; (□) 0.49 H₂SO₄ digestion glucose; (+) 0.22 H₂SO₄ digestion glucose; (◆) 0.98 H₂SO₄ pretreatment xylose; (◇) 0.49 H₂SO₄ pretreatment xylose; (–) 0.22 H₂SO₄ pretreatment xylose; (●) 0.98 H₂SO₄ pretreatment glucose; (○) 0.49 H₂SO₄ pretreatment glucose; (×) 0.22 H₂SO₄ pretreatment glucose; (✕) 0.98 H₂SO₄ digestion xylose; (◇) 0.49 H₂SO₄ digestion xylose; (⊕) 0.22 H₂SO₄ digestion xylose.

sugar yields of about 92.5% appeared at a logCS of about 1.5 for 0.98% and 0.49% H₂SO₄ and of 90% for a logCS of about 1.1 for 0.22% H₂SO₄.

4. Conclusions

Yield data from both our pretreatment and enzyme digestion data for water only and dilute acid pretreatment of corn stover were consistent with those reported in the literature. Different trends were observed of the effect of dilute acid and no-acid pretreatment conditions on sugar yields from Stage 1 pretreatment and subsequent Stage 2 enzymatic hydrolysis. In Stage 1, xylose sugar yields in pretreatment increased and then dropped

with increasing time while the smaller glucose yields continually increased with pretreatment time. Acid accelerated the rate of xylan solubilization relative to xylose degradation, resulting in higher maximum yields. On the other hand, glucose yields from enzymatic hydrolysis in Stage 2 increased with pretreatment times, and a significant portion of the xylan not liberated in pretreatment was released by enzymes. As a result, pretreatment times required for best sugar yields depended on which sugars and which stages were targeted. For several of the tests performed in this study, the difference in glucose and xylose profiles resulted in maximum total glucose and xylose yields occurring after the time for maximum Stage 1 xylose yield. In the past, the Stage 1 residence time for highest sugar yields were most often

selected based on Stage 1 xylose yields alone, not considering glucose in the same stage or xylose released by enzymes. In that case, the opportunity to realize higher yields would likely be missed. On the other hand, less than optimum pretreatment conditions would possibly reduce the concentration of fermentation inhibitors which could impact overall ethanol yields, and additional study is required to determine this effect.

The highest total yields of solubilized corn stover xylose and glucose from Stages 1 and 2 occurred at Stage 1 conditions with a log combined severity parameter of about 1.5. For a given acid concentration, increasing temperature in Stage 1 accelerated both xylose release and degradation with maximum xylose yields occurring at a log combined severity of about 1.0–1.75. However, the performance of individual acid/temperature series varied within this interval. A similar trend of the effect of Stage 1 temperature on Stage 2 glucose yields was found, although the yield decline was not as severe as in Stage 1, and the glucose yield maxima occurred at about a log combined severity of between 1.25 and 2.0. Again, results for individual series varied within this range.

Although useful in suggesting gross trends, the combined severity factor did not predict specific performance in this study. For example, the maximum Stage 1 xylose yield of 29% occurred at a 0.22% acid concentration with a logCS of about 0.9 but the maximum total sugar yield of 90% was found at a logCS of about 1.2. By contrast, the maximum Stage 1 xylose and total sugar yields of 35% and 93% occurred at logCS values of about 1.2 and 1.5, respectively when the acid concentration was increased to 0.98%.

Overall, the results of this study showed that up to 93% of the total sugars in corn stover were recovered for the coupled Stage 1 and Stage 2 operations at 60 FPU/g glucan at 140 °C for 40 min and a H₂SO₄ concentration of 0.98%. The maximum yield dropped to about 90% when the enzyme loading was reduced to 15 FPU/g and further to 88% for a loading of 7 FPU/g.

This study showed the benefit of xylose release by enzymes in Stage 2 to overall sugar yields, and future tests will explore the influence of xylanase addition. Furthermore, the fermentability of xylose released in Stage 2 was not evaluated, and it is important to determine how operational conditions impact fermentation yields and the operations required to overcome inhibition.

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