

Partial flow of compressed-hot water through corn stover to enhance hemicellulose sugar recovery and enzymatic digestibility of cellulose

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Available online 25 February 2005

Abstract

Flowthrough pretreatment with compressed-hot water can significantly increase the removal of xylan and lignin and enhance xylose sugar yields and cellulose digestibility, especially at high flow rates. However, continuous flowthrough operations that realize these benefits suffer from a large amount of water consumption that leads to high energy requirements for pretreatment and downstream processing. Because high flow rates are particularly effective early in hemicellulose hydrolysis and less effective later, flow with compressed-hot water was applied at selected intervals, and performance was compared with that of batch and flowthrough operations for corn stover pretreated with compressed-hot water at 200 °C. Partial flow reduced water consumption by 60% compared with continuous flowthrough operation but still achieved higher xylose sugar yields (84–89%) compared to batch pretreatment (46.6%). In addition, corn stover cellulose pretreated by partial flow had higher enzymatic digestibility (88–90%) than batch operations (~85%) at otherwise identical conditions, apparently due to much higher lignin removal for the former (40–45% vs 10–12%). Partial flow also reduced degradation, with recovery of xylose and glucose in the solids and hydrolyzate increased to 90–92% vs only about 76% for batch operation. The partial flow approach could be further improved by optimizing the operating strategy and reaction conditions, suggesting that this novel pretreatment could lead to advanced biomass pretreatment technology.

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Keywords: Pretreatment; Compressed-hot water; Xylan hydrolysis; Enzymatic hydrolysis; Partial flow; Corn stover

1. Introduction

Autohydrolysis in which biomass is pretreated with just compressed-hot water or steam is attractive because no chemicals must be added and lower cost materials of construction can be used. In this system, acetic acid released during hemicellulose hydrolysis is often credited with catalyzing pretreatment, but reaction rates are slow, perhaps due to the associated low concentration of hydrodium ions. High temperatures (200–230 °C) are generally applied to compensate but also trigger

greater sugar degradation. Consequently, yields are limited to about 65% (Heitz et al., 1991; Abatzoglou et al., 1992; Saddler et al., 1993). In addition, most of the sugars are released as oligomers that must still be hydrolyzed prior to fermentation by typical organisms (Heitz et al., 1991). Furthermore, sugar oligomers and lignin compounds solubilized during autohydrolysis precipitate when cooled and reattach to the pretreated fibers, impeding cellulose digestion. Low solids pretreatment or hot washing can reduce these compounds and enhance the digestion of the resulting fibers (Jacobsen and Wyman, 2002; Nagle et al., 2002).

Bobleter pioneered passing compressed-hot water through a stationary bed of biomass to accelerate solubilization of hemicellulose and lignin, significantly

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increasing hemicellulose sugar yields and cellulose digestibility compared to traditional autohydrolysis methods (Bobleter et al., 1983; Bobleter, 1994; Hormeyer et al., 1988). Antal and his colleagues applied flowthrough pretreatment to achieve complete xylan removal and above 90% hemicellulose sugar recovery from sugarcane bagasse (Mok and Antal, 1992; Allen et al., 1996). The flowthrough approach has also been extended to use of very dilute acid for biomass pretreatment (Torget et al., 1998) as well as other applications (Sakaki et al., 2002; Ando et al., 2000). Our previous studies showed that increasing flow rate significantly enhanced removal of hemicellulose and lignin for pretreatment with compressed-hot water or very dilute sulfuric acid at elevated temperatures (Liu and Wyman, 2003, 2004b). Further work demonstrated that fluid velocity rather than residence time was particularly influential in increasing rates, suggesting that mass transfer may play an important role in hemicellulose hydrolysis (Liu and Wyman, 2004a). High flow rates enhanced hemicellulose sugar yields, lignin removal, and cellulose digestibility, especially at elevated temperatures. Hemicellulose and lignin removal could be related at high flow rates, suggesting that lignin was released with hemicellulose but would precipitate back on the solids if held at reaction conditions (Liu and Wyman, 2003; Yang and Wyman, 2004). Unfortunately, the flowthrough approach consumes large amounts of water, resulting in very high energy requirements for pretreatment and downstream processing, especially at high flow rates that enhanced performance the most. For this reason, water consumption must be reduced if flowthrough systems are to become commercially viable for advanced pretreatment.

In this paper, a new partial flow pretreatment (PFP) approach was evaluated as a way to significantly reduce water consumption but maintain many of the best features of continuous flowthrough operation. Xylan sugar yields and recovery, hydrolyzate sugar concentration, lignin removal, and cellulose digestibility were followed for PFP pretreatment of corn stover with compressed-hot water and compared to the performance of batch and flowthrough operations at otherwise identical conditions.

2. Methods

2.1. Substrate

The National Renewable Energy Laboratory (NREL) in Golden, Colorado graciously provided corn stover from a large standard supply collected in Harlan, Iowa and maintained at NREL. Some of this material was ground to a particle size of 250–420 μm using a laboratory mill (model 4, Arthur H. Thomas Company,

Philadelphia, PA) and passed through a 35-mesh sieve to separate small enough particles to be added to our reactor system. Samples prepared in this manner were kept in a freezer ($-4\text{ }^{\circ}\text{C}$) for use in all tests. The composition of a representative sample was 36.1% glucan, 21.4% xylan, and 17.2% Klason lignin, as determined by NREL.

2.2. Pretreatment

The flowthrough system and operational procedure described previously (Liu and Wyman, 2003) were used in this study, but the flowthrough reactor volume was larger (25.4 mm OD \times 10.7 mm length, internal volume of 37.8 mL) to allow us to process 6.5 g of corn stover. All batch, flowthrough, and partial flow pretreatments were carried out in the same reactor vessel. For batch pretreatment, the solid concentration was about 20 wt%. The residual solids after pretreatment were washed with cold water, with the amount depending on the operational mode, as shown in Table 1.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated cellulose in the solid residues was performed by the same procedure as used by the other members of the team and is described in the introductory paper for this special journal issue.

2.4. Sample analysis

Liquid hydrolyzate was collected at 4 min intervals, and the pH was measured promptly on reaching room temperature. Then, a known weight of each was dried under vacuum at $60\text{ }^{\circ}\text{C}$ until weight loss ceased to determine the fraction of the mass dissolved in the liquid fraction. The rest of the sample was used for sugar analysis. The entire solid residue in the reactor was washed into a glass vial and dried at $105\text{ }^{\circ}\text{C}$ for compositional analysis. Lignin removal was calculated as the difference between the dry mass of Klason lignin in the feed material and that in the solid residue and was expressed as the percentage of the amount in the feed material.

Compositional analysis of all solids including sugar analysis and Klason lignin determination were carried out by the standard methods as reported by the National Renewable Energy Laboratory (Ruiz and Ehrman, 1996a,b; Templeton and Ehrman, 1995). Sugar monomers in the liquid portion were analyzed quantitatively by a Waters HPLC model 2695 system equipped with a 2414 refractive detector and a Waters 2695 autosampler using Millennium³² chromatography manager 3.2 software (Waters Co., Milford, MA). A Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA) was employed for analyzing sugar

Table 1

Hydrolyzate sugar concentrations for compressed-hot water pretreatment of corn stover in batch, flowthrough (FT), and partial flow (PFP) operations at 200 °C

Operations	Hot water flow, mL	Cold water wash, mL	Total water use, mL	Xylose monomer, g/L	Xylose oligomers, g/L	Total xylose, g/L
Batch, 20 min	0	60	60	0.6	10.7	11.3
Batch, 24 min	0	60	60	0.1	2.8	2.9
FT ^a	240	0	240	0.4	5.4	5.8
PFP1	40	50	90	1.2	12.3	13.5
PFP2	80	40	120	0.8	10.0	10.8

^a FT: continuous flowthrough operation at flow rate of 10 mL/min.

monomers, and xylose oligomers with a degree of polymerization (DP) of up to 10 were analyzed by a Bio-Rad Aminex HPX-42A column (Bio-Rad Laboratories, Hercules, CA). Total xylose, glucose, galactose, arabinose, and mannose concentrations in the liquid fraction were measured after post-hydrolysis of each liquid fraction sample with 4 wt% sulfuric acid at 121 °C for 1 h (Ruiz and Ehrman, 1996a,b). The concentration of xylose oligomers in the liquid samples were calculated as the difference between the total xylose concentration and monomeric xylose concentrations. These methods were as practiced by the rest of the CAFI Project team and described in the earlier introduction to this Special Issue.

3. Results and discussion

3.1. Solubilization of corn stover in the flowthrough reactor

Corn stover was pretreated with continuous flow of compressed-hot water at 200 °C at a flow rate of 10 mL/min corresponding to a nominal residence time of liquid in the reactor of 3.8 min based on the total internal volume of the reactor. Fig. 1 presents the

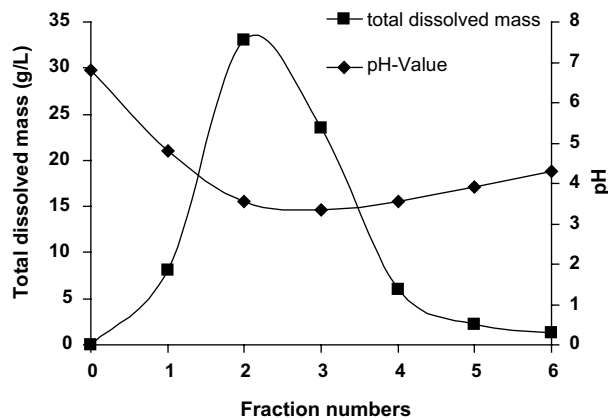


Fig. 1. The change in pH and total dissolved mass in the liquid fractions collected over 4 min intervals for compressed-water pretreatment of corn stover at 200 °C with a flow rate of 10 mL/min.

change of total dissolved mass measured for liquid samples collected over 4 min intervals. These results showed that solubilization of corn stover was relatively slow during the first 4 min, increased dramatically during the next 4 min, and decreased again after 8 min. Therefore, fractions collected in the second interval had the highest concentrations of total dissolved mass, suggesting that most of the corn stover hemicellulose is solubilized in the 4–8 min period at this temperature and flow rate. The relatively slow initial reaction could result from delayed formation of soluble oligomers, delayed heat transfer through biomass, or limited access to corn stover at the beginning. Most of the matter dissolved in the first 4 min was non-carbohydrates and color substances. After 12 min, less and less of the total mass was solubilized, suggesting that further reactions were very slow at this temperature due to less reactive material being present and the more recalcitrance nature of what was left.

The pH of the liquid fraction correlated well with the total dissolved mass concentration during biomass pretreatment with compressed-hot water, as shown in Fig. 1. The pH was high at low total dissolved concentrations but decreased as total dissolved concentration increased, reaching its lowest value when the total dissolved mass concentration was at its maximum. The good correlation between pH and total dissolved mass concentration strongly suggests that release of acetyl groups tracks breakdown of total mass. This result is different from conclusions based on batch data that acetyl groups are released first from hemicellulose and then catalyze hemicellulose hydrolysis during autohydrolysis or uncatalyzed hydrolysis of biomass hemicellulose (Lora and Wayman, 1978; Stuhler, 2002). Our data suggests that pH could be used to monitor the extent of biomass solubilization and possibly be applied to control hemicellulose hydrolysis.

Overall, these results support observations by others that autohydrolysis of biomass increases with flow rate in addition to being affected by temperature and release of acetyl groups (Bobleter et al., 1991; Mok and Antal, 1992). However, they also show that the rate of solubilization is not uniform over time and is greatest during intermediate periods.

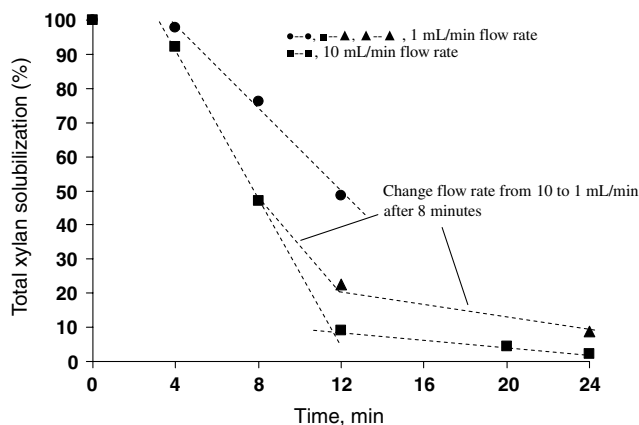


Fig. 2. The effect of flow rate on total xylan solubilization for corn stover pretreatment with compressed-hot water at 200 °C.

3.2. Effect of flow rate on Xylan solubilization

Fig. 2 shows xylan removal as functional of time and flow rate during flowthrough pretreatment of corn stover with compressed-hot water at 200 °C. As expected, xylan removal increased significantly with flow rate and time, especially in the first 12 min. For example, when flow rate was increased from 1 to 10 mL/min, xylan removal was increased from 50% to about 90% after 12 min at this temperature. However, solubilization of the residual xylan in corn stover became much slower after about 90% of xylan was removed, even at the high flow rate (10 mL/min). These results appear to support the concept that biomass hemicellulose consists of two types, a fast-hydrolyzing hemicellulose portion and a slow-hydrolyzing fraction (Kobayashi and Sakai, 1956; Kim and Lee, 1987). Based on this premise, increased flow rate appears to be only effective in accelerating solubilization of the fast-hydrolyzing hemicellulose but has less impact on the slow-hydrolyzing portion, as shown in Fig. 2. In addition, a greater fraction of biomass was more readily hydrolyzed at higher flow rates, suggesting that the percentage of fast-hydrolyzing hemicellulose in biomass is not constant but related to reaction conditions that control hemicellulose hydrolysis. A possible explanation for this observation was offered in a previous paper (Liu and Wyman, 2003).

3.3. Dissolved sugar for flowthrough operation

Post hydrolysis of each fraction from flowthrough operation at 10 mL/min and 180 °C gave the results in Fig. 3. As expected, the dissolved sugars were mostly composed of xylose with much less glucose, arabinose, galactose, and mannose. Most of the five sugars (~85%) were released between the 4 and 12 min periods for flowthrough pretreatment, indicating that hemicellulose hydrolysis occurred mainly in this time period at this temperature and flow rate. The yield of oligomers

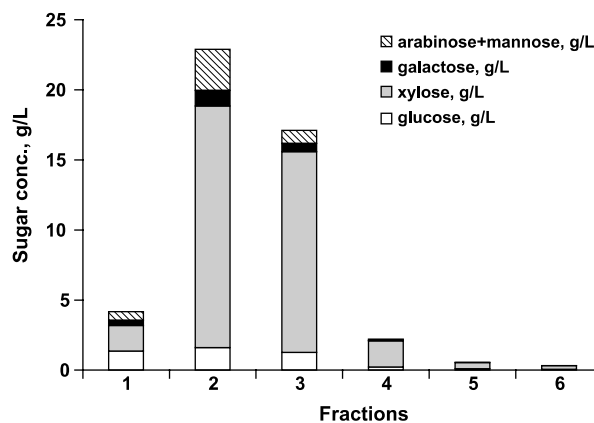


Fig. 3. Sugar concentrations in the liquid fractions collected over 4 min intervals for compressed-water pretreatment of corn stover at 200 °C for a flow rate of 10 mL/min.

measured with the Bio-Rad Aminex HPX-42A column was much lower than found by post-hydrolysis, suggesting that most of the oligomers had a DP greater than 10, consistent with our previous research (Liu and Wyman, 2003). In addition, total glucan solubilization was only about 8%, and almost no glucose or glucose oligomers were found in liquid fractions collected after 16 min, suggesting corn stover cellulose is difficult to solubilize at this temperature.

3.4. Partial flow pretreatment (PFP)

The observations that most of the hemicellulose was hydrolyzed in the intermediate time period with much less reacting initially or at the end suggested that flow for only part of the overall time could reduce water consumption while still achieving much of the performance advantages of flowthrough operation. To test this concept, two partial flow modes were developed based on the characteristics of corn stover hemicellulose hydrolysis in compressed-hot water at 200 °C, as illustrated in Fig. 4. In one, designated PFP1, the system was run in the batch mode for 4 min, followed by flowthrough operation at a flow rate of 10 mL/min for 4 min before returning to batch operation for the remaining time. In the second, PFP2, the flow period was extended to 8 min with batch operation otherwise. As before, water at room temperature was pumped through the reactor to remove solubles after quenching the reactor in the cold water bath, and the wash water was mixed with the liquid fraction collected during flowthrough operation for each run.

3.5. Comparison of water consumption and sugar concentrations

Table 1 summarizes some of our data on water consumption and sugar concentrations for hydrolyzates

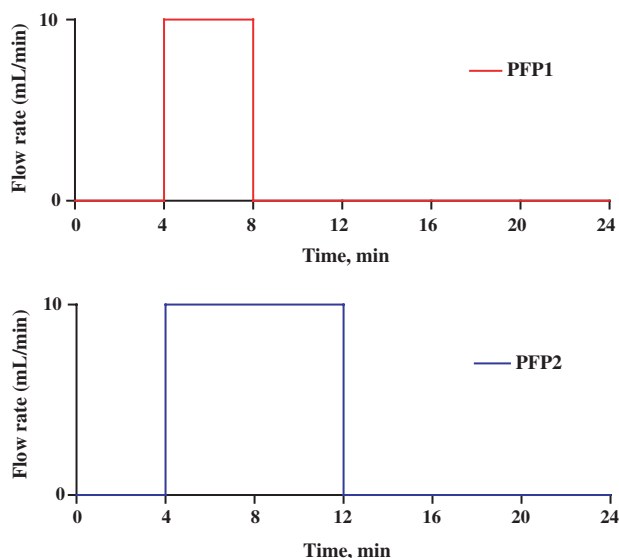


Fig. 4. Partial flow pretreatment (PFP) modes.

from batch, flowthrough, and partial flow operations at the same temperature after 24 min. Flowthrough operation resulted in the lowest total xylose concentration (5.8 g/L) because of the large amount of water used (240 mL). In comparison, the maximum concentration of total dissolved xylose in the batch runs was 11.3 g/L at the same temperature but dropped significantly at longer times because of the combined effects of reduced xylose removal and greater sugar degradation, especially at such a high temperature (200 °C). Partial flow substantially reduced water consumption but maintained relatively high xylose yields (Fig. 5), thus significantly increasing the sugar concentration. For example, the total xylose concentration in the hydrolyzate was 13.5 g/L after 24 min for mode PFP1 at 200 °C, even though 90 mL of water was used. Thus, partial flow gave greater sugar concentrations than either batch or flow-

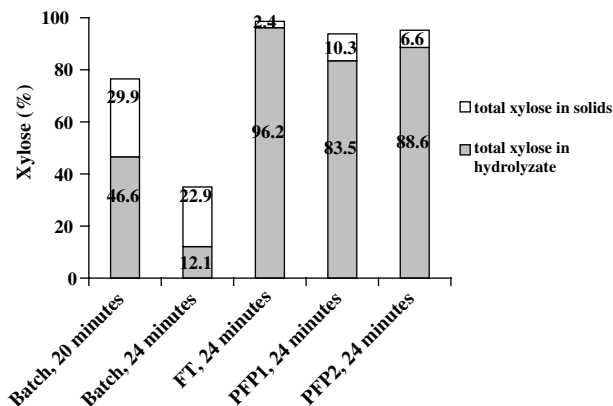


Fig. 5. Total xylose yield and recovery for compressed-hot water pretreatment of corn stover in batch, flowthrough (FT), and partial flow (PFP) operations at 200 °C.

through operations, and we believe that the hydrolyzate sugar concentration for partial flow operation can be further increased by optimizing flow times and reaction conditions.

3.6. Hemicellulose sugar yields and recovery

Fig. 5 presents the total dissolved xylose (monomer plus oligomers) yields for compressed-hot water pretreatment of corn stover by batch, flowthrough, and partial flow pretreatment at 200 °C. Also included is the total xylose recovery defined as the sum of total xylose measured in the hydrolyzate and solid residue and expressed as a percent of the total potential xylose in the corn stover feed. As expected, flowthrough operation achieved the highest total dissolved xylose yield (96.2%) and total recovery (98.6%) after 24 min. On the other hand, the total xylose yield and recovery was much lower for batch runs because of slower xylose release from the solids coupled with greater degradation of dissolved sugars at high temperature (200 °C). For example, for batch runs at the same solids concentration of about 20% (w/w), the maximum total xylose yield was only about 46%, and the total xylose recovery was less than 80%. Although not quite as good as flowthrough, partial flow operation significantly increased total xylose yields and recovery for corn stover pretreated with compressed-hot water compared to batch at the same temperature and time. For example, when corn stover was pretreated in PFP1 mode for 24 min, total xylose yield and recovery were 83.5% and 93.8%, respectively. The extension of flow time for PFP2 further increased the total xylose yield to 88.6% and recovery to 95.2% but consumed more water than the PFP1 mode.

3.7. Lignin removal

Fig. 6 summarizes some of our data on lignin removal for compressed-hot water pretreatment of corn stover in batch, flowthrough, and partial flow operations at 200 °C. Lignin removal was less than 12% for batch operation after 20 min and decreased with time apparently because of lignin condensation reactions. On the other hand, continuous flowthrough operation significantly increased lignin removal at the same conditions. For example, at a flow rate of 10 mL/min, flowthrough pretreatment of corn stover with compressed-hot water at 200 °C for 24 min removed about 60% of the lignin. Partial flow operation removed much more lignin than batch but less than flowthrough at the same conditions. For example, for compressed-hot water pretreatment of corn stover at 200 °C, lignin removal for the two partial flow modes PFP1 and PFP2 were 42% and 45%, respectively. The possible explanation for enhanced lignin removal by flowthrough pretreatment with hot water discussed in previous papers could likely explain lignin

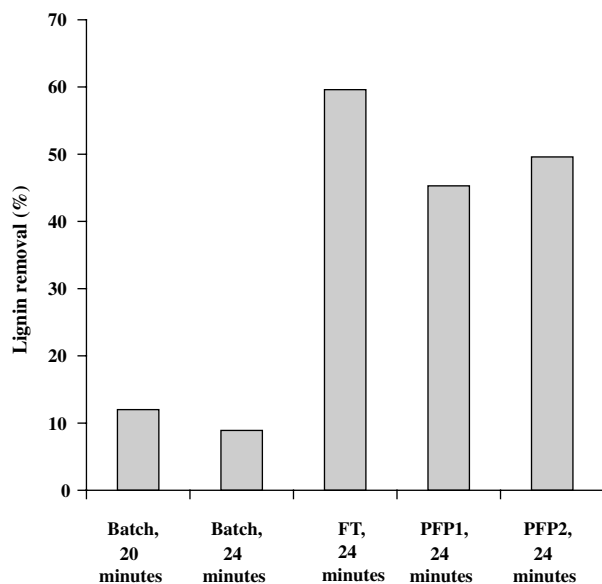


Fig. 6. Lignin removal expressed as the percent of original Klason lignin for compressed-hot water pretreatment of corn stover in batch, flowthrough (FT), and partial flow (PFP) operations at 200 °C.

removal by partial flow operations as well (Liu and Wyman, 2003, 2004a,b).

3.8. Cellulose digestibility

Cellulose digestibility was about 85% for batch, 88–90% for partial flow, and 95% for flowthrough operation, as shown in Fig. 7. The enhanced cellulose digestibility by flowthrough or partial flow operation is believed to be due to better exposure of cellulose to enzymes through increased removal of hemicellulose and particularly lignin (Converse, 1993; Yang and Wyman, 2004). In addition, less lignin is available to competitively adsorb cellulase, making more enzyme available for cellulose hydrolysis (Sutcliffe and Saddler, 1986; Converse, 1993; Yang and Wyman, 2004). As shown in Fig. 6, most of the residual xylan was hydrolyzed into xylose during enzymatic hydrolysis, implying that cellulase used for this study had some xylanase activity. Higher digestibility of residual xylan was found for FT (~70%) and PFP (~66%) pretreatment compared to batch (~60%).

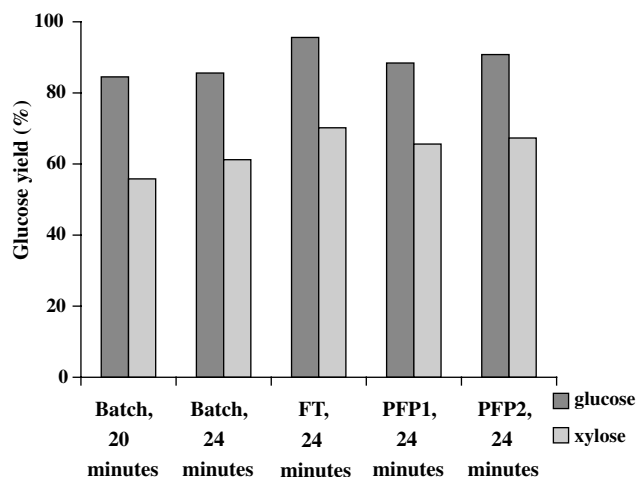


Fig. 7. Glucose yield expressed as the percent of maximum possible for enzymatic hydrolysis of pretreated-corn stover from batch, flowthrough (FT), and partial flow (PFP) operations.

3.9. Total sugar recoveries

Table 2 summarizes the total xylose and glucose recovered in the pretreatment and enzymatic hydrolysis steps. All yields reported are determined as a percent of the total glucose and xylose available in the corn stover used; on this basis, the maximum total xylose yield is 37.7% and the maximum possible yield of just glucose is 62.3%. Continuous flowthrough operation achieved the highest total sugar yield of 96%, while the maximum total sugar yield was only 76% for batch operation at the same temperature. PFP operations achieved a total sugar yield of 90–92% through increasing the total xylose yield from pretreatment and enhancing the cellulose enzymatic digestibility. Thus, PFP can obtain much higher sugar recovery than a batch operation without using any additional chemicals.

4. Conclusions

Continuous flowthrough pretreatment significantly accelerated removal of hemicellulose and lignin and achieved high hemicellulose sugar yields and recovery and high cellulose digestibility. For example, when corn

Table 2
Total xylose and glucose recovered in pretreatment and enzymatic hydrolysis steps

Operations	Pretreatment		Enzymatic hydrolysis		Total yield, %
	Xylose yield, %	Glucose yield, %	Xylose yield, %	Glucose yield, %	
Batch, 20 min	17.6	1.5	6.3	50.5	75.9
Batch, 24 min	4.5	2.3	5.3	50.1	62.2
FT	36.3	4.5	0.6	55.2	96.6
PFP1	31.5	4.3	2.6	51.2	89.6
PFP2	33.6	4.3	1.7	52.6	92.2

Notes: FT, flowthrough; PFP, partial flow.

stover was pretreated with continuous flow of compressed-hot water at 200 °C at a flow rate of 10 mL/min, the total xylose yield was about 96%, and almost 100% of the xylan was recovered after 24 min. At the same time, cellulose digestibility reached 95% after 72 h with cellulase enzyme loading of 15 FPU/g cellulose. However, the high amounts of water used resulted in xylose concentrations of less than 5.8 g/L.

This study showed that partial flow significantly reduced water consumption compared to flowthrough but still achieved relatively high hemicellulose sugar yields, lignin removal, and cellulose digestibility. For example, when corn stover was pretreated in the PFP1 mode with compressed-hot water at 200 °C for 24 min, water use was less than 40% of that for flowthrough operation at the same flow rate. Furthermore, an 84–89% total xylose yield and 94–95% xylan recovery were realized for PFP vs a maximum total xylose yield of 46.6% and recovery of 77% for batch pretreatment. Because of the higher xylose yield and decreased water use, the total xylose concentration in the hydrolyzate increased significantly to 10–13 g/L. Lignin removal also increased significantly for both partial flow approaches, e.g. 40–45% for partial flow operation vs 10–12% for batch runs. At the same time, cellulose enzymatic digestibility for partial flow operations increased to 88–90% vs about 85% for batch pretreatment. Total xylose and glucose recovery from the pretreatment and enzymatic hydrolysis steps was 90–92% for PFP, vs only 76% for batch at the same conditions. Thus, partial flow moves toward combining the best features of flowthrough and batch pretreatment and presents a promising platform on which to develop advanced biomass pretreatment systems.

Acknowledgements

We are grateful to the US Department of Agriculture Initiative for Future Agriculture and Food Systems (IFAFS) Program through contract number 00-52104-9663 for funding this research work. In addition, we appreciate the interactions with our partners in this IFAFS funded project: Y.Y. Lee from Auburn University, Bruce Dale from Michigan State University, Richard Elander from the National Renewable Energy Laboratory, Michael Ladisch from Purdue University, Mark Holtzapple from Texas A&M University, and students and others from these organizations participating in this project. We also thank the National Institute of Standards and Technology through grant number 60NANB1D0064 for supporting purchase of some of the equipment used. Finally, we acknowledge the Thayer School of Engineering at Dartmouth College for providing the facilities and some equipment used in this research.

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