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Cellulase Retention and Sugar Removal by Membrane Ultrafiltration During Lignocellulosic Biomass Hydrolysis

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Abstract

Technologies suitable for the separation and reuse of cellulase enzymes during the enzymatic saccharification of pretreated corn stover are investigated to examine the economic and technical viability of processes that promote cellulase reuse while removing inhibitory reaction products such as glucose and cellobiose. The simplest and most suitable separation is a filter with relatively large pores on the order of 20-25 mm that retains residual corn stover solids while passing reaction products such as glucose and cellobiose to form a sugar stream for a variety of end uses. Such a simple separation is effective because cellulase remains bound to the residual solids. Ultrafiltration using 50-kDa polyethersulfone membranes to recover cellulase enzymes in solution was shown not to enhance further the saccharification rate or overall conversion. Instead, it appears that the necessary cellulase enzymes, including β-glucosidase, are tightly bound to the substrate; when fresh corn stover is contacted with highly washed residual solids, without the addition of fresh enzymes, glucose is generated at a high rate. When filtration was applied multiple times, the concentration of inhibitory reaction products such as glucose and cellobiose was reduced from 70 to 10 g/L. However, an enhanced saccharification performance was not observed, most likely because the concentration of the inhibitory products remained too high. Further reduction in the product concentration was not investigated, because it would make the reaction unnecessarily complex and result in a product stream that is much too dilute to be useful. Finally, an economic analysis shows that reuse of cellulase can reduce glucose production costs, especially when the enzyme price is high. The most economic performance is shown to occur when the cellulase enzyme is reused and a small amount of fresh enzyme is added after each separation step to replace lost or deactivated enzyme.

Index Entries: Saccharification; corn stover; cellulase; glucose; ultrafiltration; vacuum filtration.

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Introduction

Alternative energy sources are being investigated owing to limited reserves of nonrenewable petroleum-based fuels. One such alternative is ethanol derived from agricultural or industrial lignocellulosic biomass. However, economic considerations have so far hindered the construction of lignocellulosic biomass-to-ethanol conversion facilities on a large scale. One way to reduce the cost of these processes is to reuse the expensive cellulase enzyme, which was estimated in 1999 to comprise 20% of the total cost of ethanol production *(1)*. While a more recent economic model *(2)* suggests that the price of cellulase may now be as low as 9% of the total ethanol production cost, significant cellulase activity still remains after saccharification. Recovery and reuse of this remaining enzyme may prove profitable.

Previously, we reported a method of recovering and reusing cellulase enzyme during the hydrolysis of ground yellow poplar using a two-part separation *(3–4)*. First, inclined sedimentation was used to remove most of the solid particles from the 2.5–10% (w/w) yellow poplar suspension. Second, an ultrafiltration unit was used to retain and concentrate the relatively large cellulase enzymes while water, sugars, and other small molecules passed through the membrane. While this separation strategy works reasonably well for ground yellow poplar at low solids concentrations, inclined settling does not effectively remove liquids from a suspension of pretreated corn stover at more realistic solids concentrations of about 15% (w/w). At these concentrations, pretreated corn stover has a mudlike consistency, and it does not settle effectively.

Many reseachers have previously studied the use of ultrafiltration to recycle cellulase during saccharification *(5–22)*. However, much of this previous work involved either purified cellulosic substrates, such as Solka-Floc, which are not representative of feedstocks in large-scale operations, or lignocellulosic substrates at concentrations that would be much too low to be economical in a full-scale process.

In the present study, we explored enzyme reuse and sugar production at reagent concentrations typical of a full-scale biomass-to-ethanol design *(2)*, using pretreated corn stover as the lignocellulosic substrate. We investigated the advantages of using technologies such as vacuum filtration and ultrafiltration to recover and reuse cellulase enzymes in a saccharification process. Because it is known that a significant fraction of cellulase enzymes remains bound to the spent hydrolysate *(23)*, we used vacuum filtration to extract inhibitory reaction products such as glucose and cellobiose, while retaining solid particles and bound enzymes. This vacuum filter cake was then contacted with fresh pretreated corn stover, encouraging additional saccharification without the use of additional enzymes. Such a separation also creates a sugar stream that can be processed for a variety of end uses. Because some cellulase enzymes such as β-glucosidase may remain in solution and pass through the vacuum filter, we also investigated whether it is necessary to recover these enzymes using ultrafiltration as an additional

processing step. Finally, an economic model of a large-scale saccharification process was developed using data measured in the laboratory. Such a model can be used to consider the reuse of cellulase across a range of enzyme prices, especially useful since the cost of cellulase continues to decline.

Materials and Methods

Pretreated Corn Stover and Cellulase Enzyme

Pretreated corn stover containing approx 59% cellulose and 41% lignin by dry weight was obtained from a pilot facility at the National Renewable Energy Laboratory (NREL) in Golden, CO. Because of the pretreatment process, the corn stover contains sulfuric acid, which must be removed prior to saccharification experiments. Hence, the corn stover was washed with deionized water and centrifuged six times. The supernatant was discarded after each washing. The resulting corn stover slurry had an insoluble solids concentration of 15±1% (w/w), measured by drying samples in an oven. Washed corn stover was stored frozen until use. Cellulase used in these experiments is from a commercial preparation made specifically for NREL by Iogen (Ottawa, Ontario, Canada). It is supplied as a liquid solution reported to contain a filter paper activity of 55 filter paper units (FPU)/mL *(24)*.

Saccharification

Saccharification took place in 250-mL Erlenmeyer flasks incubated at 45°C in a shaker. Each flask was initially charged with 50 g of washed corn stover slurry. Citrate buffer (50 m*M*) was used to maintain a pH of 4.7. Sodium azide (0.1% [w/v]) was used to inhibit microbial growth. Cellulase was loaded at 10 or 20 FPU/g of cellulose. The contents of each flask were then mixed with a spatula, and a sample was taken to measure background glucose concentrations. Samples were taken using the top end of a Pasteur pipet and were then centrifuged. Glucose concentrations in the supernatants were determined using a YSI 2700 Select Biochemistry Analyzer (Yellow Springs Instrument, Yellow Springs, OH). Cellobiose concentrations in samples taken at the beginning and end of selected saccharification experiments were determined by high-performance liquid chromatography using a Hewlett Packard model HP1090 high-performance liquid chromatograph and a standard methodology based on the use of Bio-Rad HPX-87H and HPX-87P columns. Cellulose is converted to these sugars with the following stoichiometries:

$$(\text{Glucan})_n + n\,\text{H}_2\text{O} \rightarrow n\,\text{Glucose} \quad (1)$$

$$2\,(\text{Glucan})_n + n\,\text{H}_2\text{O} \rightarrow n\,\text{Cellobiose} \quad (2)$$

in which the glucan monomers of cellulose are converted into glucose and cellobiose. Cellulose conversion to glucose (moles of glucose produced per mole of initial glucan) is calculated based on the measured amounts of glucose generated from the glucan originally present in each flask, with additional conversion to cellobiose included in selected experiments.

The original mass of glucan is calculated based on the measured 15% (w/w) total insoluble solids fraction and the reported 59% (w/w) of these solids consisting of cellulose.

Vacuum Filtration and Ultrafiltration

Vacuum filtration was used to remove liquids containing glucose, cellobiose, and other dissolved solutes from the saccharification flask, while retaining spent hydrolysate solids and the associated bound enzymes. Whatman no. 41 filter paper (pore size: 20–25 μm) was used because of its relatively large pores and high permeability. Vacuum filtration was run until filtrate was no longer being collected (about 5 min), and the resulting filter cake was scraped off and returned to the saccharification flask. In a few experiments, ultrafiltration was used to recover any soluble enzymes (such as β-G) present in the vacuum filtrate. In these experiments, 3.8-cm-diameter polyethersulfone Biomax membrane disks from Millipore (Bedford, MA) with a 50-kDa molecular weight cutoff were used in a stirred-cell apparatus. In all cases, the stirred cell was pressurized with nitrogen to its maximum rated pressure of 70 psi (4.8 bar). Ultrafiltration was run until about 7 mL of retentate remained in the stirred cell. The retentate was then returned to the saccharification flask. Protein transmission during vacuum filtration and protein rejection during ultrafiltration were measured by spectrophotometry using a Coomassie Blue protein assay (Pierce, Rockford, IL). Glucose concentrations were measured between all steps of the separation.

Batch and Semibatch Saccharification

Saccharification experiments were divided into three types: control, semibatch, and diafiltration. During the control experiments, saccharification progress was monitored as already described over 15 d. No attempt was made to remove glucose using filtration.

During the semibatch experiments, vacuum filtration was applied at 4 d and 8 d after the start of saccharification, to remove the sugar product as filtrate. In selected semibatch experiments, ultrafiltration was applied to the vacuum filtrate to recover soluble enzymes. In other semibatch experiments, the vacuum filter cake was washed extensively with deionized water to remove any enzymes not bound to the solids. After filtration, pretreated corn stover slurry and 7 mL of solution (the ultrafiltration filtrate, or citrate buffer when ultrafiltration was not used) were added to the residual solids and bound enzymes, to replace the volume removed as filtrate during the ultrafiltration step. The additional substrate promotes further saccharification by reusing the cellulase enzymes. To promote further saccharification in a final set of semibatch experiments, additional cellulase at a specific activity of 5 FPU/g of fresh cellulose was added along with the fresh corn stover after vacuum filtration.

Diafiltration experiments were conducted to determine whether enough inhibitory reaction products could be removed by filtration to enhance the saccharification rate. During these experiments, vacuum fil-

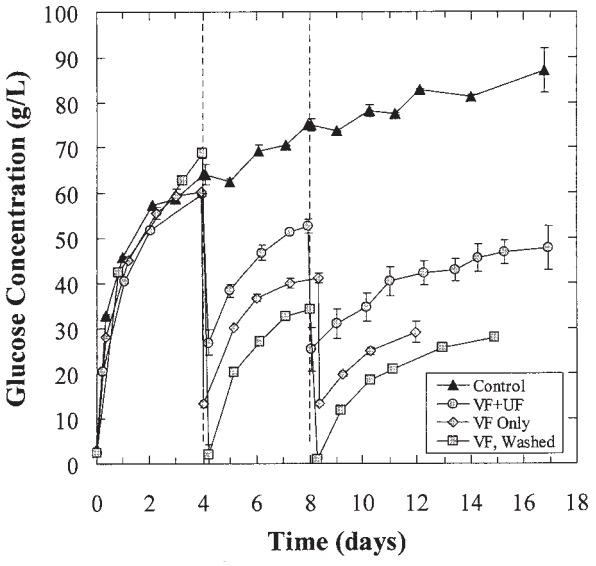

Fig. 1. Glucose concentration over time for semibatch saccharification experiments at 15% (w/w) initial insoluble solids concentration and 20 FPU/g of cellulose enzyme loading. Filtration was applied at two different times to remove glucose (vertical dashed lines). VF Only: vacuum filtration was applied alone; VF+UF: ultrafiltration was also applied following vacuum filtration; VF, Washed: Vacuum filtration was applied and the filter cake was washed with water. Error bars represent averages ± 1 SD for two repeated experiments.

tration followed by ultrafiltration was applied at 4 h, 12 h, 24 h, and 4 d after the start of saccharification, as described in the previous section. Buffer was added to the flasks after each of the four separations, to restore the liquid volume removed as permeate. Filtration applied in this manner is expected to remove inhibitory products such as glucose and cellobiose, while retaining corn stover and cellulase enzymes.

Results and Discussion

Semibatch Saccharification

Glucose concentrations measured during the semibatch saccharification experiments at a cellulase loading of 20 FPU/g of cellulose are shown in Fig. 1. Removal of sugar in the vacuum filtrate resulted in glucose concentrations lower than in the control experiment, as expected. Concentrations in semibatch experiments with ultrafiltration tended to be higher because of glucose returned in the retentate. By contrast, glucose was nearly absent just after filtration in the semibatch experiments with washed filter cakes owing to the washing away of the supernatant. To determine the effectiveness of cellulase reuse, the total amount of glucose generated per liter of initial reactants was calculated for all semibatch experiments.

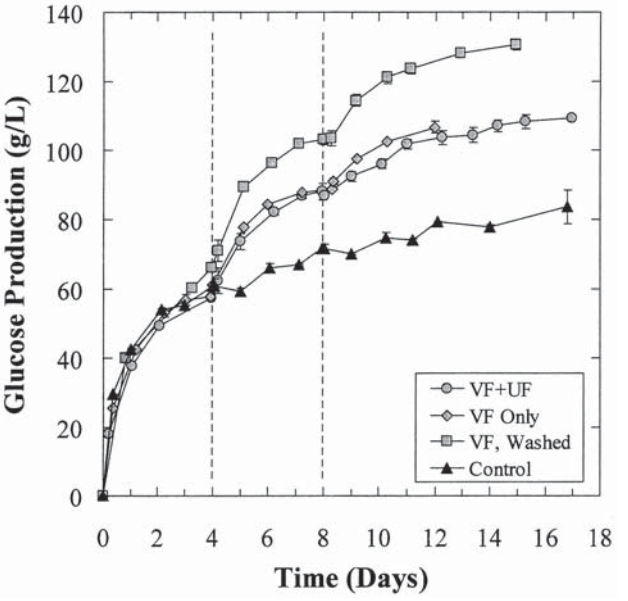

Fig. 2. Glucose production per liter of reactants over time for same experiment as shown in Fig. 1. (definitions of symbols and vertical dashed lines are the same). Error bars represent averages ± 1 SD for two repeated experiments.

Results at a cellulase loading of 20 FPU/g of cellulose are shown in Fig. 2. Immediately apparent is that additional glucose was generated using recycled cellulase and fresh cellulose, though the rate of glucose production declined after each filtration and addition step. In the economic analysis presented later, we examine the trade-off between the savings generated by reusing cellulase enzymes and the costs of adding fresh cellulose.

It is also important to note from Figs. 1 and 2 that the use of ultrafiltration did not result in the generation of any additional glucose over the case in which vacuum filtration was applied alone. Moreover, the experiments in which the corn stover was washed during vacuum filtration generated as much or more glucose than observed for the other experiments. These results suggest that sufficient β-glucosidase activity is adsorbed onto corn stover particles, so that hydrolysis continues when fresh corn stover is added even without recovering soluble enzymes in the supernatant. This hypothesis is supported by the work of Kadam and Knutsen *(23)*, who reported a strong β-glucosidase adsorption affinity toward pretreated corn stover. Furthermore, it should be noted that glucose production was accelerated shortly after the addition of fresh pretreated corn stover at 4 and 8 d after the start of saccharification. This effect is probably owing to the abundance of amorphous cellulose present in the fresh pretreated corn stover, which is more easily converted than is the remaining cellulose from the original charge.

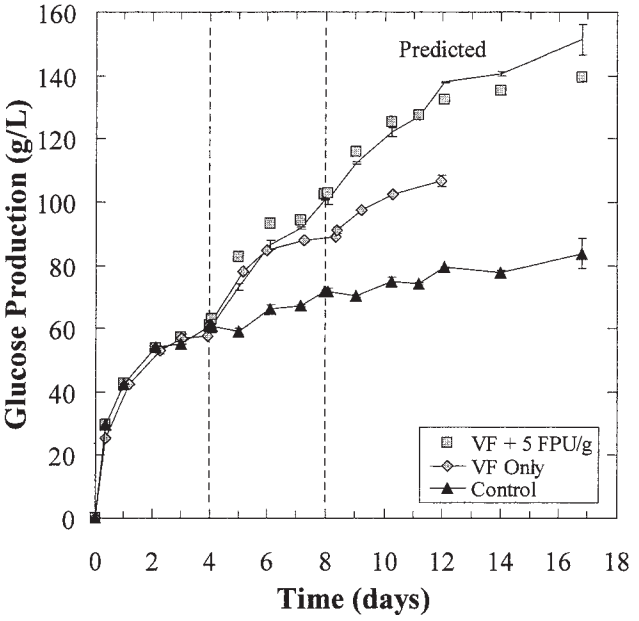

Fig. 3. Glucose production per liter of reactants over time for semibatch saccharification experiments at 15% (w/w) initial insoluble solids concentration and initial enzyme loading of 20 FPU/g of cellulose. Vacuum filtration was applied at two different times to remove glucose (vertical dashed lines). Fresh pretreated corn stover was added to replenish the volume removed by filtration. Additional enzyme was loaded at 5 FPU/g of fresh cellulose. The predicted curve was calculated by summing the amount of glucose generated by additional control experiments at 20 and 5 FPU/g of cellulose. Error bars represent averages ± 1 SD for two repeated experiments.

Irreversible binding of enzymes to lignin or recalcitrant cellulose may limit the production of glucose, and it may be the cause of the reduced production rate observed in each successive step in Fig. 1. In an attempt to increase the production of glucose, additional cellulase at 5 FPU/g of cellulose was added along with fresh corn stover after vacuum filtration. Glucose production during this experiment is shown in Fig. 3. The additional cellulase results in a marked increase in glucose production over the original batch and semibatch experiments. The improved performance occurs because both the residual and new cellulosic solid are converted to sugars by the combined action of the initial and added enzyme during the second and third 4-d periods. The trade-off between the extra value of higher glucose production and the increased cost of using additional enzyme is examined in our economic analysis. The predicted curve is based on the total amount of glucose generated from control experiments at 20 and 5 FPU/g of cellulose. This curve is the total

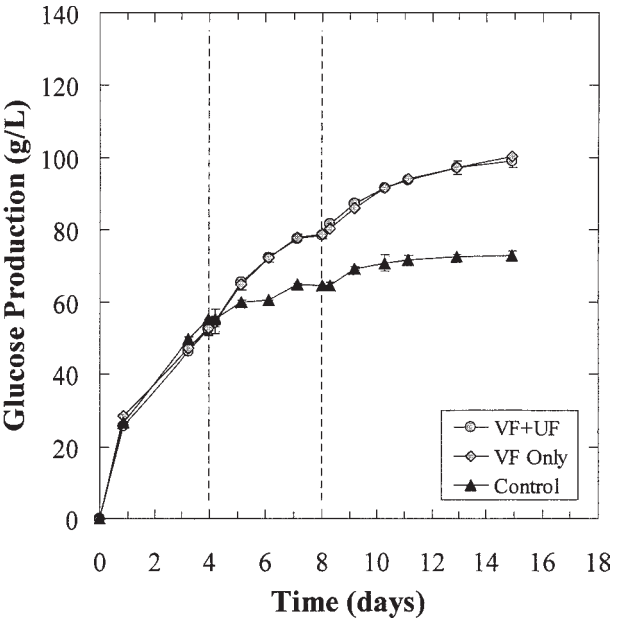

Fig. 4. Glucose production per liter of reactants over time for similar semibatch experiment as shown in Fig. 2, but at a lower enzyme loading of 10 FPU/g of cellulose (definitions of symbols and vertical dashed lines are the same). Error bars represent averages ± 1 SD for two repeated experiments.

amount of glucose that would be generated if the semibatch experiment were divided into three separate control experiments, with 50 g in control flask 1 and a mass in control flasks 2 and 3 equal to the amount of fresh corn stover added after each filtration in the semibatch experiment. Semibatch glucose production lower than this predicted curve might be attributed to cellulase deactivation. Fortunately, it appears that cellulase is only minimally deactivated by filtration, since the semibatch data closely match the predicted curve up to 11 d of saccharification and are only slightly lower thereafter.

To determine whether similar trends would be observed at lower enzyme loadings, semibatch experiments were conducted at an initial cellulase concentration of 10 FPU/g of cellulose, with glucose productions shown in Fig. 4. The use of ultrafiltration to recover β-glucosidase again appears unnecessary. Additionally, glucose productions after 12 d of saccharification at the lower enzyme loading were only slightly lower than those reported at the higher enzyme loading, suggesting that the use of cellulase beyond 10 FPU/g of cellulose might be unnecessary if long-term cellulose conversion is the process goal.

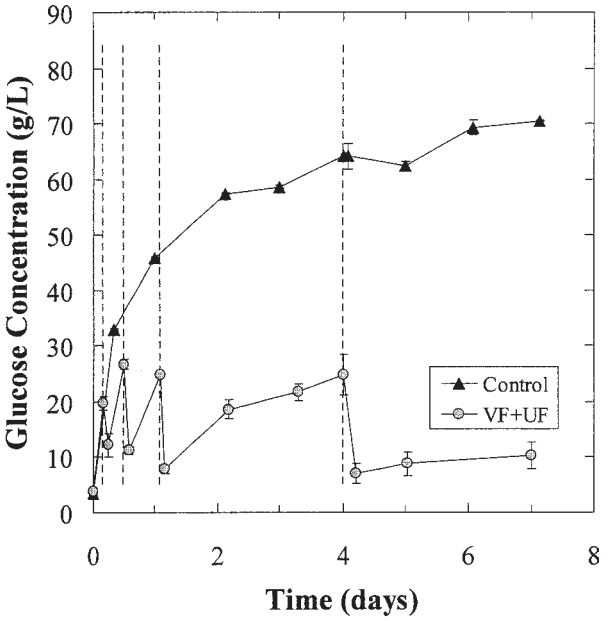

Fig. 5. Glucose concentration over time for diafiltration saccharification experiments at 15% (w/w) initial insoluble solids concentration and 20 FPU/g of cellulose enzyme loading. Vacuum filtration and ultrafiltration were applied at four different times to remove glucose (vertical dashed lines). Filtration was not applied to the control flasks. Error bars represent averages ± 1 SD for three repeated experiments.

Saccharification with Diafiltration

Glucose concentrations measured during diafiltration saccharification experiments at a cellulase loading of 20 FPU/g of cellulose are shown in Fig. 5. Removal of sugar as permeate resulted in glucose concentrations that are much lower than for the control experiments, as expected. Cellulose conversions to glucose were calculated using these concentrations, and the glucose removed as permeate was also included. It was anticipated that the lower glucose concentrations might result in an increased cellulose conversion, because saccharification kinetics are known to be product inhibited *(5,6)*. However, as shown in Fig. 6, the lower glucose concentration did not result in a higher conversion-both the diafiltration and control experiments show just over 60% conversion to glucose after 7 d. When cellobiose production is also considered, the conversion in both experiments after 7 d is increased to about 65%. It is possible that the glucose concentrations after filtration, which never fell below 10 g/L without washing, were still too high to result in a significant increase in the rate of conversion. Lower glucose concentrations could be obtained with more frequent filtration or washing, but the resulting product sugar stream would then be too dilute for economic use.

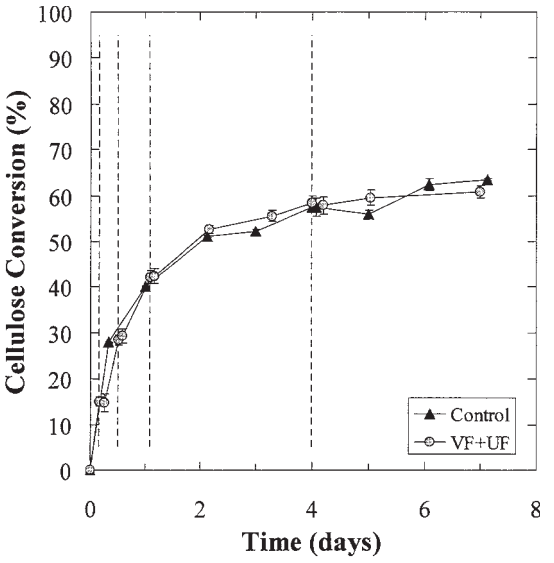

Fig. 6. Cellulose conversion over time for the same experiments as shown in Fig. 5. The data represent cellulose conversions based on glucose production alone. Cellobiose measurements taken at the end of the experiments account for an additional conversion of about 4%. Error bars represent averages ± 1 SD for three repeated experiments.

Economic Analysis

An economic analysis of a hypothetical full-scale lignocellulosic saccharification process was carried out to determine possible cost savings owing to enzyme reuse. The production cost of glucose is compared in three different operating configurations: batch, semibatch, and semibatch with additional cellulase. In this section, the batch-operating configuration is similar to the "control" experiments discussed in previous sections. These designs are based on the 2002 Lignocellulosic Biomass to Ethanol Process Design report from NREL *(2)*, which uses a continuous operation. However, to simulate the experiments in our laboratory, the present economic analysis uses only batch or semibatch operation. Hence, the production costs reported here are expected to be higher than in a large-scale continuous operation and were calculated primarily as a means of comparing the relative production costs across the three operating configurations.

The initial steps for all three configurations are the same. Raw corn stover enters the plant at a rate of nearly 100,000 kg/h and is washed, milled, and sent to the pretreatment unit. During pretreatment, the milled corn stover is exposed to high-pressure sulfuric acid, converting most of the hemicellulose into soluble sugars by hydrolysis reactions. In addition, these conditions "expose" the cellulose for the subsequent enzymatic saccharification. Following pretreatment, the stover is conditioned and the pH is adjusted to 4.5. In all configurations, an average of 430,000 kg/h of pretreated corn stover is sent to the saccharification vessels. Capital costs for these three initial processes are shown in Table 1.

Table 1
Economic Model Parameters and Glucose Production Costs for Batch and Semibatch Saccharification Processes

	20 FPU/g Batch	20 FPU/g Semibatch	20+5 FPU/g Semibatch	10 FPU/g Batch	10 FPU/g Semibatch
Biomass flow (kg/h)	98,000	98,000	98,000	98,000	98,000
Average hydrolysate flow (kg/h)	429,000	429,000	429,000	429,000	429,000
Average cellulose flow (kg/h)	28,400	28,400	28,400	28,400	28,400
Average cellulase flow (L/h)	11,400	4200	6000	5700	2100
Average cellulase loading (FPU/g)	20.0	7.4	10.5	10.0	3.7
Reactor volume (L)	48,000,000	55,000,000	56,000,000	48,000,000	55,000,000
No. of saccharification vessels	14	16	16	14	16
No. of filtration Units	6	7	7	6	7
Capital costs					
Feed handling	$7,500,000	$7,500,000	$7,500,000	$7,500,000	$7,500,000
Pretreatment	$19,000,000	$19,000,000	$19,000,000	$19,000,000	$19,000,000
Neutralization/conditioning	$7,800,000	$7,800,000	$7,800,000	$7,800,000	$7,800,000
Saccharification cost	$9,100,000	$10,400,000	$10,400,000	$9,100,000	$10,400,000
Filtration cost	$12,700,000	$14,800,000	$14,800,000	$12,700,000	$14,800,000
Total capital investment	$56,100,000	$59,500,000	$59,500,000	$56,100,000	$59,500,000
Annual expenses					
Biomass feedstock	$23,200,000	$23,200,000	$23,200,000	$23,200,000	$23,200,000
Cellulase	$12,100,000	$4,500,000	$6,400,000	$6,000,000	$2,200,000
Labor	$2,150,000	$2,150,000	$2,150,000	$2,150,000	$2,150,000
Amortized capital payment	$6,600,000	$7,000,000	$7,000,000	$6,600,000	$7,000,000
Total expenses	$44,050,000	$36,850,000	$38,750,000	$37,950,000	$34,550,000
Cellulose conversion to glucose	58 ± 4%	48 ± 4%	62 ± 4%	52 ± 4%	49 ± 3%
Cycle time (d)	5	15	15	5	15
Glucose production (g/[L·cycle])	45 ± 3	98 ± 7	129 ± 6	40 ± 3	101 ± 6
Annual glucose production (kg)	$1.50 \pm 0.08 \times 10^8$	$1.26 \pm 0.08 \times 10^8$	$1.6 \pm 0.1 \times 10^8$	$1.34 \pm 0.08 \times 10^8$	$1.30 \pm 0.08 \times 10^8$
Glucose production cost (¢/kg)	29 ± 2	29 ± 2	24 ± 2	28 ± 2	27 ± 2

During saccharification, pretreated corn stover is sent to several 950,000-gal (3600-m^3) vessels, with an associated capital cost of $650,000 per vessel. Cellulase is then added at 10 or 20 FPU/g of cellulose, and the reaction proceeds. The number of required vessels is calculated based on the total volume required to process 430,000 kg/h of pretreated corn stover over the reaction cycle, which is 5 d for batch operation and 15 d for semibatch operation. The total volume for the semibatch processes is slightly higher than the batch processes owing to inert solids that build up during the 15-d processing time.

Every 4 d, the reactor's contents are sent to a series of pressure filters to remove residual solids from the product sugar stream. In the batch configurations, the filter cake is discarded and the saccharification vessels are charged with fresh pretreated corn stover and enzyme. In the semibatch configuration, the filter cake is returned to the saccharification vessels, and fresh corn stover (either with or without additional cellulase at 5 FPU/g of cellulose) is added to restore the mass of products removed as filtrate. After every third filtration, the filter cake is discarded and the saccharification vessels are loaded with fresh pretreated corn stover and enzyme.

Each filtration unit can process about 100,000 kg/h of slurry, and the effluent is to be processed in 1 d. To economize on the number of filters, saccharification is divided into four staggered batches so that the filters are operating nearly continuously. The number of required filtration units for each operating condition is shown in Table 1. The capital cost associated with each filtration unit is $2,110,000.

The total capital investment associated with feed handling, pretreatment, neutralization/conditioning, saccharification, and filtration is amortized over the 20-yr design life of the plant at a fractional interest rate of 10%. Annual labor costs are estimated by NREL to be $2,150,000 *(2)*. These costs were estimated using data from similar ethanol plants.

The production of glucose from each reaction cycle is calculated from cellulose conversions measured in the laboratory, assuming that cellulose conversion remains independent of reactor size. The model also assumes that cellulose conversion remains independent of the initial glucan concentration, because corn stover tested in the laboratory had a solids fraction of 15 ± 1% (w/w), while the design report specifies a lower initial solids fraction of 11.2% (w/w). The amount of glucose generated in one reaction cycle is calculated by multiplying the molar flow rate of glucan into the reactor by the cellulose conversion measured in the laboratory. Conversions measured at 4 d and 12 d are used in the batch and semibatch process models, respectively. The cycle times of 5 d and 15 d are longer for the two process models because filtration will require 1 day after every 4 d of saccharification. The glucose productions listed in Table 1 are lower than those measured experimentally in the laboratory because of the lower initial solids fraction. The annual glucose production is calculated by assuming that the processes are operated 350 d/yr.

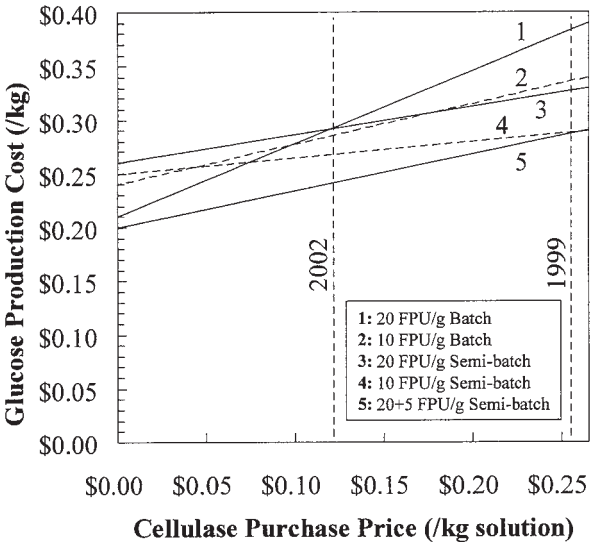

Fig. 7. Glucose production cost as a function of cellulase purchase price for three process configurations: batch, semibatch, and semibatch with additional cellulase. Vertical dashed lines are cellulase purchase prices in 1999 (26.6¢/kg) and in 2002 (12.2¢/kg).

The only chemical expenses considered are from the biomass feedstock and cellulase enzyme; all other chemical costs are small and essentially the same for the batch and semibatch models. NREL estimates the annual cost of corn stover to be $23,200,000 *(2)*. The annual cellulase flow rate is calculated based on the enzyme loading in the saccharification vessels and the specific activity in the cellulase solution of 50,000 FPU/L. The rate of cellulase consumption in all operating configurations is shown in Table 1. The price of cellulase solution has dropped rapidly in the past several years. In 1999, the price of cellulase solution was 25.6¢/kg *(1)*. In 2002, however, the price fell to only 12.2¢/kg, and it is expected to continue to decline *(2)*. Because of this variability, the glucose production costs were calculated for a range of cellulase prices and are shown in Fig. 7 for enzyme loadings of 10 and 20 FPU/g of cellulose. Glucose production costs listed in Table 1 are based on the price of enzyme in 2002. The uncertainties in glucose production and, hence, cost shown in Table 1 are primarily owing to the uncertainties in the solids concentrations used in the laboratory experiments.

From Table 1 and Fig. 7, there are several trends worth noting. First, using the lower enzyme concentration of 10 FPU/g of cellulose is economical only at high enzyme costs. Second, when the enzyme price declines below about 10¢/kg, the use of 20 FPU/g of cellulose becomes more economical for the batch case, because the higher conversion of substrate is more important than the reduced enzyme use. Third, enzyme reuse by employing the semibatch process is economically attractive only for

enzyme prices of 5 and 12¢/kg for 10 and 20 FPU/g of cellulose, respectively. Finally, the most economical process appears to be that for which a small concentration of new enzyme is added after each filtration step in the semibatch case. In this process, the enzyme costs are relatively low and yet high conversion of the relatively expensive cellulose feedstock is obtained. The cost savings are only modest (about 5¢/kg of glucose produced) at current enzyme prices, because the total biomass feedstock cost dominates over the total enzyme costs (*see* Table 1).

Conclusion

The results that we have presented show that a simple solid/liquid separation using a large-pore vacuum filtration unit is a viable method for recovering significant quantities of active cellulase enzymes bound to the solid substrate, and that these enzymes may be reused by simply mixing fresh substrate with the spent hydrolysate. Our work has also shown that reducing the concentration of inhibitory reaction products to levels low enough to enhance the saccharification rate is unproductive-the benefits of an enhanced saccharification rate are more than offset by the expenses owing to frequent filtration and the associated dilute product stream. The use of ultrafiltration to recover enzymes in solution was also shown to be unnecessary, since enzymes appear to be tightly bound to the residual solids; even when the filter cake is thoroughly washed with water and then mixed with fresh substrate, glucose continues to be generated. Thus, vacuum filtration alone can be used to retain solids and enzymes in the retentate, with the filtrate then representing a sugar stream that may be used for additional processing. Finally, an economic model suggests that the reuse of cellulase is potentially favorable using current cellulase prices. However, as cellulase prices continue to decline, future biomass conversion facilities will likely use the enzyme in only one round of saccharification.

Acknowledgments

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