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Application of a slurry feeder to 1 and 3 stage continuous simultaneous saccharification and fermentation of dilute acid pretreated corn stover

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HIGHLIGHTS

- A laboratory scale slurry-fed three stage continuous fermentation system was set up.
- The performance of continuous SSF of pretreated corn stover was measured.
- Dilution rates of up to 0.25 h^{-1} could be operated stably without cell wash out.
- Productivity in 3 stage continuous SSF was 12% higher compared to single stage.

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ABSTRACT

Continuous operation is often chosen for conceptual designs of biological processing of cellulosic biomass to ethanol to achieve higher volumetric productivities. Furthermore, continuous stirred tank reactors (CSTR) can handle higher solids concentrations than possible in batch mode due to broth thinning at partial conversion in a continuous fermentor. However, experience and literature data are very limited for continuous simultaneous saccharification and fermentation (cSSF) processes. In this work, a slurry feed system was developed and applied to a 3-stage bench-scale cSSF train to convert pretreated corn stover to ethanol and determine the effects of dilution rate and number of fermentation vessels on overall volumetric productivity. The highest productivity of 0.4 g L^{-1} h^{-1} was achieved in a single cSSF vessel with an 8 h residence time. Furthermore, productivity at identical total residence times was 12% higher for operation with 3 cSSF stages than for a single CSTR stage for pretreated corn stover.

1. Introduction

Production of sustainable fuels from lignocellulosic materials including agricultural and forestry residues (e.g., corn stover and sawdust, respectively) and herbaceous (e.g., switchgrass) and woody (e.g., poplar) plants is widely accepted as a means to reduce non-biogenic CO_2 release by the transportation sector, reduce the nearly total dependence of transportation fuels on petroleum, improve energy security, create domestic jobs, and reduce trade deficits (Claassen et al., 1999; Dornburg et al., 2010; Lal, 2005). Biological routes offer the advantages of the high product yields that are essential to low unit fuel costs, high selectivities to intended products that reduce waste treatment and toxicity issues, and low temperature operations that result in low containment costs. Enzyme mediated saccharification of the carbohydrates in lignocellulosic biomass has been widely researched because of its ability to realize these advantages (Gray et al., 2006; Himmel et al., 2007). Furthermore, rapid fermentation of the sugars released by enzymes to ethanol in the same fermentation vessel in the so-called simultaneous saccharification and fermentation (SSF) configuration is often favored to remove sugars whose accumulation would otherwise severely inhibit enzyme activity and limit yields at industrially attractive sugar concentrations (Olofsson et al., 2008; Spindler et al., 1988; Takagi et al., 1977; Wright et al., 1988). Although the SSF process can improve enzyme effectiveness, enzyme loadings to achieve high yields still tend to be high and therefore expensive, and reducing the amount of enzyme required to realize high yields has the greatest leverage in lowering ethanol production costs (Lynd et al., 2008).

Most experiments that establish enzyme loadings required to achieve high yields are based on batch fermentations of cellulosic biomass to ethanol, in which the enzyme loadings are defined based on the cellulose available at the start of fermentations

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(Dowe and McMillan, 2008). For example, the typical loading of 30 mg of cellulase protein per gram of glucan corresponding to an activity of around 15 FPU per gram of glucan that has been found to be needed means just that: 30 mg of enzyme will be consumed for every gram of cellulose added to a batch fermentor. This demand translates into at least 30 g of enzyme being consumed for every liter of ethanol produced even when close to theoretical ethanol yields are assumed and credit is included for the ethanol produced from hemicellulose sugars. On the other hand, because fermentations in a continuous stirred tank reactor (CSTR) would operate with some of the cellulose consumed, less enzyme must be added to achieve the same ratio of enzyme to unreacted cellulose in the CSTR as would be required at the start of a batch reaction. For example, if a CSTR operates at a 50% cellulose conversion, then adding only 15 mg of enzyme/g cellulose in the CSTR feed would still achieve a loading of 30 mg of enzyme per gram of unreacted cellulose. However, as lignin, which is known to irreversibly adsorb enzymes thereby lowering the remaining activity, is not converted in cSSF, the enzyme concentration in the feed has to be increased accordingly to reach the same identical net enzyme loading. In addition to potential advantages in reducing enzyme loadings, continuous operation is often chosen for conceptual designs of biological processing of cellulosic biomass to ethanol for a number of other reasons (Brethauer and Wyman, 2010). For example, continuous operations can achieve higher volumetric productivities by avoiding downtime for emptying, cleaning, and filling fermentors. It is also possible to better control reaction conditions as needed to achieve high productivities, and operation in a CSTR mode can allow handling of higher solids concentrations than possible with batch due to broth thinning at the partial conversion conditions in a continuous fermentor.

Despite these important potential advantages for continuous SSF fermentations and the assumption by many of the technoeconomic evaluations of cellulosic ethanol production that continuous fermentations would be the operation of choice, almost all SSF reported results are based on batch operations, and experience and literature data are very limited for continuous SSF (cSSF) processes (Brethauer and Wyman, 2010). A number of factors limit development of such data for cSSF compared to batch operations including it is more complex to set up, requires more controls, and faces experimental challenges in small-scale delivery of solid substrates to fermentors at a constant rate. In addition, the system must be run for long periods of time to achieve steady state with the result that large amounts of pretreated biomass and enzymes are needed to feed the system, making experimentation time consuming and costly.

In this study, an automated continuous slurry feed system was developed to supply small amounts of cellulose or pretreated corn stover to 0.75 L continuous stirred tank fermentors to keep feed requirements manageable. This system was then applied to cSSF to better define its performance features and opportunities for improvements and provide data for technoeconomic evaluations. This integrated feed and fermentation system was first applied to continuous single stage enzymatic hydrolysis and fermentation of Avicel to establish its operability and gather baseline data. Then, operation was extended to 3-stage cSSF of dilute acid pretreated corn stover. After experimental validation of the system in each case, a range of solids feed rates were employed to determine the effects of the dilution rate and the number of fermentors in series on the volumetric productivity of the system and determine the maximum space velocity before washout of the fermentative organism occurred. Operation with slurries at low solids concentrations assured good operability and established key operational features, with modification to the feed system to handle higher solids in the future to understand how solids concentration impacts performance.

2. Methods

2.1. Biomass and pretreatment

Corn stover was milled through a 2 mm screen using a Wiley knife mill and soaked overnight in 0.2% H_2SO_4 at a solid loading of 5% w/w. The solids were pressed to a dry matter content of 30%, treated for 10 min at 190 °C in a steam gun, and washed thoroughly (Yang and Wyman, 2009). The pretreated corn stover was used without any further particle size screening and had an average glucan content of 58 ± 3%.

2.2. Batch and continuous enzymatic hydrolysis of Avicel

Slurries of Avicel containing 1% cellulose were enzymatically hydrolyzed in 0.05 M citric acid buffer at pH 4.8 supplemented with 10 mg L^{-1} NaN_3 employing Spezyme CP cellulase (Genencor, Palo Alto, CA, USA) and Novozyme 188 β-glucosidase (Novozyme, Frankliton, NC, USA) at a temperature of 50 °C. The cellulase loading was set to 10 FPU$(g_{glucan})^{-1}$ and 15 CBU$(g_{glucan})^{-1}$. These enzyme loadings correspond to protein concentrations of approximately 0.2 and 0.05 g L^{-1}, respectively. The reaction volume was 0.75 L for batch and one-stage continuous runs, and the mean residence time for continuous hydrolysis was set to 24 ± 1 h by adjusting the flow rate to 31.3 ± 1.4 mL h^{-1}. No batch time preceded initiation of the continuous runs.

2.3. Batch and continuous simultaneous saccharification and fermentation of corn stover

SSF was carried out at a 2% (w/w) glucan concentration employing *Saccharomyces cerevisiae* D5A at a temperature of 38 °C. A yeast preculture was grown in a shake flask overnight at 38 °C on YPD medium containing 10 g L^{-1} yeast extract, 20 g L^{-1} peptone and 50 g L^{-1} glucose. The reactor was inoculated with 10% v/v yeast preculture. The pH was set to 4.8 by using 0.05 M citric buffer, and 0.3% w/w corn steep liquor was added to the medium as nutrient. A cellulase loading of 5 FPU$(g_{glucan})^{-1}$ (Spezyme CP, Genencor) and a β-glucosidase loading of 7.5 CBU$(g_{glucan})^{-1}$ (Novozyme 188, Novozyme) were applied. The reaction was run under sterile conditions.

Batch SSF was performed in a 3 L stirred tank reactor with a reaction volume of 0.75 L. The continuous runs were performed in the above-described three stage CSTR train with 0.75 L reaction volume in each reactor. Slurry feed rates were adjusted for different experiments to give residence times of 4, 8, 12 and 24 h per reactor. No batch time preceded initiation of the continuous runs.

2.4. Multistage solid fed continuous fermentation system

The three stage continuous fermentation system for this study employed commercially available fermentation hardware and software purchased from Applikon, Inc. (Foster City, CA, USA). Three 3 L stirred tank fermentors filled with 0.75 L reaction slurry were fitted with 40 mm diameter 8-bladed impellers and connected in series by 4.8 mm inner diameter transparent silicone tubing (Masterflex, Cole Parmer, Vernon Hills, IL, USA). Enzymes along with Avicel or pretreated biomass solids were added to the first fermentor in the train. The overall system is pictured in Fig. 1, and the details are described below.

2.4.1. Feeding of biomass slurries to the fermentors

The feeding of a solid substrate (in this case, Avicel or pretreated corn stover) suspended in aqueous solutions is difficult as biomass settles very rapidly compared to the flow rate in a feed

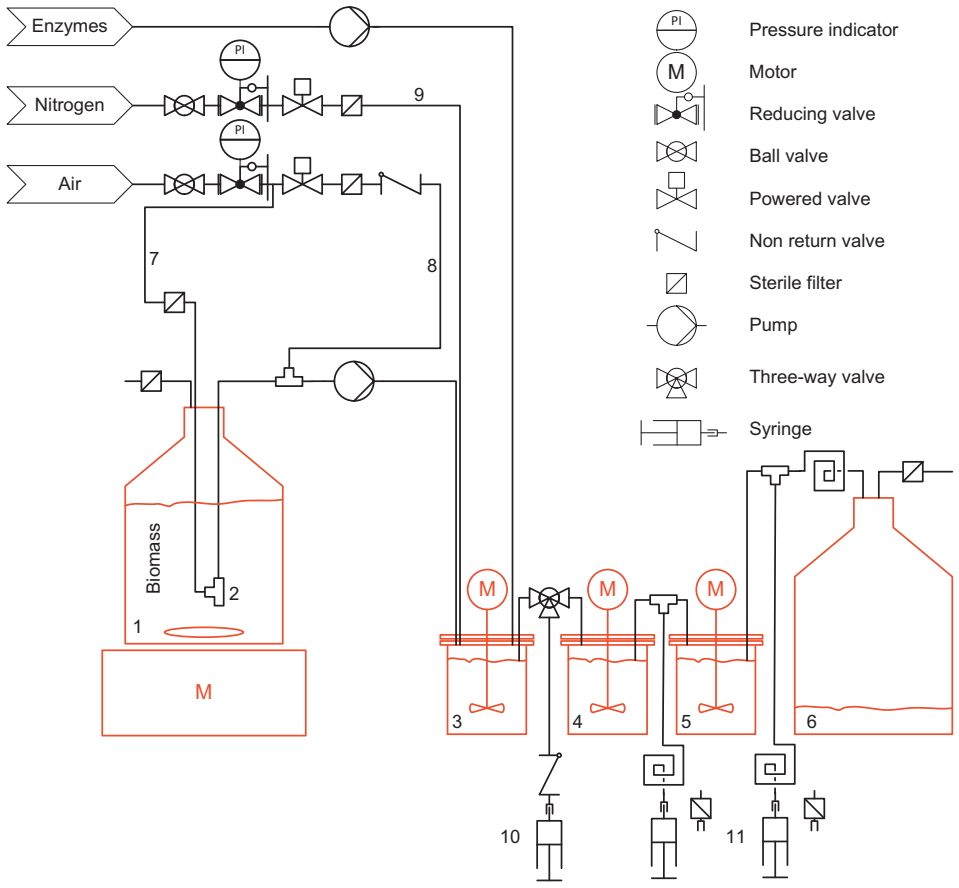

Fig. 1. Three stage laboratory continuous fermentation setup with segmented gas/liquid feeding of biomass slurry through introduction of air bubbles in the feed tube. 1: magnetically stirred feed tank, 2: feeding nozzle, 3–5: fermentor 1–3, 6: product tank, 7: air supply for bubble feeding including backpressure tubing, 8: air supply to free feeding lines, 9: nitrogen supply to transport fermentation slurry between fermenters, 10: sampling of reactor 1 through three way valve and non return valve, 11: sampling of reactors 2 and 3 with tubing to the sterile bench.

tube that could be employed to transfer biomass from a feed tank to the first fermentor. As a result, solids accumulate in the feed tank and plug the tubing. Settling can be prevented by feeding air bubbles concomitantly with the biomass because air bubbles will isolate biomass between successive bubbles in the tubing, thereby limiting settling to just the distance between bubbles and preventing accumulation of enough solids to block the tube. This segmented gas/liquid delivery method was adapted from that originally developed by Weimer et al. (1991). In our case, a 5 L bottle was used as a feed reservoir and was placed on an extra powerful magnetic stir plate (V & P Scientific, San Diego, CA, USA). The contents were mixed with a very strong magnetic stir bar (V & P Scientific), with the rotation speed set to a value of 40 as shown on the control knob of the plate. The feed bottle was equipped with a special lid that enabled introduction of air into the bottle through a sterile filter and discharge of feed from the bottle with the help of custom-made nozzle (Fig. 1). The lid was made of a screw cap that held a custom made stainless steel (ss) septum with two Teflon lined silicone linings sealing the aperture. The septum featured three connections:

- one piercing ss needle with luer lock through which to introduce air to form bubbles in the nozzle,
- one piercing piece of ss pipe with the same inner diameter as the peristaltic tubing connected on both ends, and
- one piece of pipe attached only to the upper side of the septum to hold the tubing connected to the sterile filter through which the bottle was vented.

Air bubbles were fed sideways into the lower end of the feed tube through a modified 1/8″ T-piece (Swagelok, Solon, OH, USA). The T-piece was bored out on both of the parallel ends to allow welding of two 1/4”pipes with the same inner diameter as the L/S 16 tube. Furthermore, the flow channel in between the two parallel ends was bored out to the same inner diameter as the L/S 16 tube. A 1/8″ to 1/16″ reducing union was mounted on the branch end to which the air tube was connected. The air was fed into the bottle from a pressurized air supply line in the laboratory to which a reducing valve was connected to adjust pressure to 25 psi. In order to appropriately match the air flow rate and the slurry feed flow rate, the 1/8″ Teflon tube coming from the air supply was connected to 5 ft of backpressure tubing (1/16″ outer diameter, 0.005” inner diameter, Dionex, now Thermo Scientific, Sunnyvale, CA, USA). The backpressure tubing was then connected to a sterile filter mounted to the lid of the feed reservoir bottle.

In order to prevent clogging during extended runs, the part of the feeding tube inside the feed bottle had to be emptied of any remaining biomass particles between feed intervals. To this end, a modified 1/8″ T-piece with the same design as the nozzle was mounted just above the feed bottle and equipped on the branch end with a 1/8″ non-return valve that was connected with a tube through a sterile filter to the pressurized air supply of the reactor control unit. The feed tube was cleared about 30 s after the end of each feeding period by the reactor control unit opening the air valve.

The peristaltic pump typically used for feeding antifoam agent under control of the fermentor instrumentation package was

employed to govern biomass transport from the feed reservoir to the first fermentor. This control system included a level sensor that employed two electrodes that were bridged by liquid or foam when the level rose too high. To avoid overfeeding, a feed period (called the pulse time) can be set to prompt pump flow followed by a user defined dead time, during which the pump stopped long enough to leave time for foam to collapse. For our purpose of regularly feeding biomass slurry, the two electrodes were joined, and the feed flow rate was adjusted by altering the pulse time. Feeding was initiated every 6 min, with the dead time calculated to be 360 s minus the corresponding cycle time. The flow rate was monitored once a day by weighing the feed bottle on a balance.

2.4.2. Slurry transport between fermentors

The fermentation broth was pneumatically transported from one fermentor to the next and to the final product bottle through dip tubes that were inserted into each fermentor, with their heights adjusted to correspond to the desired liquid volume of 0.75 L. To this end, 20 s long pulses of nitrogen were applied 10 times per hour to the first fermentor in the train approximately three minutes after the feeding of fresh substrate as determined by the reactor controller. The fermentors were connected with L/S 25 tubing (Masterflex, Cole Parmer, Vernon Hills, IL, USA).

2.4.3. Enzyme addition

Enzymes were diluted with 19 times their volume of 0.05 M citric acid buffer (pH 4.8) and then sterile filtered. To avoid thermal enzyme deactivation, the enzyme solution was kept in a refrigerator. The lid of the enzyme bottle was outfitted with a connection to the feed tube and a filter for sterile venting. A peristaltic tube with an inner diameter of 0.25 mm (Fisherbrand Manifold tubing, Fisher Scientific, Pittsburgh, PA, USA) was used to feed the extremely low enzyme flow rates ($\sim$1–7 mL h^{-1}). A peristaltic pump with a variable speed option (Watson-Marlow, Wilmington, MA, USA) transferred the enzyme solution into the first fermentor. A 1/16″ ss capillary was used to connect the tiny enzyme feed tube to the larger diameter tubes from the enzyme feed bottle and to the fermentor.

2.4.4. Fermentor sampling and exchange of waste or feed bottles

To allow sterile sampling of fermentor contents, two types of sampling ports were inserted in the tubes connecting these vessels. A 1/4″ ss three-way valve (Swagelok) was inserted in the tube connecting the first and second fermentors and further outfitted with a 1/4″ non-return valve followed by a 1/4″ ss pipe and a reducing union to connect to a 1/8″ ss needle with luer lock. When a sample of the slurry from the first fermentor was desired, the three-way valve was opened to feed a syringe inserted into the luer connection. To ensure a representative sample, the first few mLs were discarded. During a run, the sampling port was immersed in a tube containing 70% isopropanol as a sterile barrier that does not interfere with the target ethanol quantification. For sampling the second and third fermentors, a 1/4″ ss T-piece (Swagelock) was inserted in the transport lines between these vessels, and a L/S 16 tube long enough to reach the sterile bench was connected to the T-piece branch. A luer connector (Fisher) equipped with a sterile filter was attached to the end of the sampling tube. During normal operation, the tube was closed with a hose clamp directly after the branch in the Tee. When a sample was desired, this same hose clamp was moved to close the tube from one fermentor to the next or from the last fermentor to the final product bottle. To sample fermentor slurry, the sample tubing was placed on the sterile bench to avoid contamination of the system, the sterile filter was removed, and a syringe was connected to withdraw material. After sampling, the luer connector was cleaned of biomass by rinsing it with 70% isopropanol, and the sterile filter was reconnected. Air was then pushed through the sterile filter by a syringe to force the sample line contents back into the fermentor.

Refilling of the feed tank and emptying of the waste bottle were performed in the sterile bench, which could be reached through the sufficiently long connecting tubings.

2.5. Single stage solid fed continuous reaction system

Prior to running the entire 3 stage continuous train of fermentors, a single stage continuous fermentor was run with the solid feeder to establish operability. The single fermentor was fed with slurry from the feed tank in the manner described above. Because the citric acid buffer kept the fermentation pH constant, the pH control of the fermentor control system could be used to continuously pump out the fermentor contents through a dip tube. In this case, the pH set point of the fermentor controller was simply set to a target value of 1, resulting in the acid pump running continuously so it could remove slurry whenever the level of the fermentor contents reached the opening in the dip tube, thereby keeping the level.

2.6. Analytical methods

Ethanol, glucose and cellobiose concentrations were analyzed using high performance liquid chromatography (HPLC) (Alliance 2695, Waters) equipped with a refractive index detector (2414, Waters, Milford, MA, USA) and an Aminex HPX-87H column (BioRad, Hercules, CA, USA) with 5 mM sulfuric acid as the eluent flowing with 0.6 mL min^{-1} in an isocratic mode at 65 °C.

2.7. Kinetic modeling

As the yields of batch and continuous enzymatic hydrolysis of Avicel cannot be compared directly, a kinetic model was developed. Batch data from enzymatic Avicel hydrolysis were used to fit model parameters that were then used to simulate the continuous system. Two very similar SSF models explained in detail by their developers (Pettersson et al., 2002; Philippidis and Hatzis, 1997) were adapted to enzymatic hydrolysis of lignin free biomass assuming that cellulose is converted to cellobiose at a volumetric rate r_1 and the cellobiose in turn reacts at a volumetric rate r_2 to glucose. Thus, the mass balances on cellulose, cellobiose, and glucose become:

$$\frac{d(C)}{dt} = -r_1 + D((C)_{\text{feed}} - (C)) \tag{1}$$

$$\frac{d(CB)}{dt} = 1.056r_1 - r_2 - D(CB) \tag{2}$$

$$\frac{d(G)}{dt} = 1.053r_2 - D(G) \tag{3}$$

where (C) is the cellulose concentration, $(C)_{\text{feed}}$ is the cellulose concentration in the feed, (CB) is the cellobiose concentration, (G) is the glucose concentration, t is time and D is the dilution rate.

The rate expressions for batch and continuous reactions are as follows:

$$r_{1,batch} = \frac{\frac{k_1 \cdot enzc}{K_{eq}+enzc}(C)e^{-\lambda t}}{1+\frac{(CB)}{K_{1CB}}+\frac{(G)}{K_{1G}}} \tag{4a}$$

$$r_{1,continuous} = \frac{\frac{k_1 \cdot enzc}{K_{eq}+enzc}(C)\frac{1}{1+\lambda\tau}}{1+\frac{(CB)}{K_{1CB}}+\frac{(G)}{K_{1G}}} \tag{4b}$$

$$r_2 = \frac{k_2 \cdot enzg \cdot (CB)}{K_M(1+\frac{(G)}{K_{2G}})+(CB)} \tag{5}$$

Parameters and notations beyond those explained above are described in Table 1. To model batch reactions, the dilution rate D is set to zero. This set of equations was solved with the Berkley Madonna algorithm using a 4th order Runge–Kutta integration, and the parameters k_1, k_2, and λ were fit to the batch hydrolysis data with the curve fit function of the program.

3. Results and discussion

Available data on continuous enzymatic hydrolysis or cSSF of lignocellulosic biomass are limited presumably due to the inherent experimental complexity of feeding small amounts of solid substrate at a defined constant rate. Feeding a solid substrate suspension is difficult as the biomass settles rapidly in the feed tubing, resulting in unwanted solids accumulation in the solids feed tank and clogging of the tubing. Detrimental settling can be prevented by feeding air bubbles concomitantly with biomass in such a way that biomass is trapped between successive air bubbles and cannot block the tubing. This approach, described in more detail in Section 2, was adapted from that developed by Weimer et al. (1991) and is illustrated in Fig. 1. The resulting feed system allowed the controlled feeding of Avicel or pretreated corn stover over 6 min intervals, thereby providing virtually continuous slurry feeding compared to even the shortest continuous fermentor residence times of 4 h. Fig. 2 shows the gravimetrically measured feed rates for two different target flow rates.

3.1. Batch and one stage continuous enzymatic hydrolysis of Avicel – experimental and modeling results

In order to validate the feeding method and to gain experience, single-stage continuous enzymatic hydrolysis of Avicel was run first at a 1% w/w cellulose loading with a mean residence time of 24 h, and the results were compared to those for the batch mode of operation at otherwise identical conditions. The cellulase loading was set to 10 FPU$(g_{glucan})^{-1}$ and 15 CBU$(g_{glucan})^{-1}$ for both batch and continuous operations. For a batch run, a yield of 56% was achieved after 24 h reaction time. In the continuous run, a stable steady state was achieved approximately three residence times after start up and could be maintained over a time period of 7 days. The average glucose yield achieved was 37% of the theoretical maximum possible based on the Avicel cellulose content. The ability to achieve stable steady state results over an extended period of 216 h representing 9 residence times in which approximately 6.75 L of Avicel slurry was consumed indicated that overall the system performed very reliably. To further validate the system, we used a modeling approach to compare batch and continuous results which is not possible directly, as in a CSTR the product is diluted by incoming unconverted substrate. Thus, a simple kinetic model (Eqs. 1, 2, 3, 4a, 4b, 5) was employed based on literature data (Pettersson et al., 2002; Philippidis and Hatzis, 1997) with

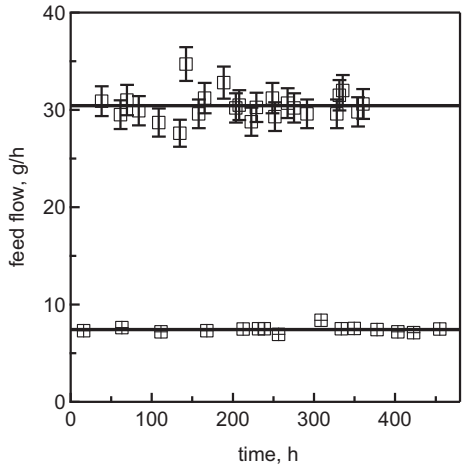

Fig. 2. Targeted and measured feed flow rates. The lower data are for feeding Avicel at a residence time of 96 h, and the upper data are for feeding corn stover for a residence time of 24 h. The solid lines represent the target flow rates. The error bars for corn stover correspond to +/− 5% of the measured feed rate. No error bars are shown for the Avicel feed.

the parameters k_1, k_2, and λ fitted to the batch results. All the parameters used in the model are summarized in Table 1. The fitted parameters of the batch model were then applied to predict performance for continuous enzymatic hydrolysis of cellulose to glucose. As shown in Fig. 3, the simulated steady state concentration of 36.3% glucose agreed well with measured data, thereby further validating the continuous reaction system.

3.2. Batch and multistage continuous saccharification and fermentation of pretreated corn stover

After achieving satisfactory continuous enzymatic hydrolysis of Avicel, the continuous SSF system was applied in more detail to dilute acid pretreated corn stover. A train of 3 equally sized CSTRs was used with equal residence times in each fermentor of 4, 8, 12, and 24 h. This set of conditions made it possible to investigate the effect of the number of vessels in series on the important performance criteria of the productivity of the fermentation system, *i.e.,* the amount of product formed per unit of time and reactor volume. Furthermore, a batch SSF was run with all conditions other than flow the same as for the continuous runs to provide a control. As shown in Fig. 4, the dilute acid steam gun pretreated corn stover was highly reactive in the batch runs, with a 92% ethanol yield reached in 6 days at a low cellulase loading of 5 FPU$(g_{glucan})^{-1}$ The productivity declined with reaction time from about 0.5 down to about 0.05 g L^{-1} h^{-1}.

Table 1
Notations, parameter definitions, and parameter values used in the kinetics model.

Notation	Parameter	Value	Source
enzc	Cellulase activity concentration (FPUL^{-1})	100	Experimental condition
enzg	β-glucosidase activity concentration (CBUL^{-1})	150	Experimental condition
k_1	Maximum specific rate of cellulose hydrolysis to cellobiose (h^{-1})	0.34	Fit to batch data
k_2	Specific rate of cellobiose hydrolysis to glucose (g (CBU h)$^{-1}$)	1	Fit to batch data, limited to a maximum value of 1
K_{eq}	Cellulase adsorption saturation constant (FPU L^{-1})	545	Philippidis and Hatzis (1997)
K_m	Cellobiose saturation constant for β-glucosidase (gL^{-1})	10.56	Philippidis and Hatzis (1997)
K_{1CB}	Inhibition constant of cellulase by cellobiose (gL^{-1})	5.85	Philippidis and Hatzis (1997)
K_{1G}	Inhibition constant of cellulase by glucose (gL^{-1})	53.2	Philippidis and Hatzis (1997)
K_{2G}	Inhibition constant of β-glucosidase by glucose	0.62	Philippidis and Hatzis (1997)
τ	Hydraulic residence time (h)	24	Experimental condition
λ	Rate of decrease in cellulose specific surface area (h^{-1})	0.04	Fit to batch data

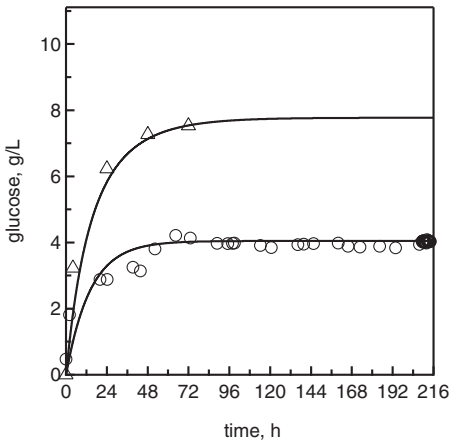

Fig. 3. Glucose concentrations measured for batch (Δ) and 24 h residence time continuous (○) enzymatic hydrolysis of Avicel cellulose at 50 °C with a 1% w/w cellulose loading as well as the corresponding modeling predictions (solid lines) for an enzyme loading of 10 FPU $(g_{glucan})^{-1}$.

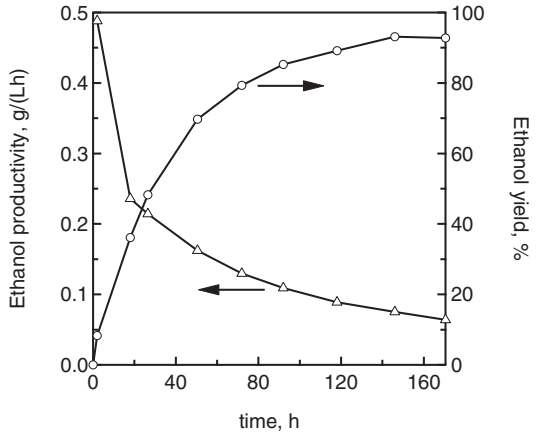

Fig. 4. Ethanol yield and productivity for batch SSF of dilute acid pretreated corn stover at a cellulose loading of 2% (w/w) and a temperature of 38 °C with an enzyme loading of 5 FPU g_{glucan}^{-1}.

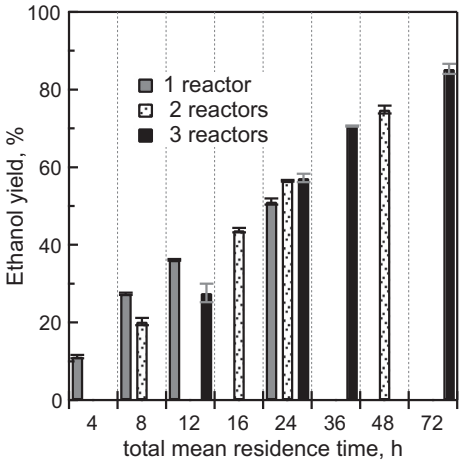

Fig. 5. Average ethanol yields from continuous simultaneous saccharification and fermentation (cSSF) of dilute acid pretreated corn stover solids for each stage in the 3-stage train. Samples were taken from each fermentor for separate runs at each residence time of 4, 8, 12 and 24 h per reactor, resulting in the displayed set of 12 steady state concentrations. For example, a total residence time of 24 h can be reached at reactor 1 (1 × 24 h), reactor 2 (2 × 12 h) or reactor 3 (3 × 8 h).

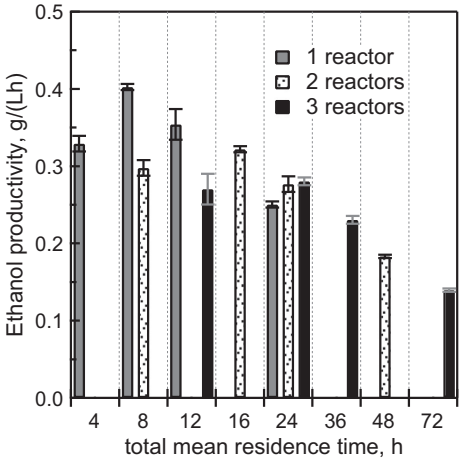

Fig. 6. Average ethanol productivities from continuous simultaneous saccharification and fermentation (cSSF) of dilute acid pretreated corn stover solids for each stage in the 3-stage train. Samples were taken from each fermentor for separate runs at each residence time of 4, 8, 12 and 24 h per reactor, resulting in the displayed set of 12 steady state concentrations.

In the continuous SSF runs, stable steady states could be achieved in all three fermentors. As shown in Fig. 5, steady state ethanol yields increased from about 10% to 85% as the residence time was increased from 4 to 24 h in each fermentor in the train. Thus, the highest yield was achieved at a combined residence time of 72 h for the 3 fermentors. Productivities corresponding to the yield results are presented in Fig. 6. Depending on the mean residence time and the number of fermentors included in calculating the overall productivity, ethanol productivities ranged from 0.12 to 0.4 g L^{-1} h^{-1}. At an equal ethanol yield of 70%, a productivity of 0.24 g L^{-1} h^{-1} was achieved in the three-stage continuous system compared to 0.18 g L^{-1} h^{-1} in the batch system. The maximum productivity was achieved with a single stage fermentor with a residence time of 8 h. At lower but also at higher dilution rates, the productivity dropped, although in the latter washout of the cells did not occur. It is interesting to note that at a total residence time of 8 h that could be reached in a single stage system with a residence time of 8 h or in a two stage system with residence times of 4 h per fermentor, the productivity was higher in the single stage system. The same was true when comparing productivity from a single fermentor with a 12 h residence time to results from a three stage system with an equal total residence time. On the other hand, at a total residence time of 24 h where the performance of single or multistage fermentors can be compared from the available data, progressively increasing the number of fermentors from 1 to 2 and then to 3 vessels increased the overall cSSF productivity from 0.25 to 0.27 to 0.28 g L^{-1} h^{-1} corresponding to improvements by 10.5% or 12%, respectively.

It is often assumed that performing a reaction in a cascade of reactors rather than a single continuous stirred tank reactor (CSTR) will lead to a higher product concentrations or higher volumetric productivities, but this is not necessarily the case as outlined by de Gooijer et al. (1996). Unfortunately, there are only few publications available investigating this important point of process design for enzymatic hydrolysis mediated reactions. Jin et al. (2013) reported the continuous saccharification and co-fermentation (SScF) of AFEX pretreated corn stover in a 5 stage system, where the first fermentor was for prehydrolysis at 50 °C and fermentors 2–5 for SScF at 30 °C. The influence of the number of fermentors in series was not discussed by the authors but can be estimated from the reported data. Total residence times of 34 and 44 h could

be reached with three or two fermentors in series, respectively. For a residence time of 34 h, the cumulative ethanol productivity was 0.65 g L^{-1} h^{-1} for 2 fermentors in series but increased to 0.725 g L^{-1} h^{-1} for 3 fermentors in the train. On the other hand, for a residence time of 44 h, increasing the number of fermentors from 2 to 3 reduced the productivity slightly from 0.675 to 0.65 g L^{-1} h^{-1}. Simulations by González Quiroga et al. (2010) of enzymatic hydrolysis in a train of ten equally sized CSTRs with residence times of 10, 30, or 50 h in each fermentor pointed in the same direction as our results. As approximately estimated from results presented in Fig. 3 of that publication, a three stage cascade of CSTRs with a total residence time of 30 h would result in about a 15% higher cellulose conversion than a single stage CSTR with the same total residence time (0.75 instead of 0.65). However, these findings were not validated experimentally.

4. Conclusions

A virtually continuous bubbling feed system reliably supplied small amounts of Avicel cellulose or pretreated cellulosic biomass to a 3 stage continuous SSF process and allowed detailed small scale process investigations. Continuous SSF of dilute acid pretreated corn stover showed greater productivity in the three-stage continuous system (0.24 g L^{-1} h^{-1}) compared to batch (0.18 g L^{-1} h^{-1}) at identical ethanol yields. The productivity peaked at a residence time of 8 h and was at a combined total residence time of 24 h higher in the multistage system compared to single stage. Future research will focus on higher solids cSSF to increase ethanol concentrations.

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