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Analysis of Conversion of Particulate Biomass to Ethanol in Continuous Solids Retaining and Cascade Bioreactors

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ABSTRACT

Design considerations for continuous conversion of biomass to ethanol using the simultaneous saccharification and fermentation (SSF) process are discussed. A previously presented and verified modeling approach is extended to describe advanced solid-substrate reactor systems incorporating solids and enzyme retention, and reaction in CSTR cascades. A single solids retaining CSTR has predicted advantages over a batch process at low-residence time and conversion. The predicted performance of a cascade of CSTR reactors for SSF approaches that of the batch system, whereas a cascade system with differential retention of solids with respect to the aqueous phase (solids residence time = 1.5 times the liquid residence time) is predicted to allow a 47% decrease in overall reactor volume relative to a batch system at high conversion (90% cellulose utilization). Further benefits are anticipated because of the effects of substrate classification in nonwell-mixed reactor configurations. Apparatus for laboratory-scale experimentation using solid substrates is presented, along with progress toward experimental verification of the reactor concepts proposed. In addition to predicting bioreactor productivity, the modeling was used to examine ethanol tolerance. In contrast to the approximately linear inhibition trend observed for soluble substrates, the ratio of dilution rates necessary to achieve a fixed conversion in the absence and presence of inhibition is essentially unity until the maximum growth rate is approached, and falls precipitously thereafter. Implications of this and other simulation results for the bioreactor design are discussed.

Index Entries: Biomass; bioreactors; ethanol; reactor design; population modeling.

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NOMENCLATURE

Symbol	Description	Units
B	β-glucosidase concentration in solution	U/L
c	Conversion-independent component in rate function	h^{-1}
C	Cellobiose concentration	g/L
DRT	Relative differential residence time of particles	Dimensionless
Et	Ethanol concentration	g/L
ES	Concentration of cellulose-cellulase complex	U/L
$E(t, \tau)$	Residence time distribution in CSTR	h^{-1}
G	Glucose concentration	g/L
k	Hydrolysis rate constant	h^{-1}
k_C	Rate constant for hydrolysis of cellobiose to glucose	g/(U·h)
k_G	Monod constant	g/L
$k_{C/G}$	Inhibition of cellobiose hydrolysis by glucose	g/L
$k_{S/C}$	Inhibition of cellulose hydrolysis by cellobiose	g/L
$k_{S/E}$	Inhibition of cellulase hydrolysis by ethanol	g/L
$k_{X/E}$	Inhibition of cell growth by ethanol	g/L
P	Particle concentration in reactor	Particles/L
Q	Volumetric flow rate	L/h
n	Exponent of the declining substrate reactivity	Dimensionless
r_i	Rate of formation of component i	g/(L·h)
S	Cellulose component of the biomass substrate remaining	g/L
t	Time	h
V_r	Reactor volume	L
X	Fractional reactor cellulose conversion	Dimensionless
X_c	Cell concentration	g/L
X_i	Cellulose conversion in reactor i	Dimensionless
X_p	Cellulose conversion of given particle within a population	Dimensionless
$Y_{X/G}$	Cell yield per substrate consumed	Dimensionless
$Y_{Et/G}$	Product yield per substrate consumed	Dimensionless
C_S	Specific capacity of cellulose component for cellulase	U/g
μ_{max}	Maximum cell growth	h^{-1}
τ	Average CSTR liquid hydraulic residence time	h
τ_p	Average CSTR particle residence time	h

INTRODUCTION

If ethanol produced from cellulosic biomass (biomass ethanol) is to become a cost-effective substitute for gasoline on a large scale (*1–3*), its cost of production will need to be reduced substantially. Studies by Wright

et al. (3) and Chem Systems (4) have identified the biotransformation step as a major contributor to the overall cost of prodution in state-of-the-art process designs. Cellulose is the primary carbohydrate fraction of lignocellulosic materials, and cellulose hydrolysis is rate-limiting in most processing scenarios for the conversion of biomass to ethanol (5,6). Simultaneous saccharification and fermentation (SSF) involves hydrolysis of cellulose and fermentation of the resultant sugars in a single bioreactor, and has been shown to have productivity and cost advantages over previous processes where these process steps were carried out independently (7). Efforts to increase the rate of SSF have been undertaken almost exclusively in batch reactors. Some of the issues addressed to increase productivity include organism selection (7,8), level of cellulase addition (7), reaction temperature profiling (9), use of supplemental enzymes (10), and differing substrate pretreatments (11–13).

Relative to batch processing, continuous systems have advantages including high cell concentrations, amenability to advanced configurations (e.g., substrate retention or product removal), and increased on-line time. Consistent with these factors, continuous reactors are the dominant mode of production in the corn ethanol industry (14). However, few experimental studies of reactors for continuous conversion of cellulosic biomass of ethanol have been reported, and an analytical framework for such reactors has yet to be established.

South et al. (15) have proposed a kinetic model for biomass hydrolysis and applied it to the SSF process. This model involves a new rate equation for hydrolysis that has the specific hydrolysis rate declining markedly with conversion, as experimentally observed by Nutor and Converse (16) and others (17–19), with the decline in specific hydrolysis rate modeled as $[(r / (ES / C_s))] = k \times (1 - X)^n + c$. In addition, the model included equations describing SSF from the literature and consideration of the residence time of particles in the reactor. When the three parameters of the rate equation were fit to batch data, the model predicted results very well for a variety of enzyme loadings. With no change in parameters, the model also closely fit the experimental data from a CSTR, provided that the reactivity of individual particle elements was allowed to vary over the time the particle spent in the reactor.

Continuous processing of particulate biomass has unique possibilities in terms of reactor design (20). Density differences between the substrate particles and the aqueous milieu allow the particles to be differentially retained relative to the liquid. In addition, reduction in particle size as substrate reacts may give additional benefits because of preferential retention of larger particles of higher relative cellulose content. In this article, we extend the SSF model of South et al. to provide analytical descriptions of high-productivity continuous bioreactor configurations, and report preliminary experimental data relevant to the predicted SSF productivity enhancements.

MATERIALS AND METHODS

Organisms and Enzymes

The yeast *Saccharomyces cerevisiae* (strain D_5A supplied by the National Renewable Energy Laboratory) was used for all SSF fermentations, with culture handling as previously described (*21*). Genencor CL cellulase (Genencor International, San Francisco), and Novozyme 188 β-glucoside (NOVO Laboratories, Wilton, CT) were used. Cellulase activity is reported as filter-paper activity units based on the IUPAC standard procedure (*22*), β-glucosidase activity was measured using the nitrophenyl-β-glucosidase (PnPGU) assay; both these activities were determined at 50°C. The activities of the cellulase and β-glucosidase solutions used in these studies were 89.7 and 250 U/mL, respectively. Cellulase activities are reported in the absence of any additional β-glucosidase.

Substrate

The lignocellulosic feedstock used in the experimental work was prepared by the National Renewable Energy Laboratory (NREL). Poplar chips were milled and screened (2 mm > chip size > 0.25 mm), heated to 160°C for 10 min in the presence of 0.73% sulfuric acid, drained, washed, and filtered prior to use. Using quantitative saccharification, the substrate was found to be approx 56 wt% cellulose. Washed and filtered wood was stored without further neutralization at 4°C until use, up to a maximum of 28 d. Wood slurries were sterilized by autoclaving for 12 h, other medium components were sterilized separately and aseptically added after the wood slurry had cooled, to give the desired final substrate concentration.

In addition to pretreated wood at approx 50 g cellulose/L, SSF medium included yeast extract (10 g/L) and peptone (20 g/L). Some reactions were run in the presence of penicillin (10,000 U/L) and streptomycin (10 mg/L). Microscopic inspection indicated that reactors and feed carboys had no biological contamination independent of antibiotic use. The model parameters used in the simulations were determined from previous data (*21*) using hardwood flour (60 mesh) pretreated for 10 s at 220°C in the presence of 1% H_2SO_4.

Experimental Conditions and Apparatus

All experiments were carried out at 37°C with pH regulated at 4.5. Temperature was maintained using water-jacketed reactors. Control of pH was by an ADI 1020 process controller (Applikon Dependable Instruments, Sheidam, Holland).

Particulate substrate was delivered to the SSF reactors intermittently from a mechanically agitated 40-L stainless-steel feed carboy by a fixed-volume sampler (Bristol Equipment Company, Yorkville, IL). To avoid ex-

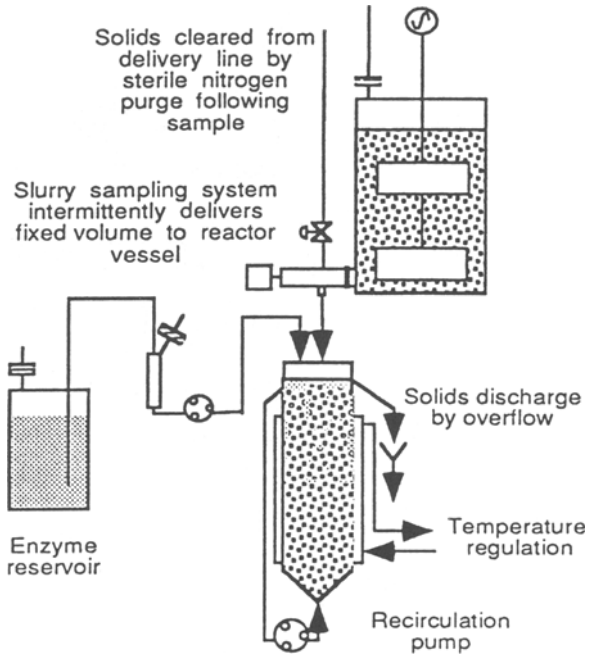

Fig. 1. Diagram of the substrate delivery system, in conjunction with the upflow reactor configuration used for experimentation.

cessive substrate attrition, the feed carboy stirrer was only run during the period the sampler was active. Blockage in the substrate delivery line was avoided by blowing the line clear using filter-sterilized nitrogen after each sample aliquot.

Custom glass SSF upflow reactors (NDS Technology Inc., Vineland, NJ) having a working vol of 1.6 L were used for the experiments in non-well-mixed configurations. These reactors had an aspect ratio of approx 3, and were run in an upflow mode with liquid being withdrawn from near the top of the reactor and recycled to the base of the reactor. Overflow was by gravity. Enzyme was filter-sterilized into a sterile 4-L carboy and delivered to the reactor by a peristaltic feed pump. To achieve the desired feed flow rates, it was necessary to run the enzyme pump intermittently, delivering enzyme immediately following the delivery of substrate. Control of the intermittent function in the reactor system was accomplished by the use of an Allen Bradley SLC-500 programmable logic controller (Oakes Electrical, Holyoak, MA). Feed was autoclaved in 20-L carboys and transferred into the feed carboy using a system previously reported (*21*). A schematic of the feed delivery system, along with the upflow reactor configuration, is shown in Fig. 1.

Batch SSF experiments were carried out in 250-mL Erlenmeyer flasks at 37°C. A 1% inoculum was grown up on a 20 g/L dextrose YPD medium for 36 h prior to inoculation. Aseptic liquid samples were taken by pipet. Stirring was by magnetically driven stir bar at approx 200 rpm.

Reactor Modeling

The model of South et al. includes partitioning of cellulase between solution and available lignin and cellulose sites by use of the Langmuir adsorption parameters of Ooshima et al. (23), a lumped description of cellulase components producing only cellobiose, with β-glucosidase in solution catalyzing conversion of cellobiose to glucose. Since the rate of hydrolysis per adsorbed enzyme is a strong function of substrate conversion, we use the rate equation presented previously (15):

$$[(r / (ES / C_s))] = k \times (1 - X)^n + c \quad (1)$$

which is consistent with the nature of reactivity decline observed by Nutor et al. Rates of cellulose hydrolysis, cellobioase hydrolysis, glucose consumption, cell growth, and ethanol production are presented in Eq. (2-6). Cellobiose and ethanol are assumed to be noncompetitive inhibitors of cellulose hydrolysis (24,25); ethanol inhibition of *S. cerevisiae* is according to the model of Ghose et al. (26), using ethanol tolerance of van Uden (27). Glucose is assumed be a competitive inhibitor of cellobiose hydrolysis by β-glucosidase (25).

$$r_S = -[k \times (1 - X)^n + c] \times (ES / C_S) \times \{[k_{S/C} / (C + k_{S/C})]\} \times \{[k_{S/Et} / (Et + k_{S/Et})]\} \quad (2)$$

$$r_C = -1.056 \times r_S - [(k_C \times C \times B / \{K_m \times [1 + (G / k_{C/G}) + C\})] \quad (3)$$

$$r_G = (-1.056 \times r_S - r_C) \times 1.053 - (r_X / Y_{X/G}) \quad (4)$$

$$r_X = [(X_C \times \mu_{max} \times G) / G + k_G] \times [1 - (Et / k_{X/Et})] \quad (5)$$

$$r_{Et} = r_X \times (Y_{Et/G} / Y_{X/G}) \quad (6)$$

The constants used in the evaluation of these equations are shown in Table 1.

Model Solution

Solution to the batch model is by direct time stepping of a fourth-order Runge Kutta solution procedure. As described in more detail elsewhere (15), solution of continuous reactor system uses a residence time distribution-based method that considers the time history of substrate particles within a reactor of spatially homogeneous aqueous composition. The composition conversion of a continuous reactor using the framework of the model is given by (7):

$$X(t) = {}^{\infty}\int_{t=0} [X_p(t) \times E(t, \tau)]\, dt \quad (7)$$

where $X_p(t)$ is the conversion of a population of substrate particles spending between t and t + δt in the reactor and

$$E(t, \tau) = (1 / \tau) \times \exp[-(t / \tau)] \quad (8)$$

is the residence time distribution (RTD) of a well-mixed CSTR.

Table 1
Parameters Used in the SSF Model

Parameters		
Symbol	Value	Source
c	0.18125 h^{-1}	(*15*)
k	2.8625 h^{-1}	(*15*)
k_C	0.0210 g/(U·h)	(*31*)
k_G	0.05 g/L	(*32*)
K_S	1.49 L/U	(*23*)
K_I	0.66 L/U	(*23*)
K_m	10.56 g/L	(*25*)
$k_{C/G}$	0.62 g/L	(*31*)
$k_{S/C}$	5.85 g/L	(*25*)
$k_{S/Et}$	50.35 g/L	(*25*)
$k_{X/Et}$	50 g/L	(*27*)
n	5.30	(*15*)
C_s	98.3 U/g	(*23*)
μ_{max}	0.4 h^{-1}	(*32*)
$Y_{X/S}$	0.09	(*32*)
$Y_{Et/S}$	0.47	(*32*)

All model predictions are conducted using 55 g cellulose/L, consistent with the recent SSF process design considered by Chem Systems (*4*). Cellulase loading was 15 U/g cellulose; kinetic parameters were as determined for pretreated mixed hardwood.

Cascade CSTRs

Multiple-reactor schemes use the output from one reactor as the feed to the remainder of the cascade. To calculate the conversion in each of a series of reactors, it is necessary to evaluate the (r / (ES / C_s)) entering each reactor in the series. Recalculating (r / (ES / C_s)) at the inlet to each of the reactors in the cascade allows a single-solution subroutine to be used to calculate the additional conversion across each reactor, X_i. Figure 2 shows a schematic diagram of (r / (ES / C_s)) of an individual particle with respect to its conversion, X_p (all conversions are implicitly a function of reaction time), in the first reactor in a cascade, and the relationship between the variables $X_{p,1}$, $X_{p,1,i}$, and $X_{p,2,i}$. An element, i, of substrate leaves reactor 1 after t_i with conversion $X_{p,1,i}$. This particle enters reactor 2. The conversion, $X_{p,2,i}$, in reactor 2 with respect to $X_{p,1}$, and $X_{p,1,x}$, is calculated by:

$$X_{p,2,i} = [(X_{p,1} - X_{p,1,i}) / (1 - X_{p,1,i})] \quad (9)$$

Rearranging Eq. (9) for $X_{p,1}$ and substituting into (1) gives (r / ES) for the particle, of conversion $X_{p,1,i}$, in reactor 2 as a function of additional conversion, $X_{p,2,i}$ (*10*).

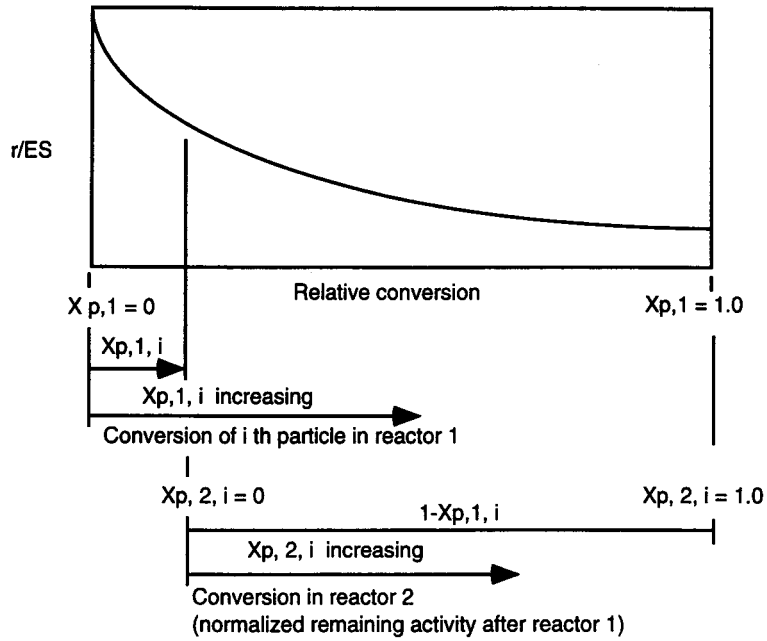

Fig. 2. Schematic diagram of (r / EC) of an individual particle with respect to conversion, X, exiting the first reactor in a CSTR cascade reactor.

$$[(r / (ES / C_s))]_{p,i} = k \times (1 - X_{p,1,i})^n \times (1 - X_{p,2,i})^n + c \quad (10)$$

Since there is a distribution of residence times in the first reactor, the average reactivity, with respect to additional conversion X_2, of a population of particles entering reactor 2 is given by:

$$[(r / (ES / C_s))]|_{X_2} = {}^{\infty}\Sigma_{i=0}(\{[k \times (1 - X_{p,1,i})^n \times (1 - X_{p,2,i})^n + c] \times E(t_i, \tau)\}) \times \Delta t = \{\Sigma^{\infty}{}_{i=0}[([k \times (1 - X_{p,1,i})^n + c] \times E(t_i, \tau)) \times \Delta t]\} \times [(1 - X_{p,2,i})^n + c] \quad (11)$$

This calculation is applied to the effluent leaving each reactor in a series to generate the average reactivity with respect to conversion for the material entering each subsequent reactor in the cascade.

As cells are introduced into all reactors other than the first in the series, cell concentration is calculated from a substrate mass balance incorporating Monod kinetics for growth (12).

$$G = \{ k_G / [\tau \times \mu_{max} \times X_{in}/(X_{out} - X_{in}) - 1]\} \quad (12)$$

Solids Retaining CSTRs

The behavior of a well-mixed stirred tank reactor that retains representative solids can be modeled by extending the analysis of the CSTR. Defining DRT as the differential residence time of the solid particles relative to the average slurry hydraulic residence time [(τ_p / τ)], the effect

of increasing the solids residence time in the reactor can be evaluated. A particle balance around the reactor is given in Eq. (13), which is rearranged to give Eq. (14) showing that the population of particles in the reactor is raised by a factor DRT.

$$\tau_p = (\#\text{ Particles in reactor} / \#\text{ Particles leaving/time}) \tag{13}$$

$$= (P_r / P_{p\,\text{out}}) \times (V_r / Q)$$

$$= (P_r / P_{p\,\text{out}}) \times \tau$$

$$(P_r / P_{p\,\text{out}}) = \text{DRT} \tag{14}$$

In the CSTR model, the RTD of the solid substrate was determined from the ideal RTD of a well-mixed CSTR for uniform solids retention (i.e., the RTD of the solid substrate is identical for all the solid components and states of conversion), the RTD of the particles within the reactor can be modeled by (15):

$$E(t, \tau) = [1 / (\text{DRT} \times \tau)] \times \exp\{[-t / (\text{DRT} \times \tau)]\} \tag{15}$$

Analysis of a cascade of CSTRs with differential solids retention (DSR) is a straightforward extension of a cascade of CSTRs without DSR, with Eq. (15) being used in lieu of Eq. (8).

RESULTS AND DISCUSSION

Ethanol Inhibition Simulations

With a verified model in hand, we are in a position to examine general features of reactor design for particulate substrates as well as to predict performance of advanced configurations. One such feature is the effect of ethanol on the overall rate of biomass hydrolysis.

As ethanol concentration increases, both the cellulase and the fermentative organism experience increasing inhibition. Of particular interest is comparison of ethanol inhibition for a process involving cellulosic biomass to that for processes utilizing soluble substrates. Figure 3 shows simulated ethanol inhibition in a single CSTR with respect to growth of S. cerevisiae, in the absence of hydrolysis limitations, hydrolysis mediated by cellulase in the absence of sugar accumulation, and the SSF system as predicted by the model. Figure 3A is in terms of relative inhibition, and 3B in terms of absolute rates. The stair-like pattern observed for the cellulosic substrate is in marked contrast to the linear pattern for soluble substrates. Thus, the degree to which ethanol retards substrate conversion in general is less for the cellulosic biomass, especially when it is considered that ethanol concentrations in excess of 50 g/L are notoriously hard to achieve with cellulosic materials (*28*). It may be seen that the

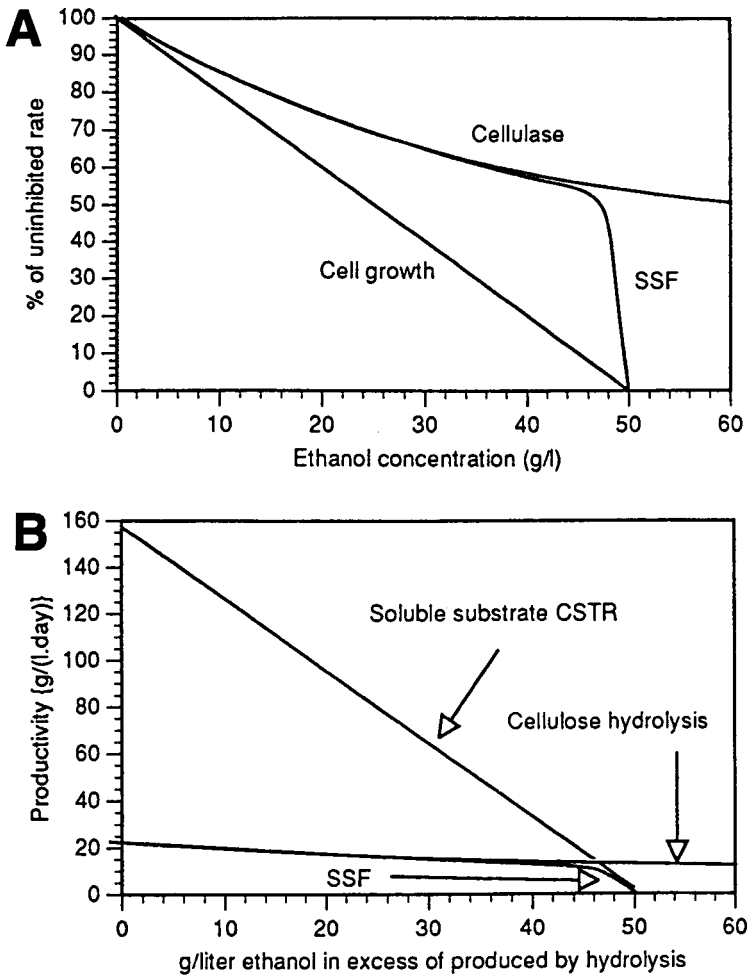

Fig. 3. Inhibition owing to the addition of ethanon on; *S. cerevisiae*, cellulase, and the SSF system as predicted by the proposed model. **A.** Relative inhibition of maximum rate. **B.** Predicted maximum rates at given ethanol concentrations. Rate calculations based on 80% conversion of available substrate.

relative ethanol tolerance of the SSF system is dominated by the tolerance of the cellulase, which is greater than that of the fermenting organism. Because of the maximum rate of the yeast system, when uncoupled from hydrolysis, is so far in excess of the hydrolysis rate, a great deal of inhibition can be experienced prior to the accumulation of hydrolytic end products significant enough to affect the rate of hydrolysis in continuous reactors. This result is also significant in the consideration of other inhibitors that may be present, such as pretreatment byproducts (*29*). The affect of such inhibitors is likely to be additive, but not significant until the overall level of cell inhibition reaches approx 90% of maximum growth rate, when significant soluble substrate buildup occurs.

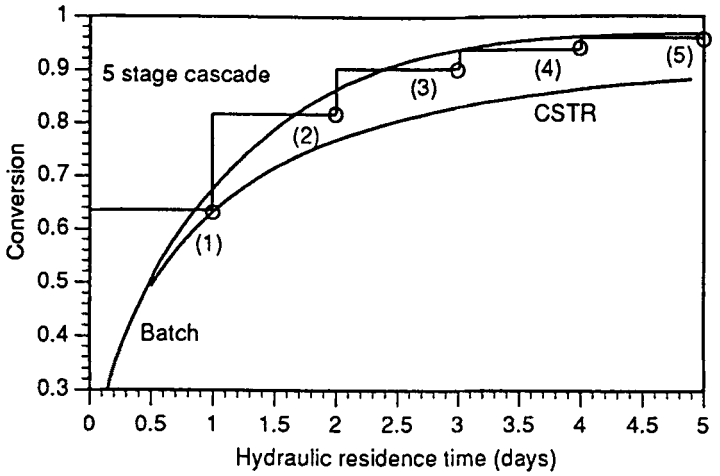

Fig. 4. Comparison of model predictions for SSF in batch, CSTR, and in a cascade of five equal-sized CSTRs.

Cascade CSTR Simulations

The productivity disadvantages of operating a single CSTR become most apparent at high conversions when the number of cellulose binding sites are low by comparison to the available lignin sites that can adsorb enzyme. Since enzyme bound to lignin is not available for hydrolysis, the overall rate of hydrolysis is affected. The effects of lignin interactions are reduced when there are small conversion changes across the reactor, such as in a CSTR cascade system. In a recent study of large-scale ethanol production using SSF, Chem Systems (*4*) considered a reactor cascade of 27 stirred tanks with seed reactors providing continuous cell addition.

Model predictions are shown in Fig. 4 for SSF in a five-CSTR cascade along with that predicted for batch SSFs. The use of a CSTR cascade is thus seen to reduce the disadvantage of continuous reactors relative to the batch process in terms of conversion of substrate, and may have substantial benefits when process availability factors and capital and operating costs associated with seed fermentors are taken into consideration. In general, SSF reactions require additional β-glucosidase to maximize rate (*10,28,30*). In the later stages of a batch reaction, when the rate of cellobiose production is low, β-glucosidase is present in excess. Since a CSTR has a nonuniform RTD (Eq. [8]), some of the low-conversion/high-reactivity substrate will leave the initial reactor in the cascade without reaction. This reactivity remains available for utilization in the later stages. Thus, use of a CSTR or CSTR cascade has the effect of lowering the burst of cellobiose production seen in the early stages of hydrolysis and thereby reducing the β-glucosidase required to prevent cellulase inhibition by cellobiose.

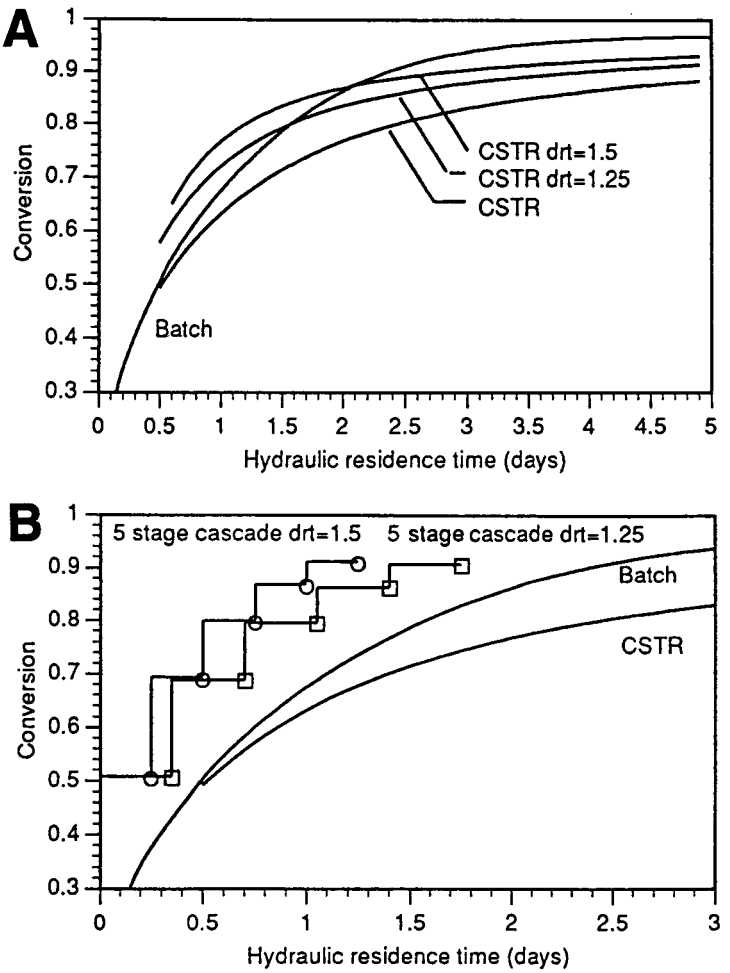

Fig. 5. Comparison between SSF in batch, and solids retaining reactors. Simulation with DRT = 1.25 and 1.5 in: **A.** Single CSTR and **B.** Cascade of five equal-sized CSTRs with 90% final conversion.

Solids Retaining Reactor Simulations

Experiments in the authors' lab have shown that partially converted (37% conversion) batch SSFs at 50 g cellulose/L substrate concentration can be settled to 14 wt% in 15 min (data not shown). These experiments indiate that for this particular feedstock, it is likely to be feasible to retain solids in a continuous bioreactor by lowering the mixing intensity such that settling is allowed to occur.

Figure 5 shows the effect of solids retention in continuous reactors. Figure 5A considers conversion in a single CSTR at two different DRTs, whereas Fig. 5B shows the same DRTs used in a CSTR cascade (with all CSTRs having equal volume) with final conversion of 90%. Available data from batch settling trials suggest that it is reasonable to expect solids retentions within the range modeled.

Solids retention increases particle concentration in the reactor and, consequently, the inventory of cellulase binding sites within the reactor, resulting in increased adsorption of cellulase from solution. Calculation of the cellulase partition followed by solution of the simultaneous differential equations describing hydrolysis yields curves of conversion as a function of differential solids residence time. The effect of solids retention is most advantageous in the low-conversion region, since at high conversion, the retained solids contain primarily lignin, which competitively binds the available cellulase, resulting in the poor performance of a single CSTR at high conversion.

Although the solids in this analysis have been modeled assuming uniform solids retention, this is unlikely to be the case. The composition of the settled solids in batch settling trials show that the material that settles most readily is that which contains the majority of the cellulose; for the batch trial at 37% conversion, the solids composition ranged from 52% cellulose at the base of the settling column to 4% in the top 10% of the column. By preferentially retaining cellulose-rich material, productivity advantages over those already predicted can reasonably be expected. Although this effect is difficult to predict in the absence of experimental data, we anticipate the magnitude may be quite large. If this is the case, then the simulated performance of the various continuous configurations herein would be conservative indications of the true potential.

Bioreactor Productivity

The productivity advantage of cascade CSTRs and solids retaining reactors can be seen from Figs. 4 and 5. To achieve 90% substrate conversion in a solids retaining (DRT = 1.5) CSTR cascade arrangement, a 47% decrease in overall reactor volume is predicted relative to a batch system.

Experimental Results

Using the upflow reactor described in the Materials and Methods section, experiments were conducted with NREL substrate to compare the conversion in this reactor configuration to that in batch. Results from initial experiments in this reactor configuration at short residence time (τ = 0.32 d) have shown substrate cellulose conversion in the upflow reactor to be 80% higher than that of the batch reaction at the same reaction time (14.0 vs 7.8%). Although the continuous reactor ran stably over the course of the experiments, significant difficulty was experienced when pumping the material recycled to the base of the reactor. Liquid distribution in the reactor was not uniform, with large channels forming and collapsing intermittently. This mode of operation is not expected to be optimal because of the presence of large dead zones, nonuniform liquid distribution, and high recycle flow negating the possible benefits of cellulose classification. However, the preliminary results are encouraging in that they support the predicted higher productivity of solids retaining continuous configurations.

CONCLUSION

A use of a verified reactor model to predict the performance of advanced reactor systems generates insights into both the importance of inhibition of the biocatalyst and benefits of several reactor configurations. The framework for analysis of continuous SSF systems presented here is applicable in any heterogeneous particulate reaction system.

Based on simulation results, soluble substrate fermentation is not rate-limiting for all but the highest tolerable ethanol concentrations. Hence, the ethanol tolerance of cellulose conversion more nearly resembles that of cellulase than of the fermenting organism for most ethanol concentrations. In general, this is expected to make ethanol tolerance a less significant reactor design issue for cellulose conversion than for conversion of soluble substrates.

When solids retention is included, significant advantage relative to batch is predicted for continuous SSF in multiple-CSTR cascades. Classification within solids retaining reactors resulting in increased cellulose retention is expected to give additional productivity benefits not analyzed here. Both modeling and experimentation show that there is no process or rate requirement for the presence of seed reactors in a continuous SSF process. Physical properties associated with the pretreated substrate that may enhance differential retention of the cellulosic portion of the substrate in continuous reactors are likely to be an important aspect in the evaluation of different pretreatment regimes.

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