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Kinetic Modeling of Cellulosic Biomass to Ethanol Via Simultaneous Saccharification and Fermentation: Part I. Accommodation of Intermittent Feeding and Analysis of Staged Reactors

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ABSTRACT: The model of South et al. [South et al. (1995) Enzyme Microb Technol 17(9): 797–803] for simultaneous saccharification of fermentation of cellulosic biomass is extended and modified to accommodate intermittent feeding of substrate and enzyme, cascade reactor configurations, and to be more computationally efficient. A dynamic enzyme adsorption model is found to be much more computationally efficient than the equilibrium model used previously, thus increasing the feasibility of incorporating the kinetic model in a computational fluid dynamic framework in the future. For continuous or discretely fed reactors, it is necessary to use particle conversion in conversion-dependent hydrolysis rate laws rather than reactor conversion. Whereas reactor conversion decreases due to both reaction and exit of particles from the reactor, particle conversion decreases due to reaction only. Using the modified models, it is predicted that cellulose conversion increases with decreasing feeding frequency (feedings per residence time, f). A computationally efficient strategy for modeling cascade reactors involving a modified rate constant is shown to give equivalent results relative to an exhaustive approach considering the distribution of particles in each successive fermenter.

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KEYWORDS: cellulose; ethanol; kinetics; reactor design

Introduction

Plant biomass is the only foreseeable sustainable source of organic fuels, chemicals and materials available to humanity (Lynd et al., 1999). Cellulosic biomass, including agricultural and forestry residues and dedicated crops, is particularly attractive in this context because it is widely available at low cost and has favorable attributes in environmental and life-cycle contexts (Lynd and Wang, 2004; Wyman, 2003). Because of these features, analysis and advancement of industrial processes based on cellulosic biomass has been a focus of considerable effort.

Enzymatic hydrolysis of cellulose is exceedingly complex with many enzymatic and substrate properties, as well as the interactions among these properties, impacting reaction rate (Zhang and Lynd, 2004). The full extent of this complexity is not represented in any quantitative model proposed to date. Among such models, some take a relatively comprehensive approach, seeking to incorporate as much information as possible in order to structure and test understanding. Other models, usually intended for design purposes, take a more minimalist approach in which only those phenomena and parameters needed to describe observed behavior are included. The model of South et al. (1995), which considers simultaneous saccharification and fermentation (SSF) of dilute acid pretreated poplar, is an example in the latter category. In particular, South et al. concluded that three features must be included in any broadly applicable model of enzymatic hydrolysis of cellulose: (1) An adsorption model that allows for either enzyme or substrate to be in relative excess; (2) Declining reactivity of enzyme-cellulose complexes with increasing cellulose coversion; (3) For non-batch reactors, a particle population model that accounts

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for variation in the reaction rate of particles of different ages.

In this study, the model of South et al. is modified and extended to accommodate discrete substrate feeding. Discrete feeding is potentially advantageous compared to continuous feeding because it: (1) lowers required enzyme loading to achieve a given substrate conversion, as shown subsequently; (2) allows the number of particle populations tracked to be significantly reduced, which is advantageous for scale-up analysis in a computational (CFD) framework. After modification to accommodate discrete feeding in a single reactor, the model is extended to cascade reactors.

Accommodation of Discrete Feeding

For the case of discrete feeding, each feed addition event gives rise to a new particle population. Considerations relevant to modifying the model of South et al. to accommodate such discrete feeding are addressed below.

Dynamic Enzyme Adsorption

A dynamic adsorption model is used here to calculate the concentration of cellulose–enzyme complex without iterative calculations, which are impractical for incorporation with CFD analysis and are required for equilibrium enzyme adsorption as assumed by South et al. (1995). For substrate population i, defined by a given discrete feeding event, the rate of enzyme adsorption to cellulose and lignin can be expressed as

$$r_{CE}(i) = k_{fc}[E_f](1+\sigma_C)[C_f(i)] - \frac{k_{fc}}{K_C}[CE(i)] \quad (1)$$

$$r_{LE} = k_{fl}[E_f](1+\sigma_L)[L_f] - \frac{k_{fl}}{K_L}[LE] \quad (2)$$

Conservation equations for cellulose, lignin and enzyme are:

$$[C_f(i)] = [C(i)] - \frac{[CE(i)]}{1+\sigma_C} \quad (3)$$

$$[L_f] = [L] - \frac{[LE]}{1+\sigma_L} \quad (4)$$

$$[E_f] = [E] - \frac{\sigma_C}{1+\sigma_C}\sum_{i=0}^{n}[CE(i)] - \frac{\sigma_L}{1+\sigma_L}[LE] \quad (5)$$

Equations (1)–(5) together comprise a dynamic model for enzyme adsorption. Variable definitions may be found in the list of symbols at the end of the text, and are similar to those used by South et al. (1995).

We use values for K_C, K_L, σ_C, and σ_L as reported by Ooshima et al. (1990), also used by South et al. (1995). This leaves the two adsorption rate constants, k_{fc} and k_{fl}, to be determined. It has been reported in many studies (Boussaid and Saddler, 1999; Chernoglazov et al., 1988; Kim et al., 1998; Lee and Woodward, 1989; Ooshima et al., 1983; Singh et al., 1991) that adsorbed enzyme reaches a constant value in $\leq$30 min. Here we assume that CE and LE complexes reach 95% of their equilibrium concentrations after 30 min, and we determine the values for adsorption rate constants using a nonlinear least square fit in Matlab. Values for adsorption parameters are presented in Table I along with other parameters used in this study.

Particle Conversion Versus Reactor Conversion

A central feature of the South et al. (1995) model, also supported by the data of Nutor and Converse (1991) as well as general experience with enzymatic cellulose hydrolysis (Lynd et al., 2002), is a pronounced decline in the specific reaction rate of CE with increasing conversion. As a result of this property, it is necessary to track the extent of conversion and reactivity of individual particle populations. In calculating the rate constant as a function of conversion, it is appropriate to employ particle conversion ($x_p(i)$, Eq. 6) instead of reactor conversion (x, Eq. 7) in the conversion-dependent reaction constant since particle conversion represents the conversion of the particle population independent of loss of particles in the reactor effluent whereas the value of the reactor conversion changes due to particle outflow as well as reaction

$$x_p(i) = \frac{([C(i)]_0/N(i)_0) - [C(i)]/N(i)}{[C(i)]_0/N(i)_0} = \frac{[C(i)]_0 - (1/R(i)) \times [C(i)]}{[C(i)]_0} \quad (6)$$

$$x = \frac{[C]_0 - \sum_{i=1}^{n}[C(i)]}{[C]_0} \quad (7)$$

Table I. Parameter values and their source.

Symbol	Value	Source
σ_C	0.0806	Ooshima et al. (1990)
σ_L	0.0123	Ooshima et al. (1990)
μ_{max}	0.4 h^{-1}	Ghose and Tyagi (1979)
c	0.18125 h^{-1}	South et al. (1995)
e	5.3	South et al. (1995)
k	2.8625 h^{-1}	South et al. (1995)
K_C	1.82 L/g	Ooshima et al. (1990)
$K_{C/Cb}$	5.85 g/L	Phillippidis et al. (1992)
$K_{C/Eth}$	50.35 g/L	Phillippidis et al. (1992)
K_{Cb}	640 h^{-1}	Gusakov and Sinitsyn (1985)
$K_{Cb/G}$	0.62 g/L	Phillippidis et al. (1992)
k_{fc}	1.84 L/(g h)	This work
k_{fl}	0.836 L/(g h)	This work
K_G	0.05 g/L	Ghose and Tyagi (1979)
K_L	0.807 L/g	Ooshima et al. (1990)
K_m	10.56 g/L	Phillippidis et al. (1992)
$K_{X/Eth}$	50.0 g/L	van Uden (1983)
$Y_{Eth/G}$	0.47	Ghose and Tyagi (1979)
$Y_{X/G}$	0.09	Ghose and Tyagi (1979)

Discrete Reaction Rate Equations

Rate equations in the model, following the form proposed by South et al. (1995) but adapted for discrete feeding are:

$$r_C(i) = -\left[k \times (1 - x_p(i))^e + c\right] \times \frac{[CE(i)]}{1 + \sigma_C} \times \frac{K_{C/Cb}}{[Cb] + K_{C/Cb}} \times \frac{K_{C/Eth}}{[Eth] + K_{C/Eth}} \quad (8)$$

$$r_{Cb} = -1.056 \times \sum_{i=1}^{n} r_C(i) - \frac{K_{Cb} \times [Cb] \times [B]}{K_m \times (1 + ([G]/K_{Cb/G})) + [Cb]} \quad (9)$$

$$r_{Xc} = \frac{[Xc] \times \mu_{max} \times [G]}{[G] + K_G} \times \left(1 - \frac{[Eth]}{K_{X/Eth}}\right) \quad (10)$$

$$r_G = \left[-1.056 \times \sum_{i=1}^{n} r_C(i) - r_{Cb}\right] \times 1.053 - \frac{r_{Xc}}{Y_{X/G}} \quad (11)$$

$$r_{Eth} = r_{Xc} \times \frac{Y_{Eth/G}}{Y_{X/G}} \quad (12)$$

Equation (8) is the rate of cellulose hydrolysis for an individual substrate particle population with particle conversion $x_p(i)$. Equations (9)–(12) represent rates of formation of cellobiose, yeast cells, glucose and ethanol, respectively.

Discrete Material Balance

For a species y fed to the reactor (y = cellulose, lignin, or enzyme), the dynamic material balance may be written as

$$\begin{cases} \frac{d[y]}{dt} = r_y & \text{at all other time} \\ [y] = \frac{I(t)}{f} \times [y] + \frac{O(t) \times (f-1)}{f} \times [y] & \text{at time of feeding/removal} \end{cases} \quad (13)$$

$$I(t) = \begin{cases} 1 & \text{at time of feeding} \\ 0 & \text{at all other time} \end{cases},$$

$$O(t) = \begin{cases} 1 & \text{at time of removal of reactor contents} \\ 0 & \text{at all other time} \end{cases}$$

For a species z not fed to the reactor (z = CE(i), LE, Cb, Xc, G, or *E*th), the dynamic material balance is

$$\begin{cases} \frac{d[z]}{dt} = r_z & \text{at all other time} \\ [z] = \frac{O(t) \times (f-1)}{f} \times [z] & \text{at time of removal} \end{cases} \quad (14)$$

Analysis of Staged Reactors

Exhaustive Method

For staged reactors, each discrete transfer of material from stage m to stage $m+1$ involves multiple particle populations. The exhaustive approach to analyze this situation tracks each particle population entering each reactor. The solution algorithm by the exhaustive method for an mth stage reactor ($m > 1$) is the same as that for a 1st stage reactor except that the overall fraction of each particle population remaining in each of the subsequent reactors needs to be calculated. Consider a specific particle population i, its particle conversion in reactor m (represented by superscript I) is

$$_p(i)^I = \frac{\frac{[C(i)]_0^I}{N(i)_0^I} - \frac{[C(i)]^I}{N(i)^I}}{\frac{[C(i)]_0^I}{N(i)_0^I}} = \frac{[C(i)]_0^I - \frac{1}{R(i)^I} \times [C(i)]^I}{[C(i)]_0^I} \quad (15)$$

where $R(i)^I = N(i)^I / N(i)_0^I$. And the particle conversion of population i in reactor $m+1$ (represented by superscript II) is

$$x_p(i)^{II} = \frac{\frac{[C(i)]_0^I}{N(i)_0^I} - \frac{[C(i)]^{II}}{N(i)^{II}}}{\frac{[C(i)]_0^I}{N(i)_0^I}} = \frac{[C(i)]_0^I - \frac{1}{R(i)^{II}} \times [C(i)]^{II}}{[C(i)]_0^I} \quad (16)$$

where $R(i)^{II} = N(i)^{II} / N(i)_0^I$. To use the same algorithm of calculating remaining fraction of particle population i in reactor one, we define a nominal remaining fraction of particle population i in reactor $m+1$, R calculated by Equation (17), where $N(i)_0^{II}$ is the number of particles of population i fed to reactor $m+1$ calculated by Equation (18)

$$R = \frac{N(i)^{II}}{N(i)_0^{II}} \quad (17)$$

$$N(i)_0^{II} = \frac{1}{f} \times N(i)^I \quad (18)$$

By substitution, we can get the overall fraction of particles remaining in reactor $m+1$.

$$R(i)^{II} = \frac{N(i)^{II}}{N(i)_0^I} \times \frac{\frac{1}{f} \times N(i)^I}{N(i)_0^{II}} = \frac{N(i)^I}{N(i)_0^I} \times \frac{N(i)^{II}}{N(i)_0^{II}} \times \frac{1}{f} = R(i)^I \times R \times \frac{1}{f} \quad (19)$$

For the exhaustive method, the number of particle populations in reactor m is in the order of n^m, which

increases rapidly as the number of reactors increases (n is the number of particle populations in reactor one).

Average Reaction Constant Method

The large number of particle populations, and hence equations, involved in the exhaustive method makes it difficult or impossible to analyze scenarios with high feeding frequency and several successive staged reactors due to limitations in computing power. To overcome this limitation, we borrowed the approach of South and Lynd (1994) by considering all particle populations fed to reactor $m+1$ to be one population, and we calculate an average reaction constant based on the reactivity of the entering particle populations. For population i in reactor $m+1$, its incremental particle conversion achieved in the reactor, $x_{p,m+1}(i) - x_{p,m}(i)$, can be normalized as $\tilde{x}_{p,m+1}(i) = (x_{p,m+1}(i) - x_{p,m}(i))/(1 - x_{p,m}(i))$ which after manipulation gives

$$(1 - x_{p,m+1}(i)) = (1 - x_{p,m}(i))(1 - \tilde{x}_{p,m+1}(i)) \quad (20)$$

For n particle populations fed at the same time into reactor $m+1$, the overall rate of reaction for cellulose is

$$r_C = \sum_{i=1}^{n} [k \times (1 - x_{p,m+1}(i))^e + c] \times \frac{[CE(i)]}{1 + \sigma_C} \times S \quad (21)$$

where $S = (K_{C/Cb}/([Cb] + K_{C/Cb})) \times (K_{C/Eth}/([Eth] + K_{C/Eth}))$. Substituting Equation (20) into Equation (21) gives

$$r_C = \sum_{i=1}^{n} [k \times (1 - x_{p,m}(i))^e \times (1 - \tilde{x}_{p,m+1}(i))^e + c] \times \frac{[CE] \times w(i)}{1 + \sigma_C} \times S \quad (22)$$

where $w(i)$ is the fraction of concentration of cellulose enzyme complex of population i, and $\sum_{i=1}^{n} w(i) = 1$. For the particles fed into reactor $m+1$ at the same time, we assume they have the same normalized incremental particle conversion since they have the same age in the reactor

$$\tilde{x}_{p,m+1}(1) = \tilde{x}_{p,m+1}(2) = \cdots = \tilde{x}_{p,m+1}(n) = \tilde{x}_{p,m+1}(i) \quad (23)$$

Substituting Equation (23) into Equation (22) gives

$$r_C = \{k' \times (1 - \tilde{x}_{p,m+1})^e + c\} \times \frac{[CE]}{1 + \sigma_C} \times S \quad (24)$$

where $k' = \left[\sum_{i=1}^{n} k \times (1 - x_{p,m}(i))^e \times w(i)\right]$ is the average remaining hydrolysis rate constant for the particle populations fed at the same time into the subsequent reactor.

Results

Batch SSF of dilute acid-pretreated hardwood was simulated using equilibrium and dynamic enzyme adsorptions respectively. Figure 1 shows two conversion curves predicted using Berkeley Madonna software. The predictions over the time of reaction are almost identical.

Figure 2 presents steady-state substrate conversion data obtained in a CSTR with seven sets of reacting conditions as reported by South et al. (1995). Predicted conversions are also shown for the model of South et al. as well as the reformulated model reported here with $f = 100$ (which closely approximates a CSTR). It may be observed that the results of the two models are essentially the same, and both models agree well with the experimental results.

Table II shows a comparison of reactor conversion in the second and third reactors for different feeding frequencies. There are very small differences between the results using the exhaustive and the average reaction constant methods, which suggests that average reaction constant method can be employed to substantially reduce computational intensity from the order of n^m equations to the order of n equations.

Figure 3 shows steady-state conversion versus feeding frequency for one, two, and four equal volume reactors. Reactors in a series have the same volume. For each case, conversion decreases with increasing feeding frequency, which is less pronounced as the number of staged reactors becomes larger.

Discussion

In this article, the reactor model for biological hydrolysis of cellulosic biomass presented by South et al. (1995) is modified to radically decrease the number of calculations per time step (anticipating CFD analysis) and to

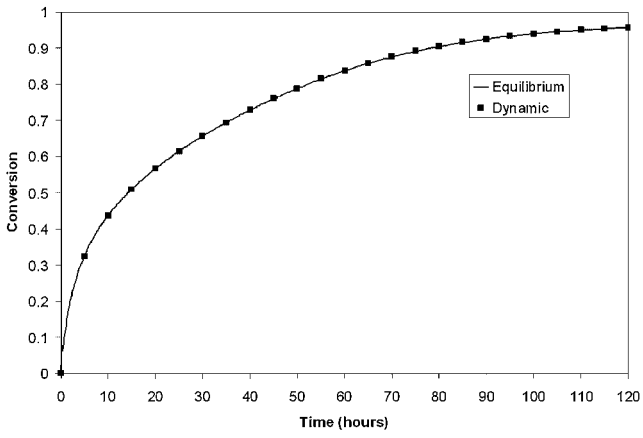

Figure 1. Comparison of batch SSF conversion using the equilibrium and the dynamic enzyme adsorption for dilute acid pretreated hardwood with a cellulose concentration of 55 g/L and an enzyme loading of 10 U/g cellulose.

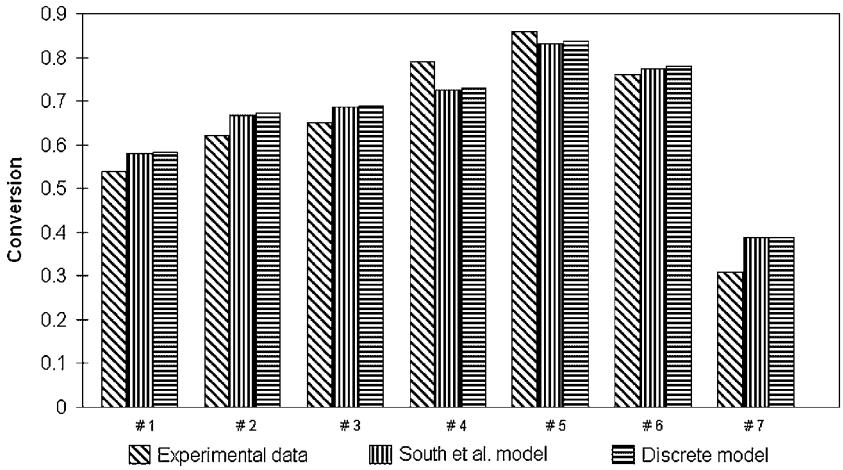

Figure 2. Comparison of experimental data and predictions from the South et al. model and the Discrete model; the experimental data are steady-state cellulose conversion reported by South et al. (1995) for dilute acid pretreated hardwood using *T. reesei* cellulase for hydrolysis and yeast stain D5A for fermentation in a media with 10 g/L yeast extract, 20 g/L peptone, 10 mg/L streptomycin, and 10,000 units/L penicillin.

accommodate discrete feeding and staged reactors. Dynamic enzyme adsorption is used instead of equilibrium adsorption, thereby avoiding iterative calculations. We apply an average reaction constant to discrete staged reactors and show that results are equivalent to an exhaustive approach in which each particle population is tracked. We note here that the correct conversion to use in the conversion dependent rate constant is the particle conversion, which is different from the reactor conversion.

South et al. (1995) calculated the mean cellulose conversion in a steady-state CSTR using the equation

$$x(\tau) = \int_0^\infty x(t) \times E(t,\tau)\,\mathrm{d}t \tag{25}$$

where $x(t)$ is the conversion of a particle population that has been in the reactor for a time t, and $E(t, \tau)$ is the residence time distribution assuming perfect mixing. Here, we calculate the mean cellulose conversion at the end of a reaction cycle initiated by a discrete feeding event by

$$\overline{x} = \sum_{i=0}^{m} x_{\mathrm{p}}(i) P(i)\,\Delta t \tag{26}$$

For perfect mixing, we have

$$P(i) = \frac{1}{f} \times \left(1 - \frac{1}{f}\right)^{(f\times t)/\tau} \times \frac{1}{\Delta t} \tag{27}$$

which reduces to $E(t, \tau)$ for infinite feeding frequency. However, the new model can accommodate imperfect mixing, for example, using experimentally or computationally determined flow fields.

As shown in Figure 3, conversion increases with decreasing f, which indicates that we can reduce the use of cellulase enzymes which are relatively expensive, but still achieve the same conversion operating at lower f as that operating at higher f. As f increases, the system more asymptotically approaches the fully continuous state. Particles begin to leave the reactor immediately after they are fed for a CSTR whereas all particles react for a minimum time of τ/f for the case of intermittently fed reactors. Therefore, more particles with very low conversion leave a CSTR as compared to an intermittently fed reactor, resulting in a lower mean conversion. This is not the familiar CSTR versus batch reactor analysis because even a CSTR enjoys high reactivity initially for enzymatic cellulose hydrolysis. The mean conversion would be much lower if reaction rate is evaluated at exit particle conversion and concentration as for soluble substrate (Fig. 4).

Intermittently fed reactors provide a continuous range of operating modes between fully batch ($f = 1$) and fully

Table II. Comparison of conversion in second and third reactor using the exhaustive approach and the average reaction constant approach for an enzyme loading of 10 U/g cellulose, a cellulose concentration of 55 g/L, and a residence time of 1 day for each reactor with the same volume.

	x			
	Second reactor		Third reactor	
f	Exhaustive (%)	Average k (%)	Exhaustive (%)	Average k (%)
1.33	78.62	78.58	89.69	89.66
2	77.73	77.62	88.72	88.65
4	76.68	76.49	87.70	87.55
8	76.11	75.91	87.21	87.05
10	76.00	75.80	87.13	86.97

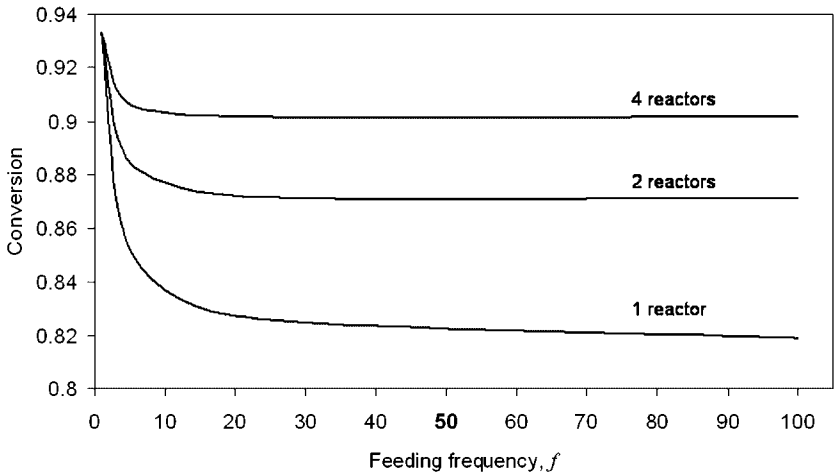

Figure 3. Cellulose conversion versus feeding frequency from prediction for one, two, and four equal volume reactors for a cellulose concentration of 55 g/L, an enzyme loading of 10 U/g cellulose, and a total residence time of 4 days.

continuous (f = infinity) operation. It is quite possible that the economic optimum f lies somewhere between these extremes, although this remains to be definitively shown. Our analysis shows that the highest single-reactor conversion will be obtained in a batch reactor. Although this is a potentially important advantage, the fact that particles maintain their high reactivity even in a CSTR substantially mitigates this advantage compared to processing a soluble substrate, and use of staged reactors can mitigate this advantage further because the probability of a reactive particle having low residence time in multiple successive reactors is small.

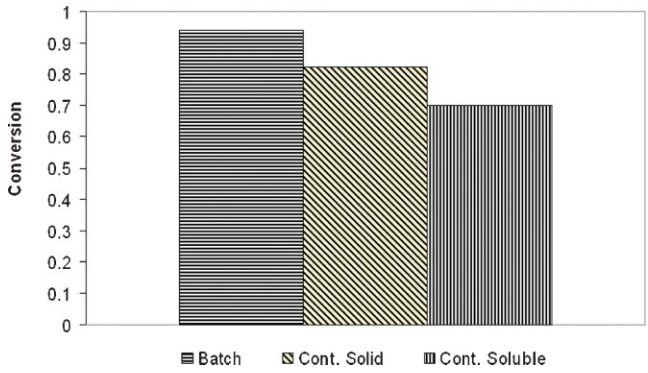

Figure 4. Comparison of conversions for batch (0.94), continuous (0.82), and continuous soluble (0.70) with a cellulose concentration of 55 g/L, an enzyme loading of 10 U/g, and a residence time of 4 days. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

Nomenclature

[]	concentration of the symbol within (g/L)
$[C]_0$	total cellulose concentration fed to the reactor (g/L)
$[C(i)]_0$	cellulose concentration fed to the reactor for population i (g/L)
σ_C	adsorption capacity of enzyme on cellulose (g/g)
σ_L	adsorption capacity of enzyme on lignin (g/g)
τ	reactor residence time (h)
μ_{max}	maximum cell growth rate (h^{-1})
B	β-glucosidase
C	total cellulose substrate; for population i if $C(i)$
c	conversion independent component in rate function (h^{-1})
Cb	cellobiose
$CE(i)$	cellulose enzyme complex for population i
$C_f(i)$	cellulose substrate not bound with enzyme for population i
E	total enzyme
e	exponent of the declining substrate reactivity
E_f	cellulase enzyme not bound to cellulose or lignin
Eth	ethanol
f	feeding frequency/feed number per residence time
G	glucose
I	index of particle population
k	hydrolysis rate constant (h^{-1})
k'	average remaining hydrolysis rate constant (h^{-1})
K_C	cellulose adsorption constant (L/g)
$K_{C/Cb}$	inhibition of cellulose hydrolysis by cellobiose (g/L)
$K_{C/Eth}$	inhibition of cellulose hydrolysis by ethanol (g/L)
K_{Cb}	rate constant for hydrolysis of cellobiose to glucose (h^{-1})
$K_{Cb/G}$	inhibition of cellobiose hydrolysis by glucose (g/L)
k_{fc}	cellulose dynamic adsorption constant (L/(g h))
k_{fl}	lignin dynamic adsorption constant (L/(g h))
K_G	Monod constant (g/L)
K_L	lignin adsorption constant (L/g)
K_m	Michaelis constant of β-glucosidase for cellobiose (g/L)

$K_{X/Eth}$	inhibition of cell growth by ethanol (g/L)
L	total lignin
LE	lignin enzyme complex
L_f	lignin not bound with enzyme
n	total number of particle population
$N(i)$	number of particles in the reactor for population i
$N(i)_0$	number of particles fed to the reactor for population i
$P(i)$	discrete residence time distribution for particle population i
$R(i)$	fraction of particles remain in the reactor for population i
r	rate of reaction for the symbol in subscript (g/(L h))
$w(i)$	fraction of concentration of cellulose enzyme complex for population i
x	reactor conversion
Xc	yeast cell
$x(\tau)$	mean cellulose conversion in a steady-state CSTR
$\bar{x}$	mean cellulose conversion at the end of a reaction cycle for discrete feeding
$x_p(i)$	particle conversion for population i
$\tilde{x}_p(i)$	normalized particle conversion for population i
$Y_{Eth/G}$	ethanol yield per substrate consumed (g/g)
$Y_{X/G}$	cell yield per substrate consumed (g/g)

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References

Boussaid A, Saddler JN. 1999. Adsorption and activity profiles of cellulases during the hydrolysis of tow Douglas fir pulps. Enzyme Microb Technol 24:138–143.

Chernoglazov VM, Ermolova OV, Klyosov AA. 1988. Adsorption of high-purity endo-1,4-β-glucanases from Trichoderma reesei on components of lignocellulosic materials: Cellulose, lignin, and xylan. Enzyme Microb Technol 10:503–507.

Ghose TK, Tyagi RD. 1979. Rapid ethanol fermentation of cellulose hydrolysate. 2. Product and substrate inhibition and optimization of fermentor design. Biotechnol Bioeng 21:1401–1420.

Gusakov AV, Sinitsyn AP. 1985. Kinetics of the enzymatic hydrolysis of cellulose. 1. A mathematical model for a batch reactor process. Enzyme Microb Technol 7:346–352.

Kim DW, Jang YH, Jeong YK. 1998. Adsorption kinetics and behaviour of two cellobiohydrolases from Trichoderma reesei on microcrystalline cellulose. Biotechnol Appl Biochem 27:97–102.

Lee NE, Woodward J. 1989. Kinetics of the adsorption of Trichoderma reesei C30 cellulase to DEAE-Macrosorb. J Biotechnol 11:75–82.

Lynd LR, Wang MQ. 2004. A product-nonspecific framework for evaluating the potential of biomass-based products to displace fossil fuels. J Ind Ecol 7(3–4):17–32.

Lynd LR, Wyman CE, Gerngross TU. 1999. Biocommodity engineering. Biotechnol Prog 15(5):777–793.

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Mol Biol Rev 66(3):506–677.

Nutor JRK, Converse AO. 1991. The effect of enzyme and substrate levels on the specific hydrolysis rate of pretreated poplar wood. Appl Biochem Biotechnol 28/29:757–771.

Ooshima H, Sakata M, Harano Y. 1983. Adsorption of cellulase from Trichoderma viride on cellulose. Biotechnol Bioeng 25:3103–3114.

Ooshima H, Burns DS, Converse AO. 1990. Adsorption of cellulase from Trichoderma reesei on cellulose and lignacious residue in wood pretreated by dilute sulfuric acid with explosive decompression. Biotechnol Bioeng 36:446–452.

Phillippidis GP, Smith TK, Wyman CE. 1992. Study of the enzymatic hydrolysis of cellulose for production of fuel ethanol by the simultaneous saccharification and fermentation process. Biotechnol Bioeng 41:846–853.

Singh A, Kumar PKR, Schugerl K. 1991. Adsorption and reuse of cellulases during saccharification of cellulosic materials. J Biotechnol 18:205–212.

South CR, Lynd LR. 1994. Analysis of conversion of particulate biomass to ethanol in continuous solids retaining and cascade bioreactors. Appl Biochem Biotechnol 45/46:467–481.

South CR, Hogsett DAL, Lynd LR. 1995. Modeling simultaneous saccharification and fermentation of lignocellulose to ethanol in batch and continuous reactors. Enzyme Microb Technol 17(9):797–803.

van Uden N. 1983. Effects of ethanol on the temperature relation of viability and growth in yeast. Crit Rev Biotechnol 1:263–272.

Wyman CE. 2003. Potential synergies and challenges in refining cellulosic biomass to fuels. Chem Power Biotechnol Prog 19(2):254–262.

Zhang YHP, Lynd LR. 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. Biotechnol Bioeng 88(7):797–824.