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Mathematical Modeling of Cellulose Conversion to Ethanol by the Simultaneous Saccharification and Fermentation Process

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ABSTRACT

Ethanol, a promising alternative fuel, can be produced by the simultaneous saccharification and fermentation (SSF) of lignocellulosic biomass, which combines the enzymatic hydrolysis of cellulose to glucose and the fermentation of glucose to ethanol by yeast in a single step.

A mathematical model that depicts the kinetics of SSF has been developed based on considerations of the quality of the substrate and enzyme, and the substrate-enzyme-microorganism interactions. Critical experimentation has been performed in conjunction with multiresponse nonlinear regression analysis to determine key model parameters regarding cell growth and ethanol production. The model will be used for rational SSF optimization and scale-up.

Index Entries: Cellulose hydrolysis; SSF modeling; biomass conversion; ethanol production.

NOMENCLATURE

a	Growth-associated ethanol formation constant (g/g)
α_s	Surface area occupied by unit mass of cellulase (m^2/g)
α_t	Available surface area of cellulose (m^2/L)

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b	Nongrowth-associated specific ethanol production rate (g/g/h)
(B)	Concentration of cellobiose (g/L)
(C)	Concentration of cellulose (g/L)
(E)	Concentration of ethanol (g/L)
(E_1)	Concentration of cellulase in solution (g/L)
$(E_1{}^*)$	Concentration of cellulase adsorbed on cellulose (g/L)
(E_2)	Concentration of β-glucosidase (g/L)
(G)	Concentration of glucose (g/L)
k_d	Specific rate of cell death (h^{-1})
k_1', k_2	Specific rate of cellulose and cellobiose hydrolysis, respectively (h^{-1})
K_e	Constant in cellulase adsorption Eq. 2 (g/L)
K_i	Constant of cell growth inhibition by the substrate (glucose) (g/L)
K_m	Michaelis constant of β-glucosidase for cellobiose (g/L)
K_{1B}, K_{2B}	Inhibition constants of cellulase and β-glucosidase by cellobiose, respectively (g/L)
K_{1E}, K_{2E}, K_{3E}	Inhibition constants of cellulase, β-glucosidase, and cell growth by ethanol, respectively (g/L)
K_{1G}, K_{2G}	Inhibition constants of cellulase and β-glucosidase by glucose, respectively (g/L)
K_{1L}, K_{2L}	Constants for cellulase and β-glucosidase adsorption to lignin, respectively (L/g)
K_3	Monod constant of glucose for cell growth (g/L)
K_4	Monod constant of glucose for ethanol synthesis (g/L)
(L)	Concentration of lignin (g/L)
m	Specific rate of substrate consumption for maintenance requirements (h^{-1})
μ_m	Maximal specific growth rate (h^{-1})
r_1, r_2, r_3	Volumetric rate of cellulose, cellobiose, and glucose utilization, respectively (g/L/h)
t	Time (h)
(X)	Concentration of cell mass (g/L)
Y_{XG}	Average yield coefficient of cell mass on substrate (glucose) (g/g)
ϕ	Cellulose reactivity coefficient (dimensionless)
Subscripts	
t	Total value

INTRODUCTION

Alternative fuels, such as ethanol manufactured from domestic cellulosic feedstocks (*1*), can reduce or eliminate the dependence of the US economy on imported petroleum. Cellulosic biomass (deciduous and coniferous woods, grasses, and agricultural residues), the most abundant renewable resource on earth with an annual production of approx 4×10^{10}

t (2), is readily available from agricultural, forestry, pulp industry, and municipal residues for production of ethanol fuel.

Lignocellulosic biomass consists primarily of cellulose, hemicellulose, and lignin at a typical weight ratio of 50:28:22. Although rich in carbohydrates (cellulose, hemicellulose), lignocellulosic biomass is an insoluble substrate with a complex structure, which makes efficient conversion of cellulose into fermentable sugars and subsequently into ethanol difficult. Extensive research has demonstrated that SSF, the simultaneous saccharification (hydrolysis) of cellulose to glucose and fermentation of glucose to ethanol, improves the kinetics (3) and economics (4) of biomass conversion through circumvention of enzyme inhibition by hydrolysis products, minimization of contamination risk because of the presence of ethanol, and reduction in capital equipment requirements.

Five major steps are employed in the current biomass conversion technology developed at the National Renewable Energy Laboratory:

1. Chemical pretreatment of lignocellulose to disrupt its structure and render it more accessible to enzymatic attack;
2. Enzyme production using cellulase-secreting organisms;
3. Fermentation of xylose, derived from hemicellulose during pretreatment, into ethanol;
4. SSF to convert cellulose into ethanol; and
5. Ethanol recovery from the SSF reactor effluent.

SSF is at the center of the bioconversion process, because cellulose is the major biomass component; it has the largest contribution (>25%) to the capital cost of the bioconversion process (5). It is therefore imperative to understand the kinetics of SSF and quantitate its dependence on variables that can be manipulated. This task can be realized in a comprehensive and rational way by developing a mathematical model that conceptualizes the performance of SSF. Through the model, issues of major importance, such as the effect of substrate and enzyme loading on ethanol productivity, the mode of operation, the effect of feedstock composition, and the desired pretreatment efficiency, can be systematically addressed in order to improve the technology.

Cellulose hydrolysis kinetics has been extensively researched, and several mathematical models have been proposed to date (*6–18*). Very few of the models, however, have attempted to simulate both hydrolysis and fermentation of cellulose as an integrated process (*19,20*). Unfortunately, the validity of these models is limited to certain biomass conversion systems and conditions, because not all the factors affecting the kinetics of SSF, as will be presented later, have been taken into consideration.

The objective of this work is to identify and analyze the steps of the SSF process, and develop a preliminary mathematical representation of its kinetics. The model will provide valuable insight into key interactions and rate limitations in SSF, and serve as a tool for rational improvement and scale-up of the bioconversion process.

SUBSTRATE AND ENZYME IDENTITY

Cellulose is an insoluble, linear polymer of D-glucose residues linked by β-1,4-glucosidic bonds (*21*). Cellulose chains, held together by hydrogen bonds among hydroxyl groups of adjacent molecules and van der Waals forces, form fibrils that are surrounded by hemicellulose and layers of lignin, which protect cellulose from enzymatic attack. Regions of dense interchain bonding form crystalline areas. Regions with less ordered structure (amorphous cellulose) are more readily hydrolyzable than crystalline areas.

Cellulases, the enzymes that degrade cellulose, consist of several components believed to function synergistically. A generally accepted enzymatic mechanism has been proposed for fungal cellulases (*22*): 1,4-β-D-glucan glucanohydrolase (endoglucanase) attacks randomly β-1,4-glucosidic bonds creating shorter-length cellulose chains; 1,4-β-D-glucan cellobiohydrolase (exoglucanase) hydrolyzes these chains from the nonreducing termini generating cellobiose residues; and β-D-glucoside glucohydrolase (β-glucosidase) finally cleaves cellobiose to glucose units.

SSF MODEL DESCRIPTION

The kinetics of SSF depends mainly on four factors:

1. Quality and concentration of the cellulosic substrate;
2. Quality and concentration of the cellulase and β-glucosidase enzyme system;
3. Mode of interaction between substrate and enzyme; and
4. Mode of interaction between enzyme and fermentative organism.

The structural characteristics of the substrate influence the susceptibility of cellulosic biomass to enzymatic degradation. The quality of the enzyme affects the activity of its components, as they hydrolyze cellulose. The enzyme–substrate interaction regulates the extent of enzyme adsorption onto the substrate and, hence, the rate of this heterogeneous reaction. Finally, the microorganism–enzyme interaction determines the growth kinetics of the cells and their ethanol productivity, as well as the potential inhibitory effect of hydrolysis (cellobiose, glucose) and metabolic (ethanol) products on enzyme activity.

Based on these considerations, a model was developed for the conversion of cellulose into ethanol (Fig. 1). Certain assumptions were made to facilitate model development and parameter estimation. Cellulase (E_1), assumed to consist of only endoglucanase and exoglucanase, adsorbs onto the solid lignocellulosic substrate and cleaves cellulose (C) to shorter-length polysaccharide chains through the action of endoglucanase (E_{1a}); simultaneously, hydrolysis of these chains to cellobiose (B) is carried out by exoglucanase (E_{1b}). However, since the concentration of the insoluble

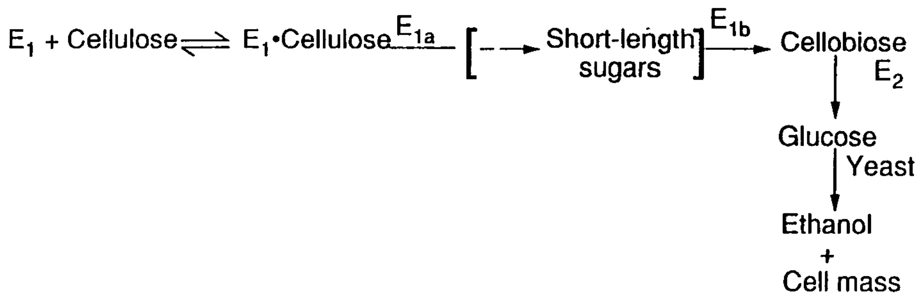

Fig. 1. Simplified schematic representation of the SSF reaction sequence (E_1: cellulase; E_{1a}: endoglucanase; E_{1b}: exoglucanase; E_2: β-glucosidase).

intermediate macromolecules cannot be determined experimentally, the reaction scheme is further simplified to the direct conversion of cellulose to cellobiose. Cellobiose diffuses into the aqueous phase, where β-glucosidase (E_2) catalyzes its hydrolysis to glucose (G), which serves as a carbon/energy source and leads to production of ethanol (E), as the major metabolic product, and cell mass (X). The model is based on the following assumptions:

1. Cellulase consists of endoglucanase and exoglucanase, but no distinction is made between them, since current analytical procedures cannot accurately distinguish the activities of the two cellulase components during SSF;
2. Cellulase and β-glucosidase are exogenously added to the SSF system;
3. Cellulose is hydrolyzed to cellobiose by cellulase with negligible formation of glucose;
4. The pH during the SSF does not vary considerably, and therefore has no significant effect on enzyme activity and cell growth;
5. Ethanol and carbon dioxide are the only major metabolic products of the organism; and
6. The growth medium provides an excess of all nutrients except for carbon source (glucose), which is derived from cellulose.

The model is not restricted to any particular SSF system or set of conditions, and refers to both anaerobic and aerobic operations.

SSF MODEL FORMULATION

Since cellulose is an insoluble substrate, the enzyme must adsorb on its surface in order to promote hydrolysis. In contrast, the hydrolysis of cellobiose to glucose by β-glucosidase is carried out in solution. Thus, the conversion of cellulose to ethanol consists of a combination of heterogeneous and homogeneous catalyses, with strong interdependence. In order to develop a comprehensive SSF model, it is imperative to identify and analyze the steps that compose the cellulose-to-ethanol conversion process (Fig. 1). These steps are:

1. Cellulase diffusion towards cellulose;
2. Adsorption of cellulase on the surface of cellulose;
3. Hydrolysis of cellulose to cellobiose catalyzed by the cellulase components;
4. Diffusion of cellobiose into the aqueous phase;
5. Hydrolysis of cellobiose to glucose catalyzed by β-glucosidase;
6. Diffusion of glucose towards the cells;
7. Uptake of glucose by the cells;
8. Glucose catabolism and ethanol synthesis; and
9. Ethanol secretion into the aqueous phase.

Enzyme, Substrate, and Product Diffusion

Experimental studies in a 50 g/L cellulose suspension have indicated that enzyme diffusion is rapid relative to cellulose hydrolysis (*23*). Moreover, since cellobiose, glucose, and ethanol are small molecules with high diffusivities in aqueous solutions (on the order of 10^{-5} cm^2/s), their diffusion should not be controlling the overall cellulose conversion rate. Therefore, no mass transfer limitations are considered in this preliminary model. Still, potential enzyme diffusion limitations at higher cellulose concentrations need to be investigated.

Cellulase Adsorption to Cellulose and Cellulose Hydrolysis to Cellobiose

The hydrolysis of cellulose is a reaction that takes place on the surface of the insoluble substrate. Therefore, principles of heterogeneous catalysis need to be taken into consideration (*24*). Accordingly, the hydrolysis rate of cellulose, r_1 will depend on the concentration of cellulase adsorbed on cellulose:

$$r_1 = k_1' (E_1^*) \tag{1}$$

where k_1' is the specific rate of hydrolysis and (E_1^*) is the concentration of adsorbed cellulase.

Endoglucanase and exoglucanase reportedly adsorb to cellulose at similar proportions (*25*), whereas β-glucosidase remains in the aqueous phase (*26*). Competitive adsorption of endoglucanases and exoglucanases on cellulose has been observed (*27*), and the kinetics of their synergism is being studied (*28*). In order to calculate the amount of cellulase adsorbed to the surface of cellulose, (E_1^*), the adsorption and desorption rates of cellulase are assumed to be at all times in equilibrium:

$$k_{ads}(\alpha_t - \alpha)(E_1) = k_{des}\alpha \rightarrow \alpha = \alpha_t (E_1) / [(E_1) + K_e] \tag{2}$$

where (E_1) is the free cellulase concentration, k_{ads} and k_{des} are the specific rates of cellulase adsorption on and desorption from the surface of cellulose, respectively, K_e is the ratio k_{des}/k_{ads}, α_t is the total surface area of the substrate, and α is the surface area of the substrate occupied by cellulase. It is assumed that the substrate surface is energetically uniform, enzyme adsorption is limited to a monomolecular level, and there is no interaction among adsorbed enzyme molecules. Taking into account Eqs. 1 and 2 and that the enzyme can occur in either the free or adsorbed form, r_1 becomes:

$$r_1 = k_1' \alpha_t(E_1)_t / \{\alpha_s[K_e + (E_1)_t] + (\alpha_t - \alpha)\} \quad (3)$$

where $(E_1)_t$ is the total cellulase concentration and α_s is the surface area occupied per unit mass of cellulase (assumed constant). At high enzyme concentrations, the substrate surface area becomes saturated with cellulase ($\alpha \rightarrow \alpha_t$) and, consequently:

$$r_1 = k_1\alpha_t(E_1)_t / (K_e + (E_1)_t) \quad (4)$$

where $k_1 = k_1'/\alpha_s$. Adsorption of cellulase to cellulose has been reported to be fast, reaching saturation within 2 min (*23*). In agreement with Eq. 4, a linear correlation between initial hydrolysis rate and initial substrate surface area has been observed with acid-pretreated hardwoods and softwoods (*29*). According to Eqs. 3 and 4, over time the hydrolysis rate decreases as the consumption of cellulose leads to a decrease in available surface area α_t. Such a rate decrease has been well documented (*13,18,30*).

The surface area, α_t, can be calculated from the pore volume accessible to dextran molecules of diameters similar to that of cellulase (*29*), using the solute exclusion technique (*31*). This method, however, only measures the pore (internal) surface area of cellulose, not the total surface area α_t. It has been proposed that surface area is proportional to (C) (*11*), the square power of (C) (*17*), or the two-third power of (C) (*13*). The dependence of surface area on (C) may vary among different cellulosic substrates and as a result of different pretreatment conditions. It is therefore necessary to determine experimentally the type of relationship between α_t and substrate concentration.

Cellulase and β-glucosidase also adsorb, although irreversibly, to lignin (*25*). This adsorption reduces the effective concentrations of the two enzymes. A linear dependence of lignin-adsorbed cellulase and β-glucosidase on lignin concentration is assumed in this preliminary mathematical model, although the surface area of the lignaceous content of biomass may be the determining factor instead of its concentration (to be investigated). Consequently, the concentrations of the two enzymes (E_1^*) and (E_2) are multiplied by the expressions:

$$1 - K_{1L}(L), 1 - K_{2L}(L) \quad (5)$$

respectively, in order to obtain the concentrations of active cellulase and β-glucosidase. K_{1L} and K_{2L} are model parameters. A linear relationship between β-glucosidase adsorbed to lignin and lignin concentration has been reported (32).

The gradual decrease in the cellulose hydrolysis rate during SSF may be owing to several factors, such as increasing crystallinity, decreasing available surface area, and enzyme inhibition/inactivation. In order to account for the effect of cellulose structure on the hydrolysis rate and distinguish it from the effects of available surface area and enzyme inhibition, a substrate reactivity coefficient ϕ is introduced in the model. The parameter ϕ is time-dependent ($\phi_0 = 1$) and accounts for the change in cellulose hydrolysis rate during SSF because of the change in accessibility of the substrate. The decrease in ϕ over time may result from the rapid conversion of easily hydrolyzed cellulose and a subsequent ''enrichment'' of cellulose with hydrolysis-resistant (crystalline) regions. As a result, the specific hydrolysis rate k_1' decreases, and the model accounts for this slow down by multiplying k_1' by ϕ. The pattern of decrease of ϕ over time may vary among lignocellulosic substrates of different origins and may be affected by the pretreatment conditions. Estimation of ϕ based on fundamental substrate characteristics will be attempted, and the use of the cellulose crystallinity index, determined by X-ray diffractometry (11), as a measure of ϕ will be examined first.

Taking ϕ into account, as well as noncompetitive inhibition of cellulase by cellobiose (B), glucose (G), and ethanol (E) (33), the rate of cellulose hydrolysis to cellobiose, r_1, becomes:

$$r_1 = (k_1 \alpha_t \phi (E_1)_t / [K_e + (E_1)_t] \{1 + [(B) / K_{1B}] + [(G) / K_{1G}]\})$$
$$[K_{1E} / K_{1E} + (E)] [1 - K_{1L}(L)] \qquad (6)$$

where K_{1B}, K_{1G}, and K_{1E} are the inhibition constants of cellulase by cellobiose, glucose, and ethanol, respectively.

Cellobiose Hydrolysis to Glucose

In contrast to cellulase, β-glucosidase remains in the aqueous phase and catalyzes the hydrolysis of cellobiose to glucose. It has been reported that the action of β-glucosidase is inhibited by its substrate, cellobiose (34), and also competitively inhibited by its product, glucose (30,34). Hence, the rate of cellobiose hydrolysis is:

$$r_2 = k_2(E_2)(B) / (K_m \{1 + [(G) / K_{2G}]\} + [B]\{1 + [(B) / K_{2B}]\})$$
$$[K_{2E} / K_{2E} + (E)] [1 - K_{2L}(L)] \qquad (7)$$

where k_2 is the specific rate of cellobiose (B) hydrolysis, K_m is the Michaelis constant of β-glucosidase (E_2) for cellobiose, and K_{2B}, K_{2G}, and K_{2E} are the inhibition constants of β-glucosidase by cellobiose, glucose (G), and ethanol (E), respectively.

Glucose Uptake and Anaerobic Fermentation to Ethanol

Glucose, the carbon/energy source of the fermentative organism, is metabolized anaerobically into cell mass (X), with concomitant synthesis of ethanol and carbon dioxide. Other metabolic products are produced in negligible amounts. A Monod kinetic expression that includes substrate and product inhibition (*35*) is used to account for the dependence of microbial growth on glucose concentration:

$$[d(X)/dt] = \mu_m [(G)/K_3 + (G) + (G)^2/K_i]\,[K_{3E}/K_{3E} + (E)]\,(X) - k_d(X) \quad (8)$$

where μ_m is the maximal specific growth rate of the microorganism, K_3 is the Monod constant for glucose (variable among organisms), K_i is the substrate inhibition constant, and K_{3E} is the inhibition constant of cell growth by ethanol. The last term of Eq. 8 represents potential cell lysis, assumed to be proportional to the cell mass concentration; k_d is the specific rate of cell death. In addition to cell mass synthesis, a portion of glucose serves as a source for cell maintenance requirements (*36*). Hence, the glucose utilization rate, r_3, by the organism is:

$$r_3 = (1/Y_{XG})\,[d(X)/dt] + m(X) \quad (9)$$

where Y_{XG} is an average yield coefficient of cell mass on the substrate (glucose), and m is the specific rate of substrate consumption for maintenance requirements. This equation does not include an additional term of glucose consumption for the formation of ethanol, since ethanol is a product directly associated with energy generation by the fermentative organism.

The ethanol formation rate consists of a growth-associated and a non-growth-associated term (*37*), and also depends directly on the concentration of glucose (G) (*20*):

$$(d(E)/dt) = \{a\,[d(X)/dt] + b(X)\}\,[(G)/K_4 + (G)] \quad (10)$$

where a is the growth-associated ethanol formation constant, b is the non-growth-associated specific ethanol production rate, and K_4 is a Monod constant for ethanol synthesis.

Model Summary

Based on Eqs. 6, 7, and 9, the profiles of cellulose, cellobiose, glucose, cell mass, and ethanol concentration during a batch SSF process are described by the following mass balance equations:

Cellulose $$[d(C)/dt] = -r_1 \quad \text{(M-1)}$$

Cellobiose $$[d(B)/dt] = 1.06\,r_1 - r_2 \quad \text{(M-2)}$$

Glucose $$[d(G)/dt] = 1.05\,r_2 - r_3 \quad \text{(M-3)}$$

Cell mass $$[d(X) / dt] = \mu_m [(G) / K_3 + (G) + (G)^2 / K_i]$$
$$[K_{3E} / K_{3E} + (E)] (X) - k_d(X) \quad \text{(M-4)}$$

Ethanol $$[d(E) / dt] = \{a[d(X) / dt] + b(X)\} [(G) / K_4 + (G)] \quad \text{(M-5)}$$

Thus, the preliminary mathematical model of SSF is composed of five differential equations, involving five dependent variables ([C], [B], [G], [X], and [E]) and one independent (time, t). In the current model formulation, enzyme deactivation is assumed negligible, and lignin concentration is considered constant, since lignin is chemically inert during SSF. The model can be easily modified to account for different SSF conditions and modes of operation, such as multiple metabolic products, simultaneous SSF and enzyme synthesis, aerobic fermentations, and fed-batch and continuous operations.

DETERMINATION OF MODEL PARAMETERS

The parameters of the SSF model will be determined through critical experiments, each one examining the kinetics of a particular step of the SSF process isolated to the maximal feasible extent from all other steps. Nonlinear regression of the model equations to experimentally obtained kinetic data will be used to determine the parameter values. The goal of the regression is to estimate those values of the parameters that minimize the weighed sum of squared residuals. During the iterative procedure, the parameter correction vector $\Delta \underline{P}$ is:

$$\Delta \underline{P} = [\sum_{j=1}^{m} w_j(\underline{J}_j^T \underline{J}_j + k_j\underline{I})]^{-1} [\sum_{j=1}^{m} w_j \underline{J}_j^T (\underline{Y}_j - \underline{Y}_j^*)] \quad (11)$$

where $\underline{Y}_j$ is the vector of experimental measurements of the jth dependent variable, $\underline{Y}_j^*$ is the vector of model predictions for this variable, w_j is the weighing factor of $\underline{Y}_j$, $\underline{J}_j$ are the Jacobian matrices of the model, ($\underline{J}_j^T$ is the transpose matrix of $\underline{J}_j$ and k_j are parameters of the algorithm. A nonlinear regression algorithm was developed based on the fast-converging Levenberg-Marquardt least squares minimization procedure, which is a hybrid of the Gauss-Newton and the steepest descent methods (*38*).

A critical experiment, designed to determine the parameters of cell growth and ethanol synthesis, demonstrates the methodology used for parameter determination. For the production of ethanol, *Brettanomyces custersii* CBS 5512 (patent application filed) was cultivated in 10 g/L of yeast extract, 20 g/L of peptone, and 10 g/L of glucose under batch anaerobic conditions at 38.5°C. The conditions and medium composition were selected to resemble closely the actual SSF process. During the course of the fermentation, the concentration profiles of glucose, dry cell mass, and ethanol were monitored (Fig. 2). After 5 h of lag phase, the cells exhibited

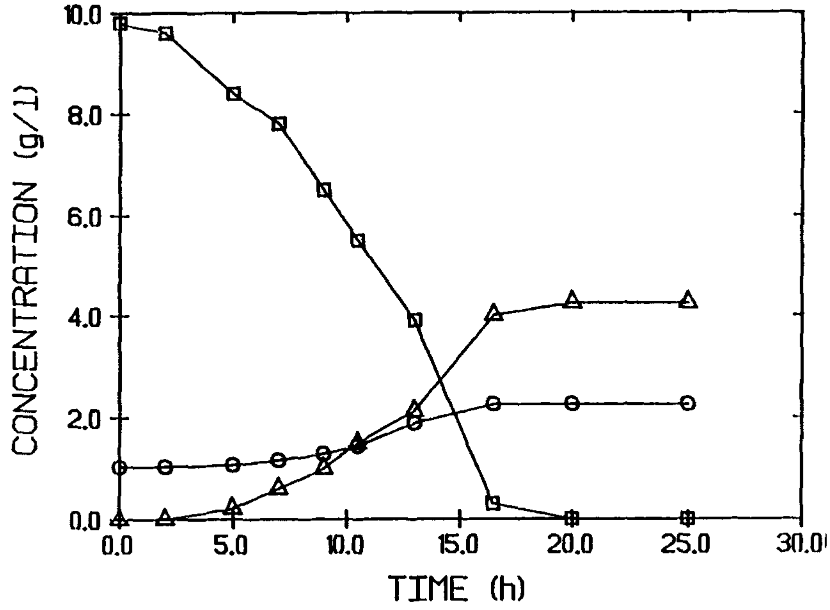

Fig. 2. Cell growth and ethanol production by *Brettanomyces custersii* CBS 5512 cultivated in a medium composed of 10 g/L yeast extract, 20 g/L peptone, and 10 g/L glucose under batch anaerobic conditions at 38.5°C (○ dry cell mass; □ glucose; △ ethanol).

exponential growth, followed by the stationary phase, where no further increase in cell mass and ethanol concentration was observed.

A mathematic model that describes the glucose fermentation process consists of model equations M-3 through M-5, with $r_2=0$ (no glucose formation). The glucose fermentation model consisting of these three ordinary differential equations includes one independent variable (t), three dependent variables ($[X]$, $[G]$, and $[E]$), and nine parameters (μ_m, K_3, K_i, k_d, Y_{XG}, m, a, b, and K_4). Accordingly, 27 variational equations ($\partial \underline{Y}_j/\partial \underline{P}$) were prepared to formulate the Jacobian matrices of the Levenberg-Marquardt algorithm and estimate $\Delta \underline{P}$.

The simplistic representation of cell physiology by equations M-3, M-4, and M-5 does not allow them to simulate the adaptation (lag) period of cell growth. Hence, observations obtained during the lag phase were not taken into consideration. At a statistical confidence level of 95%, convergence to a minimum weighed sum of squared residuals was achieved at the following parameter values:

$\mu_m = 0.142\ h^{-1}$	$K_3 = 0.171$ g/L	$K_i = 33.8$ g/L
$k_d = 0.0505\ h^{-1}$	$Y_{XG} = 0.310$ g/g	$m = 0.211\ h^{-1}$
$a = 0.00269$ g/g	$b = 0.198\ h^{-1}$	$K_4 = 0$ g/L

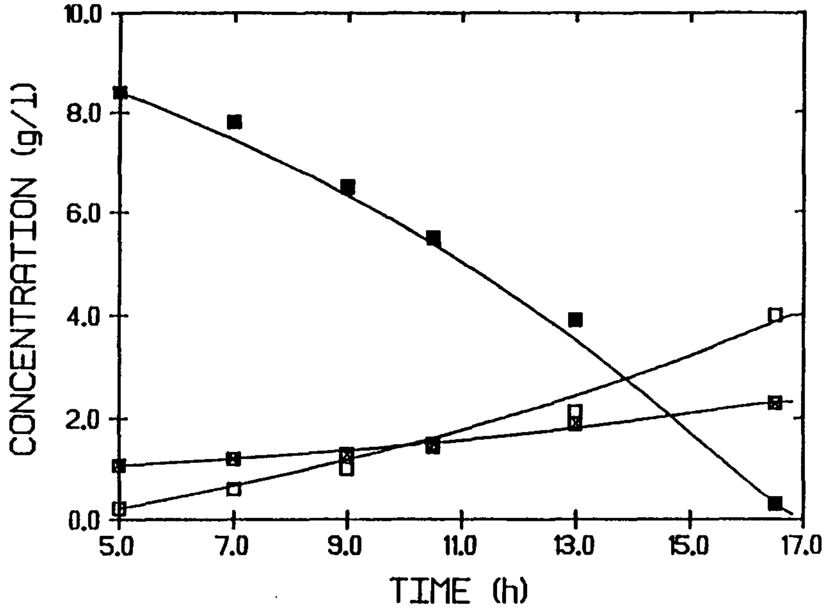

Fig. 3. Multiple nonlinear regression of the exponential and stationary phase experimental data of Fig. 2. The symbols represent the experimental measurements of the model variables (⊠ dry cell mass; ■ glucose; □ethanol concentration), whereas the continuous lines depict the optimal model predictions for the profile of the corresponding variable.

The satisfactory regression of the model equations to the experimental data is demonstrated in Fig. 3. The small value of a indicates that ethanol production is practically nongrowth associated. The null value of K_4, on the other hand, suggests that ethanol synthesis is not directly dependent on glucose concentration as was originally assumed in Eq. 10. The value of Y_{XG} is also close to those typical of other fermentative organisms (*39*) and accounts for only the portion of glucose that is used to support cell growth in Eq. 9.

The presented analysis yielded the optimal values of nine of the SSF model parameters and showed that the fermentation model, which is a subset of the preliminary SSF model, can describe the batch production of ethanol by a fermentative microorganism in a satisfactory way (Fig. 3). At the same time, the application of nonlinear regression to the first experimental observations has decreased the number of model parameters.

CONCLUSION

A mathematical model was developed to depict the kinetics of the SSF process. The model takes into account characteristics of the biomass substrate and the enzyme, as well as substrate-enzyme-microorganism interactions. Critical experimentation was performed to determine the parameters of cell growth and ethanol production, and additional experiments are under way to calculate the other model parameters. The model will be subsequently validated against independent SSF experiments to verify the validity of the parameter values, and used to assess the impact that SSF variables and parameters have on ethanol productivity, identify rate-limiting steps that need to be improved, and serve as a guide in process scale-up and commercialization.

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