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Cybernetic Modeling of Simultaneous Saccharification and Fermentation for Ethanol Production from Steam-Exploded Wood with *Brettanomyces custersii*

SHIN, DONGGYUN, AHRIM YOO, SEUNG WOOK KIM, AND DAE RYOOK YANG*

Department of Chemical and Biological Engineering, Korea University, Seoul 136-701, Korea

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Abstract The simultaneous saccharification and fermentation (SSF) process consists of concurrent enzymatic saccharification and fermentation. In the present cybernetic model, the saccharification process, which is based on the modified Michaelis-Menten kinetics and enzyme inhibition kinetics, was combined with the fermentation process, which is based on the Monod equation. The cybernetic modeling approach postulates that cells adapt to utilize the limited resources available to them in an optimal way. The cybernetic modeling was suitable for describing sequential growth on multiple substrates by *Brettanomyces custersii*, which is a glucose- and cellobiose-fermenting yeast. The proposed model was able to elucidate the SSF process in a systematic manner, and the performance was verified by previously published data.

Key words: *Brettanomyces custersii*, cybernetic modeling, ethanol production, simultaneous saccharification and fermentation, steam-exploded wood

Ethanol production from biomass is difficult because of the stability of biomass, the variety of feedstock, the choice of suitable catalysts converting biomass into sugar, and the cost of collection and storage of a low-density biomass feedstock. There are two basic approaches for converting biomass into sugars before ethanol can be produced from the fermentation of sugar by yeast. In the early 1990s, the NREL (National Renewable Energy Laboratory) examined acid hydrolysis, but the cost of acid and post-processing of the acid or the reactor cost was too high compared with the conventional petrochemical counterparts. Enzymatic hydrolysis does not require acid; however, the cost of commercial cellulase is high and the process is too slow. The enzymes used in ethanol production are cellulase and β-glucosidase, and their activities are reduced by high sugar concentration. If fermentation occurs simultaneously with saccharification, the hydrolysis rate can be maximized by reducing the level of sugar inhibition. Therefore, this simultaneous saccharification and fermentation (SSF) has been suggested as the most suitable process for the enzymatic conversion of cellulose to ethanol [12, 14]. However, one drawback is that an efficient operation of SSF is difficult because of a large difference in the optimum temperatures for cellulase and yeast: The maximum operating temperature of yeast is about 37°C, where the activity of cellulase is low.

Some kinetic models that can describe SSF have been proposed to design, optimize, and control the SSF process [3, 4]. Philippidis *et al.* [10] proposed a SSF model using a lignocellulosic biomass as a substrate, and their result could predict the kinetics of cellulose hydrolysis. However, the model has a limitation in applying it to a real feed-stock process, because the model assumes the substrate as mostly pure cellulose [10]. South *et al.* [13] improved the model of the SSF process by considering various concentrations of substrates. However, this model is also difficult to optimize or control a SSF process because of its complexity. Moon *et al.* [5] proposed a simplified model applicable to optimize the SSF process with the cellobiose-fermenting yeast, *Brettanomyces custersii*. Considering the characteristics of the fermenting yeast, their model could explain the effect of inhibitory compounds and the properties of the yeast strain. Their model consists of two different models, saccharification and fermentation. They introduced exponential terms and parameters to consider the time-dependent variation in physical properties of a substrate. They optimized the parameters to describe the nonlinear behavior, using experimental data. However, the model equation could not describe the diauxic growth caused by sequential substrate utilization in the fermentation stage.

Recently, the cybernetic perspective of microbial growth suggested that the metabolic regulation of a biochemical process could be controlled by enzymatic synthesis and activity [11]. The cybernetic model developed by Kompala

*Corresponding author
Phone: 82-2-929-9613; Fax: 82-2-926-6102;
E-mail: dryang@korea.ac.kr

et al. [1, 2] described the dynamics associated with the diauxic growth, or the sequential utilization of substrates with the preferential utilization of the substrate supporting a higher growth rate. Narang *et al.* [7] analyzed the cybernetic model for diauxic growth dynamically. Namjoshi and Ramkrishna [6] showed that the steady-state multiplicity occurs from the ability of microbial cultures to regulate their metabolic pathways in response to the environment. Cybernetic modeling approach could be used to capture the metabolic regulations associated with switching between multiple steady states. The model postulates that microorganisms optimize the utilization of available substrates to maximize their growth rate. Therefore, the cybernetic model makes it possible to accurately describe the diauxic growth of cells in batch, fed-batch, and continuous cultures without artificial and complicated factors.

In this study, the cybernetic modeling approach was applied to model the SSF process with the cellobiose-fermenting yeast, *Brettanomyces custersii*, which exhibits a typical sequential growth on multiple substrates [5]. The aim of this study was to better elucidate the behavior of the SSF process, using the cybernetic modeling approach, and to verify the performance of the proposed cybernetic model compared with experimental results published in the literature [5, 8]. The results showed that the proposed approach could effectively account for the natural consumption preference of the substrates as well as the inhibitory effects on various enzyme kinetic pathways.

DEVELOPMENT OF SSF PROCESS MODEL

The modeling of the SSF process can mainly be divided into two parts, the saccharification process and the fermentation process. The saccharification model of SSF is based on the Michaelis-Menten kinetics and enzyme inhibition kinetics. The fermentation model of the SSF is based on the Monod equation and cybernetic modeling. It has been reported that the complex compounds in SEW (steam-exploded wood) are inhibitory on microorganism, and their relative digestibility can be reduced [5]. Therefore, to introduce the cybernetic modeling, a modified kinetic model for SSF from the SEW is suggested in this study. Figure 1 shows a schematic diagram for the reaction of the proposed model.

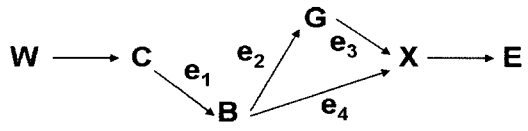

Fig. 1. Schematic diagram of the SSF reaction pathway in the proposed model.
W: steam-exploded wood; C: cellulose; B: cellobiose; G: glucose; X: yeast; E: ethanol; e_1: cellobiose; e_2: β-glucosidase; e_3: enzyme on glucose fermentation; e_4: enzyme on glucose fermentation.

Saccharification Model

The saccharification is a process for producing glucose from SEW via cellulose and cellobiose in the absence of ethanol as an inhibitor. The cellulose content of SEW is approximately 54.5% on a dry weight basis [5]. The concentrations of lignin and the other components, W, as well as cellulose, C, are:

$$W=(1-0.545)W_0 \tag{1}$$

$$C=0.545W_0 \tag{2}$$

where W_0 denotes the total concentration of SEW charged in a reactor. The rates of the concentration change for cellulose, cellobiose (B), and glucose (G) can then be expressed as follows:

$$\frac{dC}{dt}=-r_1C \tag{3}$$

$$\frac{dB}{dt}=\frac{r_1}{Y_{CB}}C-r_2B \tag{4}$$

$$\frac{dG}{dt}=\frac{r_2}{Y_{BG}}B \tag{5}$$

where Y_{CB} and Y_{BG} represent the average yield coefficients of cellulose on the cellobiose and of cellobiose on glucose, respectively. The saccharification rates of cellulose and cellobiose are represented by r_1 and r_2 including the inhibition by W, and the activity of the two enzymes (e_1 and e_2) for cellulase and β-glucosidase is as follows:

$$r_1=r_1^{max}\frac{\frac{C}{K_{1C}}}{1+\frac{B}{K_{1B}}+\frac{G}{K_{1G}}+\frac{E}{K_{1E}}+\frac{C}{K_{1C}}\left(1+\frac{B}{K_{1B}}+\frac{G}{K_{1G}}+\frac{E}{K_{1E}}\right)}(1-I_1W)\left(\frac{e_1}{e_1^{max}}\right) \tag{6}$$

$$r_2=r_2^{max}\frac{\frac{B}{K_{2C}}}{1+\frac{G}{K_{2G}}+\frac{E}{K_{2E}}+\frac{B}{K_{2B}}\left(1+\frac{B}{K_{2BI}}+\frac{E}{K_{2E}}\right)}(1-I_2W)\left(\frac{e_2}{e_2^{max}}\right) \tag{7}$$

where r_1^{max} and r_2^{max} represent the maximum specific saccharification rates of cellulose and cellobiose, respectively. K_{1x} and K_{2x} denote the inhibition constants, and K_{1xI} and K_{2xI} denote the noncompetitive inhibition constants for x, cellulose (C), cellobiose (B), and ethanol (E). These proposed inhibition kinetic equations include the noncompetitive inhibition of cellulase by cellobiose, glucose, and ethanol, and of β-glucosidase by ethanol; the competitive inhibition of β-glucosidase by glucose; and the substrate inhibition by cellobiose [5, 9, 10]. The deactivation of the two enzymes, cellulase and β-glucosidase, can then be expressed as follows:

$$\frac{d(e_1/e_1^{max})}{dt}=-b_1\frac{e_1}{e_1^{max}} \quad (8)$$

$$\frac{d(e_2/e_2^{max})}{dt}=-b_2\frac{e_2}{e_2^{max}} \quad (9)$$

where b_1 and b_2 represent the deactivation constants of cellulase and of β-glucosidase, respectively.

This saccharification model includes the inhibition factors for lignin and akin components, cellulose, cellobiose, and ethanol. In addition, the deactivation of the two key enzymes is introduced.

Fermentation Model

The growth rates of *Brettanomyces custersii* on glucose and cellobiose, r_3 and r_4, were modeled according to the modified Monod rate equation, including the substrate and product inhibition [10]. The synthesis rates of the key enzymes are also modeled based on the Monod equation including substrate inhibition [10], as follows:

$$r_3=r_3^{max}\frac{\frac{G}{K_{3G}}}{1+\frac{E}{K_{3E}}+\frac{G}{K_{3G}}\left(1+\frac{G}{K_{3GI}}\right)}(1-I_3W)\left(\frac{e_3}{e_3^{max}}\right) \quad (10)$$

$$r_4=r_4^{max}\frac{\frac{B}{K_{4B}}}{1+\frac{E}{K_{4E}}+\frac{B}{K_{4B}}\left(1+\frac{B}{K_{4BI}}\right)}(1-I_4W)\left(\frac{e_4}{e_4^{max}}\right) \quad (11)$$

$$\frac{d(e_3/e_3^{max})}{dt}=r_{e3}^{max}u_3\frac{\frac{G}{K_{e3}}}{1+\frac{G}{K_{e3}}}-b_3\frac{e_3}{e_3^{max}} \quad (12)$$

$$\frac{d(e_4/e_4^{max})}{dt}=r_{e4}^{max}u_4\frac{\frac{G}{K_{e4}}}{1+\frac{G}{K_{e4}}}-b_4\frac{e_4}{e_4^{max}} \quad (13)$$

where r_3^{max} and r_4^{max} represent maximum specific saccharification rate of cellulose and cellobiose. r_{e3}^{max} and r_{e4}^{max} represent maximum specific synthesis rates of the key enzymes. b_3 and b_4 are the specific decay rates of the key enzymes.

In the cybernetic modeling framework [2], the cellular regulatory action of the activity and the expression of the key enzymes were introduced into the model equations with the cybernetic variables u_i and v_i, representing optimal strategies for enzyme synthesis and activity, respectively. For instantaneous growth rate r_i along the two available pathways, the optimal strategies for u_i, v_i and the growth rate of the cell r_g are defined as follows:

$$u_i\frac{r_i}{\sum r_i} \quad (i=3, 4) \quad (14)$$

$$v_i\frac{r_i}{\max r_i} \quad (i=3, 4) \quad (15)$$

$$r_g=r_3v_3+r_4v_4 \quad (16)$$

Using these cybernetic variables, the balance equations of glucose, cellobiose, cell mass, and ethanol in a batch reactor are presented as:

$$\frac{dG}{dt}=-\frac{r_3v_3}{Y_{XG}}X \quad (17)$$

$$\frac{dB}{dt}=-\frac{r_4v_4}{Y_{XB}}X \quad (18)$$

$$\frac{dX}{dt}=(r_g-r_d)X \quad (19)$$

$$\frac{dE}{dt}=\alpha\frac{dX}{dt}+\beta X \quad (20)$$

where Y_{XG} and Y_{XB} represent the average yield coefficients of the cell mass on glucose and cellobiose, respectively. The ethanol formation rate consists of a growth-associated and a non-growth-associated term [10].

Simultaneous Saccharification and Fermentation Model

In SSF, glucose and cellobiose, which are consumed as multiple substrates by *Brettanomyces custersii*, are the intermediate products from cellulose. Unlike the previous studies, simultaneous productions and consumptions of the intermediate components are considered for the pathway modeling of the ethanol product process. The complete dynamic models of SSF are as follows:

$$\frac{dC}{dt}=-r_1C \quad (21)$$

$$\frac{dG}{dt}=\frac{r_2}{Y_{BG}}B-\frac{r_3v_3}{Y_{XG}}X \quad (22)$$

$$\frac{dB}{dt}=\frac{r_1}{Y_{CB}}C-r_2B-\frac{r_4v_4}{Y_{XB}}X \quad (23)$$

$$\frac{dX}{dt}=(r_g-r_d)X \quad (24)$$

$$\frac{dE}{dt}=\alpha\frac{dX}{dt}+\beta X \quad (25)$$

SIMULATION METHODS

The processes used for simulation were saccharification, fermentation, and SSF. An optimization technique was used to determine the model parameters. The method is a multidimensional unconstrained nonlinear minimization of the squared error sum between the experimental data and the simulation result, which was based on a modification of the Nelder-Mead algorithm. The experimental data of

saccharification, fermentation, SSF, and relevant methods have been reported elsewhere [5]. The model equations for saccharification, fermentation, and SSF were solved by non-stiff differential equations and the medium order method (fourth-order Runge-Kutta algorithm).

RESULTS AND DISCUSSION

Saccharification

The factors affecting the reduction of saccharification rate include the inhibition of enzymatic reaction by glucose and cellobiose, and the deactivation of cellulase and β-glucosidase. The experimental data for saccharification for three types of feed, such as α-cellulase, ball-milled SEW, and untreated SEW have been reported by Moon *et al.* [5]. Figures 2A and 2B show the results of the parameter

Table 1. The sum of the squared modeling error for saccharification in the Moon's model and the proposed model.

Feed	Moon's	Proposed
α-Cellulose	20.610	5.731
Ball-milled SEW	16.180	4.712
Untreated SEW	6.542	2.986

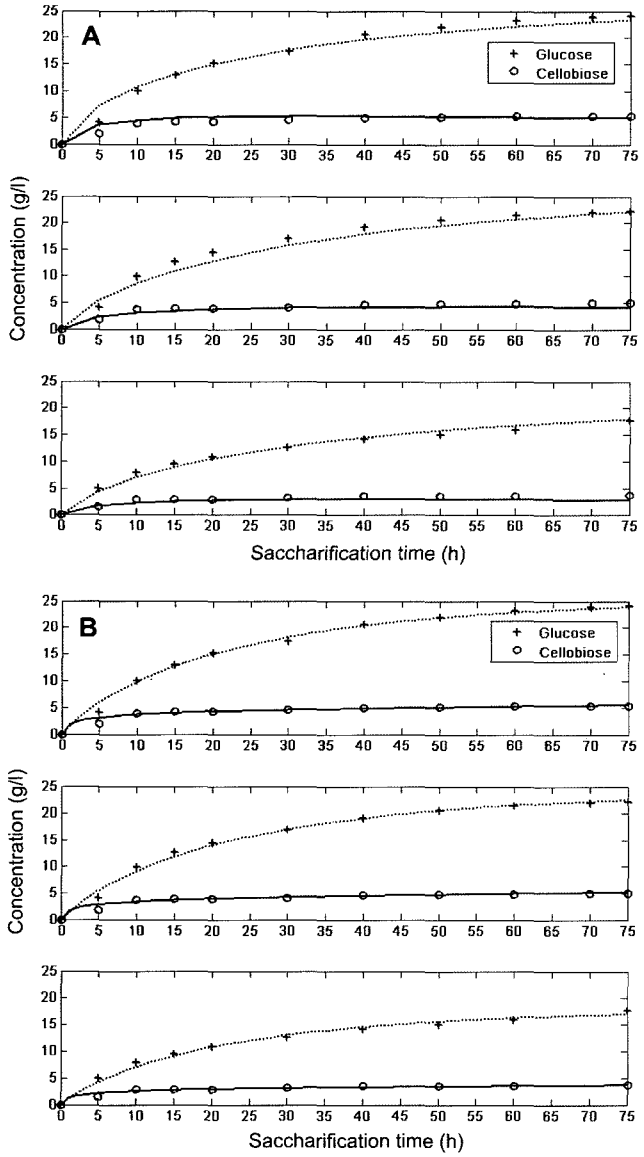

Fig. 2. Simulation result of saccharification for 80 g/l α-cellulose (upper), 80 g/l ball-milled SEW (middle), and 80 g/l untreated SEW (lower) as a substrate feed in the Moon's model (**A**) and the proposed model (**B**) (○: cellobiose; □: glucose).

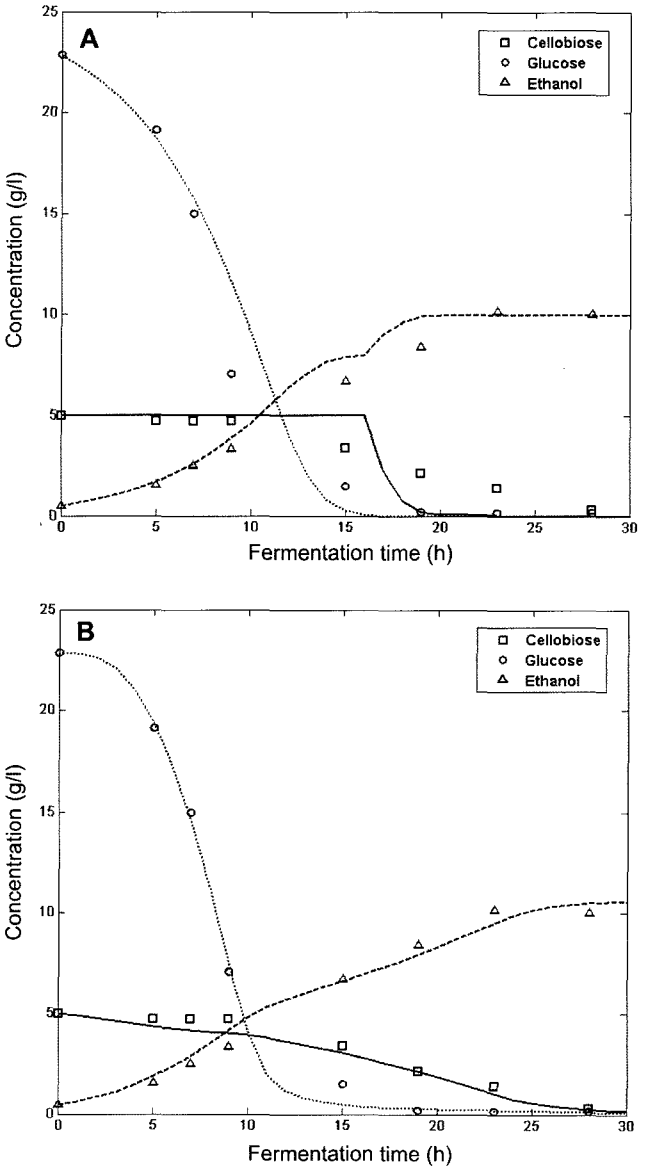

Fig. 3. Simulation result of fermentation for glucose (○), cellobiose (□), and ethanol (△) in the Moon's model (**A**) and the proposed model (**B**).

Table 2. Sums of squared modeling error for fermentation in the Moon's model and the proposed model.

Components	Moon's	Proposed
Cellobiose	4.110	1.833
Glucose	8.660	1.406
Ethanol	22.716	1.190

fitting for the saccharification in Moon's model [5] and the proposed model, respectively. The error between the experimental and simulation data was reduced from 45.62% up to 27.81% by the presently proposed model.

The cellulose content of the SEW was approximately 54.5% of the dry weight. α-Cellulose is composed of approximately 100% cellulose. However, the cellulose content of untreated SEW is unknown. Therefore, it was designated as an optimization variable in order to reduce the square error. Its value was calculated as 28.68 g/l, assuming that the SEW concentration was 80 g/l. Table 1 lists the sums of the squared error for the different feeds.

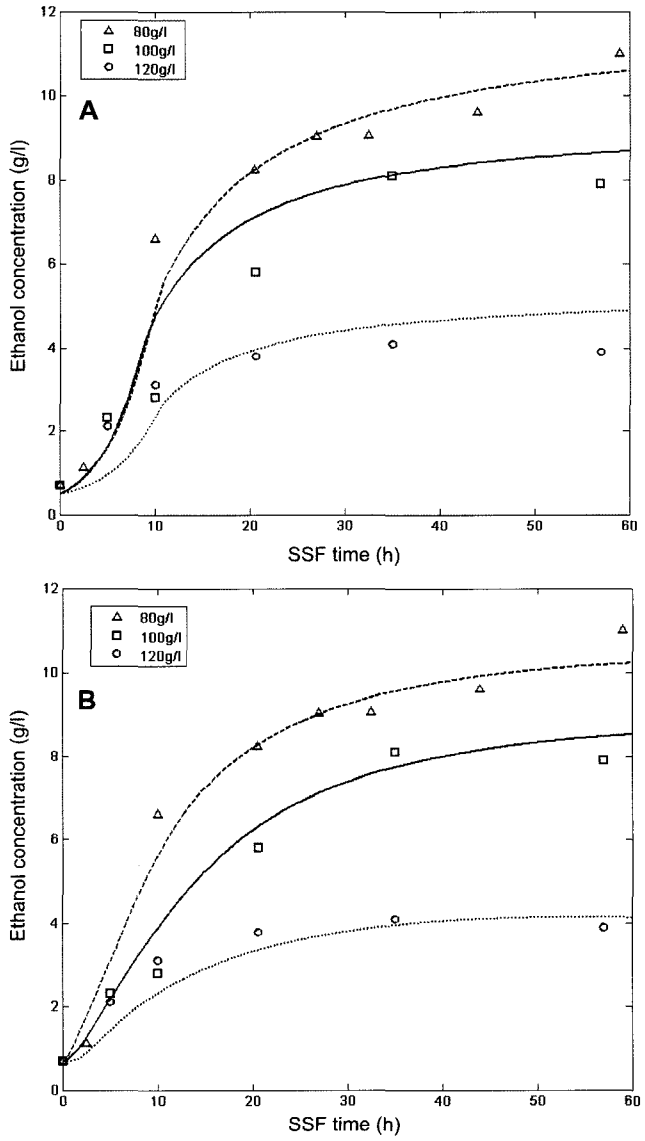

Fig. 4. Simulation result of SSF for 80 g/l (△), 100 g/l (□), and 120 g/l (○) ball-milled SEW in the Moon's model (**A**) and the proposed model (**B**).

Fermentation

Cybernetic modeling was applied to the fermentation model with cellobiose and glucose as substrates. After saccharification, *Brettanomyces custersii* was added to the vessel. Figures 3A and 3B show the experimental data and simulation result of Moon's model [5] and the proposed model, respectively. In Fig. 3, the diauxic growth of ethanol production from the sequential utilization of glucose and cellobiose is seen. Moon *et al.* used the modified Monod equation and artificial parameters to describe the sequential utilization. As shown in Fig. 3A, a change of growth pattern occurred abruptly at 16 h. This unnatural behavior was attributable to the artificial factors: In the case of cybernetic modeling, the diauxic growth could be explained using only two parameters; the enzyme synthesis and activities. Consequently, errors between the experimental and simulation data were reduced from 44.60% to 5.24%, compared with the previous model. Sums of squared error in the modeling for the time profiles of the different components are listed in Table 2. The time profile of the ethanol concentration could be precisely predicted, compared with the result of Moon *et al.*. This result clearly verified the validity of using the cybernetic approach for the fermentation kinetics.

Simultaneous Saccharification and Fermentation

The SSF model combines the saccharification model and fermentation model with ethanol inhibition in saccharification and the combined concentration rates of cellobiose and glucose. Figures 4A and 4B show the ethanol concentration profiles for the different ball-milled SEW feed concentrations of 80 g/l, 100 g/l, and 120 g/l, using Moon's model [5] and the proposed model, respectively. Error between the experimental and simulation data was reduced from 63.02% to 28.55%, compared with the previous model. Sums of squared error of the modeling for different feed conditions are listed in Table 3.

The ethanol concentration decreased as SEW concentration was increased. SEW inhibition can considerably affect

Table 3. Sums of squared modeling error for SSF in the Moon's model and the proposed model.

Feed concentration	Moon's	Proposed
80 g/l ball-milled SEW	3.586	2.260
100 g/l ball-milled SEW	6.672	1.905
120 g/l ball-milled SEW	3.143	1.340

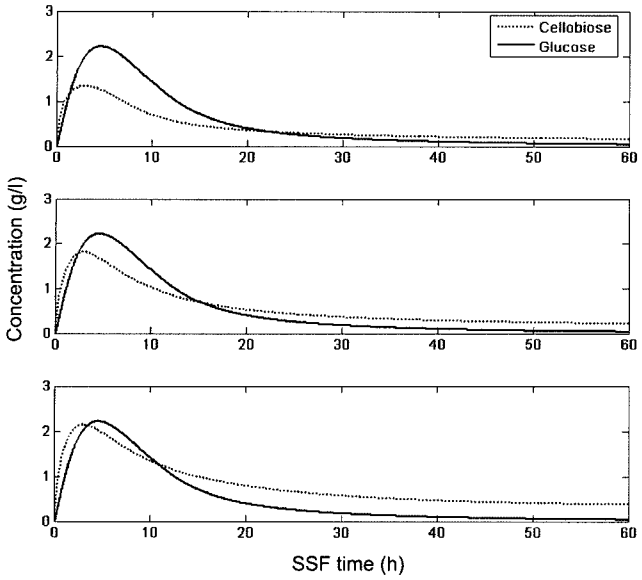

Fig. 5. Cellobiose and glucose concentration profile for 80 g/l (upper), 100 g/l (middle), and 120 g/l (lower) ball-milled SEW in the proposed SSF model.

ethanol production. In SSF, the cellobiose and glucose concentrations are shown in Fig. 5. As the SEW concentration increases, the cellobiose concentration in a vessel increases, but glucose concentration is almost constant. All the model parameters are shown in Table 4. Y_{CB} and Y_{BG} were adopted from previous studies [5].

In this study, the modeling of saccharification was carried out according to the modified Michaelis-Menten kinetics and the specific enzyme inhibition kinetics. In addition, the deactivation of the key enzymes, cellulase and β-glucosidase, was considered. The modeling of fermentation was performed using the Monod equation and the cybernetic modeling approach. Through the cybernetic modeling approach, nonconventional terms, such as exponential factors, and branching logics were not required to explain the peculiar kinetic behavior. The cybernetic approach could satisfactorily predict the kinetic behavior of sequential growth on multiple substrates found in fermentation.

This SSF model could simultaneously account for saccharification, fermentation, and SSF processes within a single framework. This was established by considering the inhibitory effects of SEW, cellulose, cellobiose, glucose, and ethanol. Furthermore, the deactivation of the enzymes and the specific enzymatic characteristics were incorporated in the model. Therefore, a more precise modeling of ethanol production via SSF was possible, and comparisons have been made against the previous results [5].

Table 4. Estimated values of parameters used in simulating the experimental data.

Parameters	Values	Parameters	Values	Parameters	Values
r_1^{max}	3.68e-1	K_{3E}	3.95e-3	r_d	6.20e-4
r_2^{max}	1.91e1	K_{3GI}	2.58e-1	a	4.50e0
r_3^{max}	2.40e-1	K_{4B}	1.89e-4	b	4.20e-5
r_4^{max}	4.56e0	K_{4E}	1.62e-4	b_1	2.20e-2
K_{1C}	8.44e-8	K_{4BI}	8.89e-2	b_2	3.89e-2
K_{1B}	2.83e-1	I_1	3.75e-3	b_3	5.50e-4
K_{1G}	8.91e+3	I_2	2.60e-7	b_4	3.32e0
K_{1E}	2.43e-1	I_3	4.30e-4	r_{e3}^{max}	3.43e-1
K_{2B}	1.08e-2	I_4	1.83e-2	r_{e4}^{max}	6.40e-1
K_{2G}	4.96e-4	Y_{CB}*	1/1.056	K_{e3}	1.20e-6
K_{2E}	2.32e+1	Y_{XB}	3.42e-1	K_{e4}	7.35e-1
K_{2BI}	2.31e-1	Y_{BG}*	1/1.053		
K_{3G}	2.56e-1	Y_{XG}	3.51e-2		

NOMENCLAUTURE

b_1 : deactivation constant of cellulase, h^{-1}
b_2 : deactivation constant of β-glucosidase, h^{-1}
b_3 : deactivation constant of enzyme on glucose fermentation, h^{-1}
b_4 : deactivation constant of enzyme on cellobiose fermentation, h^{-1}
B : cellobiose concentration, g/l
C : cellulose concentration, g/l
e_i : concentration of ith enzyme
e_i^{max} : maximum concentration of ith enzyme
E : ethanol concentration, g/l
G : glucose concentration, g/l
I_1 : inhibition constant of SEW on cellulase, l/g
I_2 : inhibition constant of SEW on β-glucosidase, l/g
I_3 : inhibition constant of SEW on glucose fermentation, l/g
I_4 : inhibition constant of SEW on cellobiose fermentation, l/g
K_{1C} : inhibition constant of cellulase for cellulose, g/l
K_{1B} : inhibition constant of cellulase for cellobiose, g/l
K_{1G} : inhibition constant of cellulase for glucose, g/l
K_{1E} : inhibition constant of cellulase for ethanol, g/l
K_{2B} : inhibition constant of β-glucosidase for cellobiose, g/l
K_{2G} : inhibition constant of β-glucosidase for glucose, g/l
K_{2E} : inhibition constant of β-glucosidase for ethanol, g/l
K_{2BI} : noncompetitive inhibition constant of β-glucosidase for cellobiose, g/l
K_{3G} : inhibition constant on glucose fermentation for glucose, g/l
K_{3E} : inhibition constant on glucose fermentation for ethanol, g/l
K_{3GI} : substrate inhibition constant on glucose fermentation for glucose, g/l

K_{4B} : inhibition constant on cellobiose fermentation for cellobiose, g/l
K_{4E} : inhibition constant on cellobiose fermentation for ethanol, g/l
K_{4BI} : substrate inhibition constant on cellobiose fermentation for cellobiose, g/l
K_{e3} : inhibition constant of enzyme on glucose fermentation, g/l
K_{e4} : inhibition constant of enzyme on cellobiose fermentation, g/l
K_m : Michaelis-Menten constant, g/l
S : concentration of substrate, g/l
r_1^{max} : maximum specific saccharification rate of cellulose, g/l·h
r_2^{max} : maximum specific saccharification rate of cellobiose, g/l·h
r_3^{max} : maximum specific fermentation rate of glucose, g/l: h
r_4^{max} : maximum specific fermentation rate of cellobiose, g/l·h
r_{e3}^{max} : maximum specific enzyme-synthesis rate on glucose fermentation, h^{-1}
r_{e4}^{max} : maximum specific enzyme synthesis rate on cellobiose fermentation, h^{-1}
r_d : specific rate of cell death, g/l·h
r_{ei}^{max} : maximum specific rate of the ith key enzyme, g/l·h
r_i : specific rate, g/l·h
r_g : specific rate of cell growth, g/l·h
u, v : cybernetic variables, dimensionless
X : biomass concentration, g/l
Y_{CB} : average yield coefficient of cellulose on cellobiose, g cellulose /g cellobiose
Y_{XB} : average yield coefficient of cell mass on cellobiose, g cell mass/g cellobiose
Y_{BG} : average yield coefficient of cellobiose on glucose, g cellobiose /g glucose
Y_{XG} : average yield coefficient of cell mass on glucose, g cell mass/g glucose
W : steam-exploded wood concentration, g/l
W_0 : initial steam-exploded wood concentration, g/l

Greek Symbols

α : growth-associated constant, dimensionless
β : non-growth-associated constant, dimensionless

REFERENCES

1. Jones, K. and D. Kompala. 1999. Cybernetic model of the growth dynamic of *Saccharomyces cerevisiae* in batch continuous culture. *J. Biotechnol.* **71:** 105–131.
2. Kompala, D., D. Ramkrishna, N. Jansen, and G. Tsao. 1986. Investigation of bacterial growth on mixed substrate: Experimental evaluation of cybernetic models. *Biotechnol. Bioeng.* **28:** 1044–1055.
3. Lin, J. Q., S. M. Lee, and Y. M. Koo. 2004. Model development for lactic acid fermentation and parameter optimization using genetic algorithm. *J. Microbiol. Biotechnol.* **14:** 1163–1169.
4. Anonymous. 2005. Modeling and simulation of simultaneous saccharification and fermentation of paper mill sludge to lactic acid. *J. Microbiol. Biotechnol.* **15:** 40–47.
5. Moon, H. J., J. S. Kim, K. K. Oh, S. W. Kim, and S. I. Hong. 2001. Kinetic modeling of simultaneous saccharification and fermentation for ethanol production using steam-exploded wood with glucose- and cellobiose-fermenting yeast, *Brettanomyces custersii. J. Microbiol. Biotechnol.* **11:** 598–606.
6. Namjoshi, A. and D. Ramkrishna. 2001. Multiplicity and stability of steady states in continuous bioreactors: Dissection of cybernetic models. *Chem. Eng. Sci.* **56:** 5593–5607.
7. Narang, A., A. Konopka, and D. Ramkrishna. 1997. Dynamic analysis of the cybernetic model for diauxic growth. *Chem. Eng. Sci.* **52:** 2567–2578.
8. Park, S. W., Y. K. Hong, S. W. Kim, and S. I. Hong. 1999. Development of strain fermenting the glucose/cellobiose mixed sugar for simultaneous saccharification and fermentation of cellulosic material. *Korean J. Appl. Microbiol. Biotechnol.* **27:** 145–152.
9. Philippidis, G., T. Smith, and C. Wyman. 1993. Study of the enzymatic hydrolysis of cellulose for production of fuel ethanol by the simultaneous saccharification and fermentation process. *Biotechnol. Bioeng.* **41:** 846–853.
10. Philippidis, G., D. Spindler, and C. Wyman. 1992. Mathematical modeling of cellulose conversion to ethanol by the simultaneous saccharification and fermentation process. *Appl. Biochem. Biotechnol.* **34/35:** 543–556.
11. Ramkrishna, D. 1982 A cybernetic perspective of microbial growth, pp. 161–178. *Foundations of Biochemical Engineering: Kinetics and Thermodynamics in Biological Systems.* American Chemical Society, Washington, DC, U.S.A.
12. Schell, D. and P. Walter. 1991 Simultaneous saccharification and fermentation of corn stover hydrolysate to ethanol, pp. 72, *Biotech. Symp. for Fuels and Chemicals*, Colorado Springs.
13. South, C. R., D. A. L. Hogsett, and L. R. Lynd. 1995. Modeling simultaneous saccharification and fermentation of lignocellulose to ethanol in batch and continuous reactors. *Enzyme Microb. Technol.* **17:** 797–803.
14. Spindler, D., G. Philippidis, and C. Wyman. 1991. Key parameters in simultaneous saccharification and fermentation of biomass to ethanol, pp. 74, *Biotech. Symp. for Fuels and Chemicals*, Colorado Springs.