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Kinetic Modeling of Simultaneous Saccharification and Fermentation for Ethanol Production Using Steam-Exploded Wood with Glucose- and Cellobiose-Fermenting Yeast, *Brettanomyces custersii*

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Abstract A mathematical model is proposed that can depict the kinetics of simultaneous saccharification and fermentation (SSF) using steam-exploded wood (SEW) with a glucose- and cellobiose-fermenting yeast strain, *Brettanomyces custersii*. An expression to describe the reduction of the relative digestibility during the hydrolysis of the SEW is introduced in the hydrolysis model. The fermentation model also takes two new factors into account, that is, the effects of the inhibitory compounds present in the SEW hydrolysates on the microorganism and the fermenting ability of *Brettanomyces custersii*, which can use both glucose and cellobiose as carbon sources. The model equations were used to simulate the hydrolysis of the SEW, the fermentation of the SEW hydrolysates, and a batch SSF, and the results were compared with the experimental data. The model was found to be capable of representing ethanol production over a range of substrate concentrations. Accordingly, the limiting factors in ethanol production by SSF under the high concentration of the SEW were identified as the effect of inhibitory compounds present in the SEW, the enzyme deactivation, and a limitation in the digestibility based on the physical condition of the substrate.

Key words: SSF, ethanol, steam-exploded wood, modeling, inhibitory compounds, cellobiose-fermenting yeast

Simultaneous saccharification and fermentation (SSF) involves the enzymatic saccharification of cellulose and fermentation of sugars by yeast to ethanol in the same vessel. More importantly, this process eliminates the product inhibition on cellulase activities by sugars. This is the mechanism of why SSF offers a high ethanol production compared to separate hydrolysis and fermentation (SHF). Up to now, much research has been conducted to achieve a low enzyme loading, faster rate of hydrolysis, and high production yields in SSF [1, 6, 7, 14, 17]. However, a kinetic model that can describe SSF is needed to design, optimize, and control a SSF process, in which saccharification and fermentation occur. The kinetic modeling of hydrolysis for cellulose to develop a kinetic model of SSF has already been extensively studied. Philippidis *et al.* suggested a mathematical model for SSF using a lignocellulosic biomass as the substrate, by considering the quality of the substrate and enzyme, and the substrate-enzyme-microorganism interactions [19]. The model was found to satisfactorily predict the kinetics of cellulose hydrolysis [20]. Oh *et al.* obtained an optimal temperature profile for maximizing ethanol production using a kinetic model, considering the effect of temperature on the hydrolysis and microorganism growth [13, 14]. However, previous work on the kinetic modeling of SSF has been devoted to an SSF process using mostly pure cellulose as the substrate. Therefore, these kinetic models have never been applied to a lignocellulosic biomass as a real feed-stock for economic ethanol production. South *et al.* developed model equations for a lignocellulosic biomass and validated the predictability of the model over a range of substrate concentration, cellulase loadings, and reactor configuration [21]. However, the model equations are difficult to apply to optimize and/or control a SSF process, because the algorithm to solve these model equations includes an iteration step. The model equations for a SSF process should be simple and amenable to optimize and/or control, while detailed enough to describe the key characteristics of the SSF process.

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Steam-explosion pretreatment has been widely used as an economically competitive pretreatment for a lignocellulosic biomass. The material is heated by high pressure steam for a few minutes and then suddenly released into atmospheric pressure for a steam explosion, thereby expelling and defibricating the lignocellulosic biomass. Unfortunately, hemicellulose and lignin derived products formed during the steam-explosion can inhibit fermentation [2, 12, 15, 23]. In this case, the SSF model should be modified on the basis of the individual substrate properties. The effects of these inhibitory compounds due to pretreatment have never been reported in the kinetic model of SSF.

Brettanomyces custersii has been identified as a promising glucose- and cellobiose-fermenting yeast for SSF from cellulose [18, 22]. A SSF model with the cellobiose-fermenting yeast, *Brettanomyces custersii,* must consider the property of the fermenting yeast.

Accordingly, this paper attempts to improve earlier models of the SSF process and test the adequacy of the modified model for correlating experimental results. SEW as the substrate and *Brettanomyces custersii* as the fermenting yeast were used for the SSF experiments. The model incorporated the effect of the inhibitory compounds and the properties of the yeast strain for the first time.

MATERIALS AND METHODS

Substrates

The oak wood chips used as the substrate were obtained from the Korea Institute of Energy Research (KIER, Daejon, Korea), soaked in 0.5% (w/w) H_2SO_4 solution (Samchun Pure Chemical Industries Ltd., Bucheon, Korea) for 24 h, and then heated by direct steam up to the desired explosion temperature of 215°C, which was maintained for 3 min [9]. Thereafter, the material was fragmented by a sudden release of pressure. The collected sample was then washed thoroughly with tap-water and dried at 80°C for 24 h, and was used as untreated SEW in the current study. Ball-milled SEW was obtained by milling the untreated SEW with a ball-mill (Il Shin Engineering Co., Seoul, Korea) using various sizes of stainless steel balls with diameters ranging from 10–60 mm for 24 h, which resulted in sieved wood flour with a particle size of 0.2 mm or less. The cellulose content of the treated oak wood chips was about 54.5% on a dry weight basis.

Enzymes

Cellulase (Celluclast 1.5-l, Novo Co., Denmark) and β-glucosidase (Novozyme 188, Novo Co., Denmark) were used without further purification. Their activities were 200 FPU (filter paper unit)/ml and 350 CBU (cellobiose unit)/ml, respectively [5].

Microorganism and Media

The *Brettanomyces custersii* H1-39 was derived through genetic improvements of *Brettanomyces custersii* CBS 5512, a promising glucose- and cellobiose-fermenting yeast for the SSF of celluose for ethanol production [18]. It was maintained by transferring each month to fresh agar plates and storing at 4°C. The growth medium for the seed culture contained 10 g/l yeast extract (Difco, Detroit, U.S.A.), 20 g/l bacto peptone (Difco, Detroit, U.S.A.), and 20 g/l glucose (Samchun Pure Chemical Industries Ltd., Bucheon, Korea).

Enzymatic Hydrolysis of SEW

The enzymatic hydrolysis of SEW was run in a 500-ml Erlenmeyer flask with 200 ml working volume at 40°C. The SEW suspension was prepared by mixing each substrate with 0.05 M citrate buffer at pH 4.8. Saccharification was conducted by adding the mixed enzyme solution to SEW slurries at 40°C. Initial substrate concentration of the slurries was 44 g/l, based on the cellulose content of the substrate. Cellulase and β-glucosidase loadings were 30 FPU/g substrate and 18 CBU/g substrate, respectively.

Fermentation with Hydrolysates of SEW

The hydrolysates of SEW were obtained by enzymatic hydrolysis under the same conditions as for the hydrolysis of SEW. The ethanol fermentation of the hydroysates was conducted in a 500-ml Erlenmeyer flask with 200 ml working volume at 40°C. The fermentation medium contained 10 g/l yeast extract and 20 g/l peptone with each hydrolysate. The reducing sugar concentration of hydrolysates was fixed to 28 g/l. The inoculum for the fermentation was prepared by aerobically growing the culture in a flask containing 10 g/l yeast extract, 20 g/l peptone, and 20 g/l glucose for 24 h at 30°C. The fermentation was initiated by adding 10% (v/v) inoculum.

SSFs

The SSFs were conducted in a 500-ml Erlenmeyer flask with 200 ml working volume at 40°C. The medium of the SSFs consisted of 10 g/l yeast extract and 20 g/l peptone with SEW. Cellulase and β-glucosidase loadings were 30 FPU/g substrate and 18 CBU/g substrate, respectively. Temperature was maintained at 40°C during the SSFs. The inoculum was the same as in the batch fermentation of the hydrolysates.

Analytical Methods

The total reducing sugar was determined by the dinitrosalycylic acid (Sigma, St. Louis, U.S.A.) method with glucose as the standard [8]. The glucose was measured by the glucose oxidase/peroxidase method (Glucose-E kit, Yeongdong Pharm. Co., Seoul, Korea). Since glucose and cellobiose were the major components of the reducing sugars, the

amount of cellobiose was estimated by subtracting the amount of glucose from the total reducing sugars. The ethanol concentration in the supernatant was determined by gas chromatography (YongLin M600D, YungLin, Anyang, Korea) equipped with a flame ionized detector using isopropanol (Sigma, St. Louis, U.S.A.) as the internal standard. The microorganism concentration in the fermentation was assayed by measuring absorbance of a sample at 600 nm with a spectrophotometer (Spectronic 20, Spectronic Instruments, Rochester, U.S.A.).

Determination of Model Parameters and Model Simulation

An optimization program subroutine, LMDIF1, that minimizes the sum of the squares of the nonlinear functions in the variables, which were based on a modification of the Levenberg-Marquardt algorithm, was used for estimating the model parameters [4]. The appropriate forms of expression were regressed to the collected kinetic data using nonlinear regression algorithms to determine the parameters and examine the predictive ability of the respective model equation. The Runge-Kutta fourth-order method was used to solve the ordinary differential equations describing the kinetic model of the SSF used in this study.

RESULTS AND DISCUSSION

In general, SSF consists of two different models, a hydrolysis model and a fermentation model. The hydrolysis model of SSF is based on the combination of enzyme adsorption and Michaelis-Menten kinetics. Typically, the microbial fermentation model is based on the Monod equation with ethanol inhibition. The noncompetitive inhibition of cellulase by cellobiose and glucose, and β-glucosidase by glucose, plus growth inhibition by ethanol are included in the previous model equations [20]. However, the inhibitory effect of the complex compounds, existing in the SEW, on the microorganism and cellobiose-fermenting ability of *Brettanomyces custersii* has not been considered and the reduction of the relative digestibility has been neglected in the previous model. Therefore, a modified kinetic model for SSF from SEW is suggested in the present work. The reaction schemes for the proposed and previous models are shown in Fig. 1.

Hydrolysis of SEW

The physical properties of the substrate used for SSF affect the hydrolysis of cellulose. The enzymatic hydrolysis of β-cellulose, untreated SEW, and ball-milled SEW was carried out as shown in Fig. 2. The resulting reducing sugar concentrations from the runs of α-cellulose, ball-milled SEW, and untreated SEW were 31.5, 26.7, and 21.5 g/l,

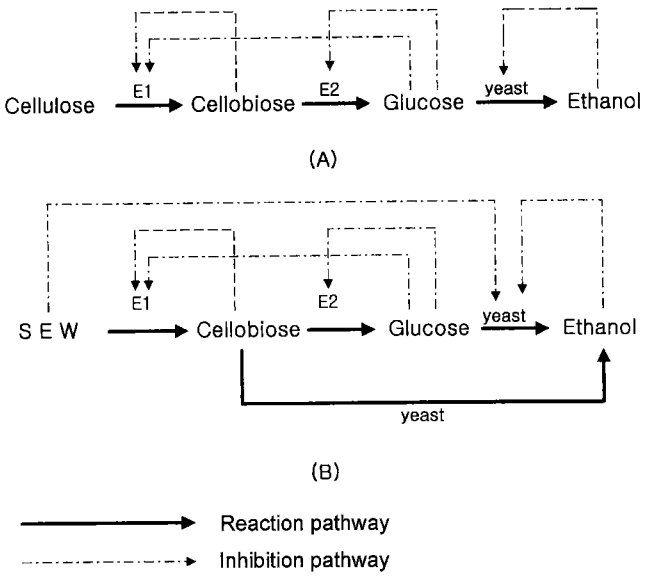

Fig. 1. Simplified schematic representation of the SSF reaction sequence.
E1, cellulase; E2, β-glucosedase. (A) previous model; (B) proposed model.

respectively. These values were, respectively, 71.6, 60.7, and 48.8% of the theoretical yield. In the case of hydrolysis of untreated SEW, the glucose yield was actually lower than the others due to poor accessibility of the substrate to the enzymes. For the ball-milled SEW, the digestibility of the substrate was improved by the ball-milling pretreatment. These results clearly indicated that the physical condition of the substrate during the hydrolysis must be considered in the model equations.

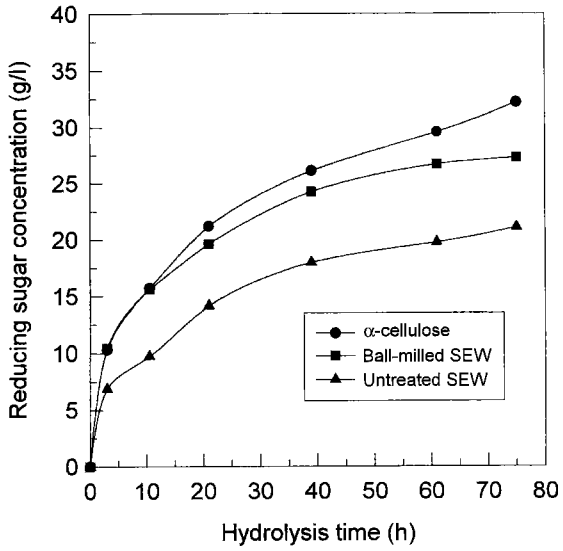

Fig. 2. Reducing sugar concentration in hydrolysis of α-cellulose, ball-milled SEW, and untreated SEW at 40°C.
Substrate concentration, 40 g cellulose/l; cellulase activity, 30 FPU/g substrate.

Hydrolysis Model

The mass balance equations are as follows [20].

The cellulose concentration:

$$\frac{dC}{dt}=-r_1 \quad (1)$$

The cellobiose concentration:

$$\frac{dB}{dt}=1.056r_1-r_2 \quad (2)$$

The glucose concentration:

$$\frac{dG}{dt}=1.053r_2 \quad (3)$$

with the rates of the hydrolytic action of cellulase and β-glucosidase represented by r_1, and r_2, respectively. Certain modifications to express the character of SEW were required in this model as follows.

The hydrolysis rate of cellulose:

$$r_1=\frac{k_1C[1-M\{(C_0-C)/C_0\}^N]E_1\exp(-\lambda_1 t)}{\{K_E+E_x\exp(-\lambda_1 t)\}(1+B/K_{1B}+G/K_{1G})} \quad (4)$$

The hydrolysis rate of cellobiose:

$$r_2=\frac{k_2B\exp(-\lambda_2 t)}{K_m(1+G/K_{2G})+B} \quad (5)$$

The model equations suggested by Philippidis *et al.* include noncompetitive inhibition of cellulase by cellulose and glucose, noncompetitive inhibition of β-glucosidase by glucose, substrate inhibition by cellobiose, and enzyme deactivation. The lignin concentration is fixed as constant, because lignin is not degraded under the employed SSF conditions. This model failed to correctly predict the long-term profiles of cellulose and glucose as it neglects the time-dependent variation in the physical properties substrate. In reality, the hydrolysis rate decreases still further owing to the transformation of cellulose into a less digestible form [10]. The effect of the structural transformation was examined by using spent as the substrate. The spent substrates were obtained after the hydrolysis of the substrates (α-cellulose, untreated SEW, and ball-milled SEW) with different reaction times. The initial hydrolysis rates were calculated by fitting the hydrolysis results for several batches of spent substrate to polynomials and then differentiating, as shown in Fig. 3. The conversion of cellulose increased as the recovery time of spent substrate increased. All plots of the initial hydrolysis rate versus the fractional conversion showed a decline in the initial hydrolysis rate as the conversion increased. This clearly indicates that the spent substrate was less susceptible to enzymatic hydrolysis. In the case of the ball-milled SEW, the digestibility decreased drastically within 20 h of hydrolysis,

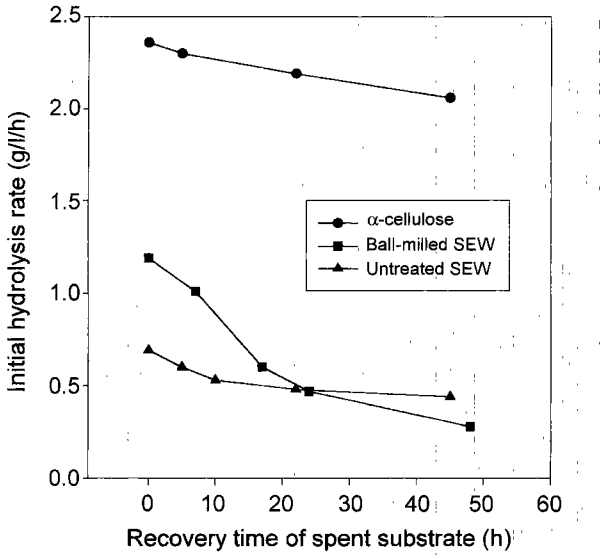

Fig. 3. Reduction of initial hydrolysis rate during hydrolysis of α-cellulose, ball-milled SEW, and untreated SEW.

and thereafter, it decreased gradually. Lee *et al.* introduced the notion of the relative digestibility of spent cellulose, φ, to lump all the structural features that contribute to the transformation of cellulose into a less digestible form [10]. φ is defined as the ratio between the hydrolysis rate of the spent cellulose and that of the original cellulose, and then correlated with the extent of conversion. The relationship between the relative digestibility and the degree of conversion of the lignocellulosic substrates was affirmed in Fig. 4. The term of $[1-M\{(C_0-C)/C_0\}^N]$ was introduced to express the reduction in the relative digestibility during hydrolysis

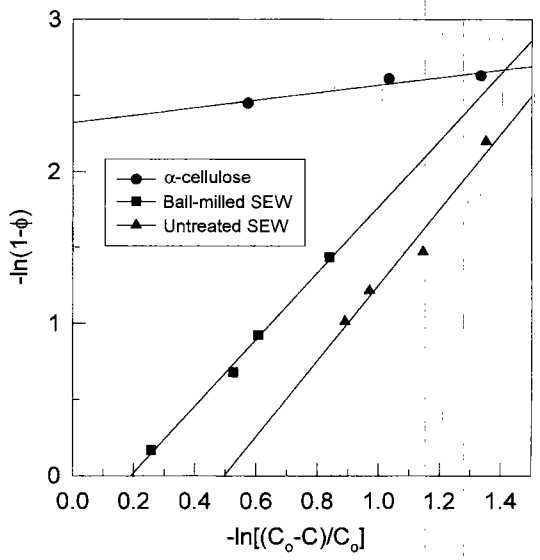

Fig. 4. Relationship between relative digestibility and degree of conversion of various substrates (α-cellulose, ball-milled SEW, and untreated SEW).

of SEW. This expression is slightly different from that suggested by Lee *et al.* [9] Two heuristic parameters, M and N, which correspond to the intercept and slope of the plot, respectively, were estimated by nonlinear regression. These parameters probably depend strongly on the initial structural features of the substrate.

Determination of Parameters used in Hydrolysis Model Equations

In the above hydrolysis model, the enzyme deactivation constants (λ_1, λ_2), product inhibition constants (K_{1B}, K_{1G}, K_{2G}), and parameters in the Michaelis-Menten equation for cellobiose degradation to glucose (k_2, K_m) can be determined regardless of the substrate conditions. Since these parameters are not sensitive to the substrate conditions, this model can adapt the values from the literatures [13, 14] when the same commercial enzymes (Celluclast 1.5-l and Novozyme 188) have been used for determining the parameters. The specific rate of hydrolysis for cellulose (k_1), the adsorption constant (K_E), and the two constants expressed for the reduction of the relative digestibility (M, N), are closely connected with the initial substrate conditions. The specific rate of hydrolysis for cellulose (k_1) and adsorption constant (K_E) were estimated using an optimization program that minimized the sum of the squares of the nonlinear functions in the variables based on modification of the Levenberg-Marquardt algorithm under each substrate condition. The results of the parameter fitting for the α-cellulose, ball-milled SEW, and untreated SEW hydrolysis data are given in Fig. 5. Parameters M and N were also estimated by nonlinear regression (Fig. 4). The parameter values used in the hydrolysis model are listed in Table 1.

Factors Affecting the Hydrolysis Rate

The factors affecting the reduction of the hydrolysis rate considered in the proposed model include the inhibition of the enzymatic reaction by glucose and cellobiose, the deactivation of cellulase, and the reduction in the relative digestibility.

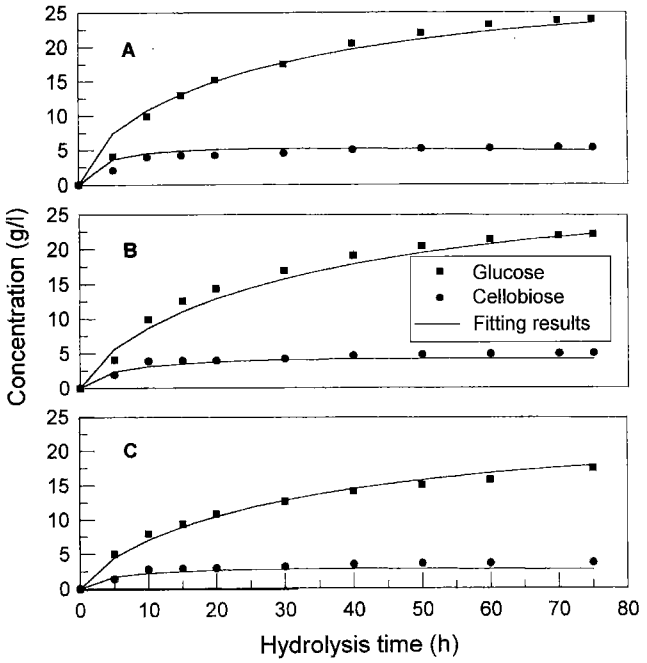

Fig. 5. Parameter fitting for α-cellulose (A), ball-milled SEW (B), and untreated SEW (C) hydrolysis data.
A: k_1=0.312, K_E=41.2; B: k_1=0.283, K_E=45.1; C: k_1=0.122, K_E=45.1.

Figure 6 shows the effects of these above-mentioned factors with regard to developing a hydrolysis model for the hydrolysis of SEW. Among the factors, inhibition by glucose is the major one, whereas the relative digestibility has a minimal effect on the hydrolysis compared with the other factors. In the SSF process, if the concentration of glucose and cellobiose were sustained at a low value, the deactivation of cellulase and reduction of the relative digestibility must be minimized to improve the hydrolysis of SEW. Therefore, both the development of cellulase whose activity can be maintained and an economical physical pretreatment are required to improve the yield of the hydrolysis in the SSF process.

Table 1. Model parameters of hydrolysis for SSF of α-cellulose, untreated SEW, and ball-milled SEW.

Parameters	Values			Sources
	α-cellulose	Untreated SEW	Ball-milled SEW	
k_1 (1/h)	0.665	0.271	0.628	This work
k_2 (g/IU/h)	52.4	52.4	52.4	Oh *et al.* [14]
K_E (IU)	45.1	45.1	45.1	This work
K_M (g/l)	0.765	0.765	0.765	Oh *et al.* [14]
K_{1G} (g/l)	12.3	12.3	12.3	Oh *et al.* [14]
K_{1B} (g/l)	6.04	6.04	6.04	Oh *et al.* [14]
K_{2G} (g/l)	9.28	9.28	9.28	Oh *et al.* [14]
λ_1 (1/h)	0.0232	0.0232	0.0232	Oh *et al.* [14]
λ_2 (1/h)	0.0144	0.0144	0.0144	Oh *et al.* [14]
M (dimensionless)	0.0840	3.454	1.516	This work
N (dimensionless)	0.2098	2.490	2.184	This work

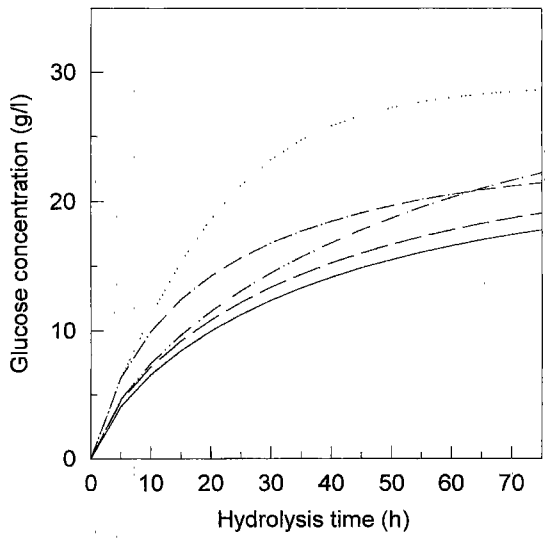

Fig. 6. Effects of various factors on the hydrolysis of untreated SEW.
—— All factors regarded; – – – reduction of digestibility not considered; –·–· deactivation of enzyme not considered; –··– inhibition by cellobiose not considered; ····· inhibition by glucose not considered.

Fermentation Model

A major problem in the bioconversion of lignocellulosic materials is the poor accessibility of the enzymes to the carbohydrate polymer. This is why pretreatment is required to increase the conversion yields of polysaccharides into monosaccharides. Although steam explosion pretreatment is one such processes that can satisfy this requirement, the by-products formed through this pretreatment work as inhibitors when the fermentation of the SEW hydrolysates occurs, thereby affecting the yeast growth and ethanol production. The inhibitors can be classified into lignin-derived phenolic compounds and sugar degradation products. The effect of inhibitors, such as vanillin and furfural, has been established as the limiting factors in the SSF process. In a traditional sense, the concentration of these toxic compounds increases as the substrate concentration in the SSF medium is increased. Yet, since the commercial enzyme solution also contains such compounds, it is important to evaluate the effect of the enzyme loading on the ethanol production in SSF. Accordingly, a series of runs were conducted under various enzyme loadings, 15–50 FPU/g substrate. The enzyme loading was found not to affect the ethanol production, and the maximum ethanol concentration was obtained within 10 h in all the runs performed (data not shown). These results suggest that reductions in the growth rate and production rate were related only to SEW concentration.

A linear dependency of reduction in the maximum specific growth rate in the presence of inhibitory compounds (μ_{max}) and the maximum specific production rate in the presence of inhibitory compounds (π_{max}) relative to the SEW concentration (W) was assumed. The model equation for μ_{max} and π_{max} is expressed in eqns (1) and (2) introducing parameters I and I' to lump all the inhibitory effects.

$$m_{max}=m_0(1-IW) \tag{6}$$

$$\pi_{max}=\pi_0(I-I'W) \tag{7}$$

where μ_0 is the maximum specific growth rate of the cells in the absence of inhibitory compounds and π_0 is the maximum production rate in the absence of inhibitory compounds. In additional consideration, the fermentation model in the SSF model is expressed as follows, deliberating the cellobiose-fermenting ability of *Brettanomyces custersii*.

The cell mass based on the uptake of glucose as the substrate:

$$\left(\frac{dX}{dt}\right)_G=\frac{\mu_0 GX}{K_S+G}\left(1-\frac{P}{P_m}\right)(1-IW) \tag{8}$$

The model in this study assumed that the specific growth rate of the microoganism due to cellobiose was the same value as that due to glucose, although the actual specific growth rates are different from each other.

The cell mass by uptake of cellulose as a substrate:

$$\left(\frac{dX}{dt}\right)_B=\frac{\mu_0 BX}{K_S+B}\left(1-\frac{P}{P_m}\right)(1-IW) \tag{9}$$

The glucose concentration:

$$\frac{dG}{dt}=-\frac{1}{Y_{X/G}}\left(\frac{dX}{dt}\right)_G \tag{10}$$

The cellobiose concentration:

$$\frac{dB}{dt}=-\frac{1}{Y_{X/B}}\left(\frac{dX}{dt}\right)_B \tag{11}$$

Brettanomyces custersii uses glucose and cellobiose in ethanol production, as such, the ethanol concentration can be expressed as follows:

$$\frac{dP}{dt}=\frac{\pi_0(B+G)X}{K_p+B+G}\left(1-\frac{P}{P'_m}\right)(1-I'W) \tag{12}$$

Determination of Parameters used in Fermentation Model Equations

In the fermentation model, the ethanol yield ($Y_{X/G}$, $Y_{X/B}$) was determined from the fermentation results using SEW hydrolysate. The maximum specific growth rate in the absence of inhibitory compounds (μ_0) and maximum production rate in the absence of inhibitory compounds (π_0) were determined from the fermentation results using α-cellulose hydrolysate. The other parameters in the fermentation model were also estimated using the optimization program. The parameter values used in the fermentation model are listed in Table 2. To validate the expression of μ_{max} and π_{max} for the reduction of fermentability by the inhibitors, the sensitivities of K_G and K_P were checked. K_G and K_P were

Table 2. Model parameters of fermentation for SSF of untreated SEW and ball-milled SEW.

Parameters	Values		Sources
	Untreated SEW	Ball-milled SEW	
μ_0 (1/h)	0.24	0.24	This work
π_0 (1/h)	0.45	0.45	This work
K_G (g/l)	0.45	0.45	This work
K_P (g/l)	0.61	0.61	This work
$Y_{X/G}$ (dimensionless)	0.127	0.127	This work
$Y_{X/B}$ (dimensionless)	0.127	0.127	This work
P_m (g/l)	80.0	80.0	Levenspiel [11]
P'_m (g/l)	100.0	100.0	Levenspiel [11]
I (l/g)	0.0048	0.0048	This work
I' (l/g)	0.00919	0.00919	This work

found not to be sensitive parameters affecting ethanol production and cell growth in the SSF modeling (data not shown). The assumption of this model, that inhibitors only affect μ_{max} and π_{max}, is reasonable when considering the reduction of fermentability due to inhibitory compounds present in the SEW.

Cellobiose-Fermenting Ability of *Brettanomyces custersii*

Brettanomyces custersii has been identified as a promising glucose- and cellobiose-fermenting yeast for the SSF of cellulose [22]. In a SSF model with a cellobiose-fermenting yeast, such as *Brettanomyces custersii,* the property of the fermenting yeast must be considered. The ability of the proposed model to describe ethanol fermentation with SEW hydrolysate is shown in Fig. 7. The match between the model prediction and the experimental data was satisfactory for the glucose and ethanol concentrations. However, there was a slight difference in the cellobiose concentration. This deviation in the consumption rate of cellobiose occurred because the actual specific growth rate based on the uptake of cellobiose is different from that based on the uptake of glucose. The exact fit for the cellobiose profile was clearly superficial. The cellobiose concentration in the SSF was maintained at a low value, therefore, the consumption rate of cellobiose did not seriously affect the ethanol production rate. The trend in the sugar consumption indicated that cellobiose was slowly consumed after a sharp decrease of glucose. When considering the characteristic of cellobiose-fermenting yeast, the simulation results matched well with the experimental data.

Relation Between Substrate Concentration and Inhibitory Compounds

Three levels of ball-milled SEW concentrations (80, 100, and 120 g/l) were investigated to evaluate the validity of the kinetic model, including inhibitors. The effect of the substrate concentration on ethanol production in SSF is shown in Fig. 8. Although the initial substrate concentration was increased, the ethanol production inversely decreased. This might have been due to severe conditions of the steam-explosion treatment. The result reaffirmed a directly proportional relationship between the substrate concentration and the amount of inhibitors. The results predicted by the proposed model, which considered inhibitory effects, agreed well with the experimental results when using 80 g/l ball-milled SEW. However, the ethanol concentration was grossly overestimated in the case of 100 and 120 g/l ball-milled SEW. Consequently, the assumption that the dependency of reduced growth is linear relative to the substrate concentration might not be correct. This could

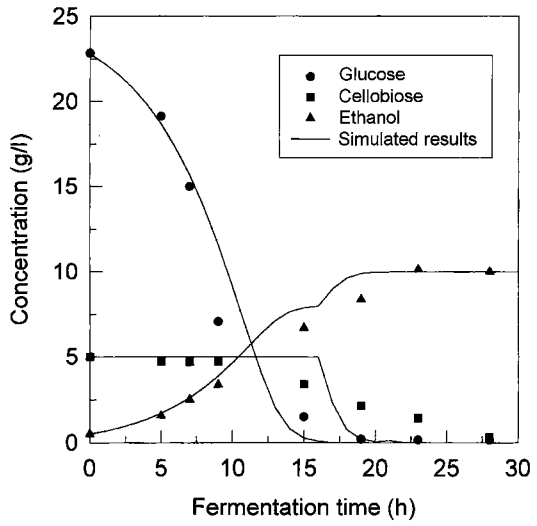

Fig. 7. Ethanol fermentation using ball-milled SEW hydrolysate.

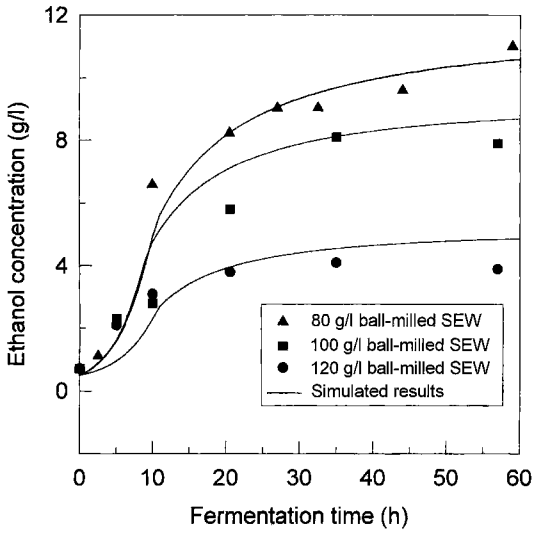

Fig. 8. Ethanol production by SSF using 80, 100, and 120 g/l ball-milled SEW.

explain the difference between the predicted value and the actual value. In addition to this explanation, the inhibition of the hydrolysis by the inhibitors produced during the steam treatment or increased enzyme adsorption to lignin as the substrate concentration increased may also be causes of the overestimation.

The inhibitory compounds present in the SEW can be removed by simple washing with water [12]. However, after washing the SEW, the inhibitory compounds still remained in the SEW. Furthermore, the washing of SEW also removes soluble pentose or pentosans derived from the hemicellulose, thereby substantially decreasing the yield of the overall conversion process [12]. The inhibitory compounds can be kept at a lower concentration if the conditions of the steam explosion are mild. Yet, the yield of the hydrolysis will be decreased because the structure of the SEW will be more difficult to hydrolyze by cellulase.

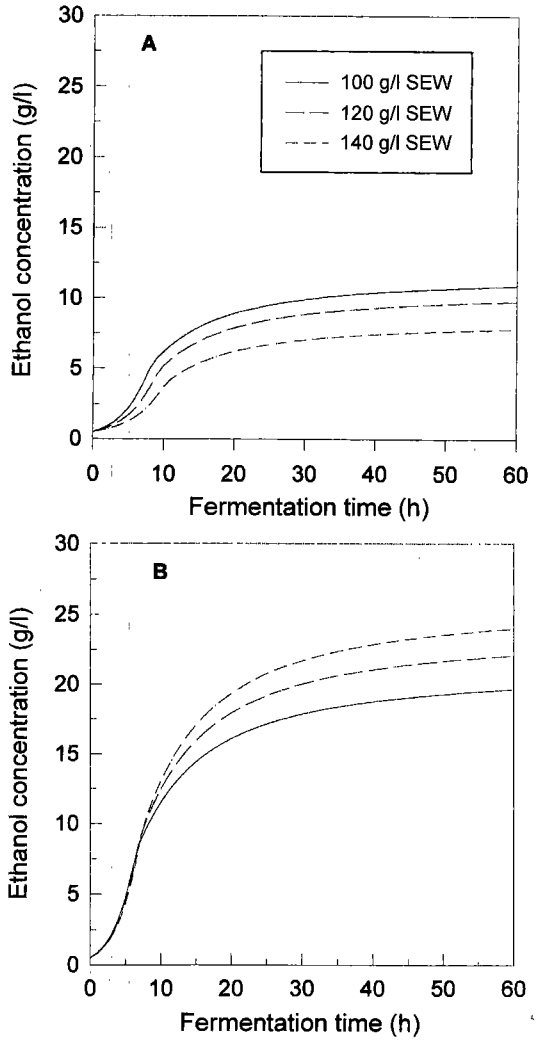

Fig. 9. Effect of inhibitory compounds on ethanol production by SSF using a high concentration of substrate.
A: I=0.002. I'=0.005; B: I=0.0005, I'=0.002.

It has already been observed that the total glucose yield increased as the pretreatment severity increased [3]. Accordingly, there needs to be a compromise between maximizing the efficiency of the hydrolysis with more severe steam treatment and minimizing the negative effect of toxic compounds on the fermentability of the cells. The inherent increase of toxic compounds with an increasing substrate concentration can not be avoided. Therefore, one of the crucial limiting factors in ethanol production by SSF, when using a high concentration of SEW, was found to be the effects of the inhibitory compounds present in the SEW on the fermentation. I and I' indicate the level of the inhibitory effect on SSF by toxic compounds present in the substrate. The proposed fermentation model can be used in the basic design of a detoxification process to remove any inhibitory compounds or to determine the pretreatment conditions. The addition of activated charcoal, extraction with organic solvents, ion-exchange, ion-exclusion, molecular sieves, overliming, and stripping have been investigated for detoxification processes [16]. Detoxification will cause lower values for I and I' in the proposed fermentation model. Figure 9B shows the ethanol concentration with an increasing substrate concentration when the values of I and I' were changed in the SSF model simulation. The ethanol concentration will not increase with an increasing substrate concentration until I and I' reach a certain value. There will be a critical value of I and I' to increase ethanol concentration with increasing substrate concentration. Overall, the SSF model, including parameters such as inhibitory compounds, can offer a guideline to determine the extent of detoxification or the severity of pretreatment like the duration and temperature of the steam treatment.

NOMENCLATURE

B : concentration of cellobiose [g/l]
C : concentration of cellulose [g/l]
C_0 : initial concentration of cellulose [g/l]
E1 : concentration of adsorbed cellulase [g/l]
E2 : free β-glucosidase concentration [g/l]
G : concentration of glucose [g/l]
I, I' : constant defined in eqns. (6) and (7) [l/g]
k_1 : specific rate of hydrolysis for cellulose [1/h]
k_2 : specific rate of hydrolysis for cellobiose [g/l IU h]
K_E : adsorption constant [1/IU]
K_{1B} : inhibition constant of cellulase for cellobiose [g/l]
K_{2B} : inhibition constant of β-glucosidase for cellobiose [g/l]
K_G : Monod saturation constant for cell growth [g/l]
K_{1G} : inhibition constant of cellulase for glucose [g/l]
K_{2G} : inhibition constant of β-glucosidase for glucose [g/l]
K_m : Michaelis constant of β-glucosidase [g/l]
K_p : Monod saturation constant for ethanol production [g/l]

M, N : constants in eq. (4) [dimensionless]
P : concentration of ethanol [g/l]
P_m : concentration of ethanol above which cells do not grow [g/l]
P'_m : concentration of ethanol above which cells do not produce [g/l]
r_1 : volumetric rate of cellulose utilization [g/l/h]
r_2 : volumetric rate of cellobiose utilization [g/l/h]
W : steam-exploded wood concentration [g/l]
X : cell concentration [g/l]
Y : yield coefficient for cells on substrate [dimensionless]

Greek Letters

λ : specific rate of enzyme deactivation [1/h]
μ_{max} : maximum specific growth rate in the presence of inhibitory compounds [1/h]
μ_0 : maximum specific growth rate of cells in the absence of inhibitory compounds [1/h]
π_{max} : maximum production rate in the presence of inhibitory compounds [1/h]
π_0 : maximum production rate in the absence of inhibitory compounds [1/h]
ψ : ratio between the hydrolysis rate of spent cellulose and of the original cellulose [dimensionless]

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