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Kinetic Modeling of Submerged Culture of *A. blazei* with Mixed Carbon Sources of Glucose and Dextrin

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Abstract A mathematical model has been proposed for the batch culture of *Agaricus blazei* with mixed carbon sources of glucose and dextrin. In the proposed model, the metabolism of *A. blazei* was divided into three parts: cell growth, exopolysaccharides (EPS) production, and another EPS production pathway activated by dextrin hydrolysis. Each pathway was described mathematically and incorporated into the mechanistic model structure. Batch cultures were carried out with six different carbon source compositions. Although parameters were estimated by using the experimental data from the two extreme cases such as glucose only and dextrin only, the model represented well the profiles of glucose, cell mass, and EPS concentrations for all the six different carbon source mixtures, showing a good interpolation capability. Of note, the lag in EPS production could be quite precisely predicted by assuming that the glucose-to-cell mass ratio was the governing factor for EPS production.

Key words: Mechanistic model, *Agaricus blazei*, mixed carbon sources

Having been reported to have an over 90% inhibition rate against Sarcoma 180 in mice, β-D-glucans from *Agaricus blazei* cell wall have received a great deal of attention as new bioactive molecules [3, 7, 9], and there is a great need to supply the market with a large amount of high quality *A. blazei* products.

A. blazei has normally been produced by solid culture, using substrates such as grain, sawdust, or wood. It usually takes several months to cultivate fruiting bodies of *A. blazei*, and product quality control is very difficult. Therefore, the production of glucans with an economically feasible productivity is hardly expected with solid culture.

For these reasons, submerged culture of *A. blazei* mycelia has recently been considered as a promising alternative for efficient production of glucans. Submerged culture has potential advantages of higher mycelial production in a compact space and shorter incubation time with lesser chance of contamination [7]. In addition, exopolysaccharides (EPS), which are also glucans and known to have biological effects, can be concurrently produced and secreted. The recovery of EPS from the culture broth requires a relatively simple process, and therefore, is less costly than the recovery of glucans from mycelia [1]. However, there still remain several problems to be solved. Although many investigators have attempted to obtain optimal submerged culture conditions for mycelial growth and EPS production by several fungi including *A. blazei*, the kinetic details of submerged cultures have not yet been clearly demonstrated [2].

In dealing with a number of engineering problems including culture condition optimization, an accurate kinetic model is necessary. The metabolism involved in *A. blazei* growth and polysaccharides synthesis is very complicated, and therefore, has not yet been well identified. In our previous studies, it was found that the glucose concentration at the beginning of the culture increased when glucose and dextrin together were used as carbon sources, and EPS productivity was higher than that with glucose as the sole carbon source [8, 10]. A significant time lag in EPS production was observed. Such complex nature of *A. blazei* culture makes it difficult to develop a mathematical model. Consequently, no model has thus far been reported for *A. blazei* culture. In this work, a mechanistic model was developed for submerged batch culture of *A. blazei*. The model was

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designed to represent the kinetics of cell growth, EPS formation, and dextrin hydrolysis.

MATERIALS AND METHODS

Microbial Strain and Stock Culture

A. blazei was kindly provided by the Rural Development Administration, Korea. Ten-ml aliquots of its mycelium suspension in 20% glycerol were stored at −80°C.

Culture Media and Conditions

The medium composition for the seed culture was (per liter) glucose, 20 g; soybean oil, 30 ml; yeast extract, 4 g; soytone peptone, 2 g; KH_2PO_4, 2 g; $MgSO_4 \cdot 7H_2O$, 0.6 g; and $FeCl_3 \cdot 6H_2O$, 0.2 mg. A 250-ml Erlenmeyer flask containing 40 ml of the seed medium was inoculated with 10 ml of mycelium suspension from the stock culture, and the culture was incubated at 28°C and 150 rpm in a rotary shaker for 5 days. Ten ml of seed culture was transferred to 90 ml of the seed culture medium in a 500-ml Erlenmeyer flask for further activation. After 2 days of incubation, the second seed culture was transferred to a 5-l jar fermentor (KoBiotech., Korea) with a working volume of 3 l. The inoculum size was 10% (v/v). The medium composition for the bioreactor culture was (per liter) glucose and dextrin, 30 g; yeast extract, 4 g; soytone peptone, 2 g; KH_2PO_4, 2 g; $MgSO_4 \cdot 7H_2O$, 0.6 g; and $FeCl_3 \cdot 6H_2O$, 0.2 mg. The temperature was maintained at 28°C, and pH was controlled at 5.0 with 2 N HCl or 2 N NaOH.

Analytical Methods

Cell concentration was determined by measuring dry cell weight (DCW). The concentration of glucose was determined by a glucose analyzer (YSI 2700, Yellow Spring Instrument, OH, U.S.A.). For quantitative analysis of EPS concentration, samples taken from the fermentor were filtered with a filter paper (Whatman #1, Whatman Inc., NJ, U.S.A.), and the filtrate was further filtered by using a membrane filter (0.2 μm, Millipore). The resulting filtrate was analyzed by a high-performance liquid chromatograph (HPLC) (L6200, Hitachi Co., Japan) with a GPC column [Ultrahydrogel 1000 column (0.78×30 cm), Waters Co., MA, U.S.A.] and an evaporated light scattering detector (ELSD) (SEDEX 75, Sedere Co., France).

RESULTS AND DISCUSSION

Batch Fermentation Experiments

In order to investigate the effect of glucose/dextrin (G/D) ratio on cell growth, glucose uptake, and EPS production by *A. blazei*, batch cultures with 30 g/l of carbon source(s) were conducted at six different G/D ratios of 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5. The time profiles of cell mass, residual glucose, and EPS concentrations are given in Figs. 1A through 1F. Two distinct phases of cell growth and EPS production were observed in all the cases. After the cell growth phase, where cell mass increased exponentially and EPS production was repressed, a period of EPS production ensued, accompanied by a repressed cell growth. Such a pattern was observed independent of the G/D ratio. The accumulation of EPS showed a trend similar to the cell growth. The final EPS concentration ranged from 2.06 g/l at a G/D ratio of 5:0 to 4.27 g/l at a G/D ratio of 1:4. The EPS production rate was proportional to the cell growth rate, as clearly demonstrated in Fig. 2. The EPS yields with the dextrin-containing media were higher than those with the glucose-only medium, suggesting that the dextrin-hydrolyzing activity of cells rather than dextrin itself might have activated another EPS production pathway.

Glucose concentration in the media with G/D ratios of 4:1 and 3:2 (Fig. 1B and 1C) did not decrease from the beginning of the culture. It even increased at the beginning, when the ratio was 2:3, 1:4, or 0:5 (Fig. 1D–1F). In all of these cases, the glucose concentration began to rapidly decrease after dextrin was depleted. It is highly possible that glucose formation from dextrin hydrolysis by the cells and glucose consumption occurred concurrently, and that the rate of dextrin hydrolysis was greater than that of glucose consumption.

In order to further examine the effects of total carbon concentration and G/D ratio on the length of lag time in EPS production, another series of batch cultures were conducted (Table 1). As indicated in Table 1, the lag time was larger, when the initial concentration of carbon source was high. The G/D ratio showed practically no effects.

Development of a Model Structure

The model was developed, based on the following hypotheses and assumption, reflecting information obtained in the experimental study mentioned earlier.

1. The key glucose metabolism of *A. blazei* as the sole carbon source consists of two parts: cell growth with the repression of EPS formation and EPS production accompanied by cell growth.

$$X+a_1G \xrightarrow{r_1} 2X \quad (1)$$

$$X+a_2G \xrightarrow{r_2} 2X+b_1P \quad (2)$$

2. When dextrin is present in the medium, it is first hydrolyzed to glucose and then metabolized instead of being directly consumed.

$$D+E \xrightarrow{r_4} b_3G+E \quad (3)$$

3. All of the enzymes in the dextrin hydrolysis pathway are dealt with as a single enzyme group. The hydrolytic

Fig. 1. Profiles of cell mass (●), residual glucose (□), and exopolysaccharide (▲) concentrations in batch culture with 30 g/l of carbon sources.
A. G/D ratio: 5:0, **B**. G/D ratio=4:1, **C**. G/D ratio=3:2, **D**. G/D ratio=2:3, **E**. G/D ratio=1:4, and **F**. G/D ratio=0:5.

activity, E, is induced by consuming a certain amount of dextrin.

$$X+a_3D \xrightarrow{r_3} X+b_2E \quad (4)$$

4. When the hydrolytic activity is induced, another EPS production pathway is activated and competes with the existing pathway, as shown in Eq. (2).

$$X+a_4G+E \xrightarrow{r_5} 2X+b_4P+E \quad (5)$$

where X, G, D, E, and P represent the cell, glucose, dextrin, hydrolytic activity, and EPS concentrations, respectively.

The material balance equations and rate expressions involved are given in Table 2. The expressions for the volumetric reaction rates of r_1 through r_5 in Table 2 were derived, based on the following assumption.

Table 1. Effects of concentration of carbon source and G/D ratio.

Run	Medium parameters		Lag time [h]	At the end of lag phase	
	Total carbon source [g/l]	G/D ratio		Cell mass [g/l]	Glucose [g/l]
1	60	1:4	36	3.99	27.1
2	60	1:9	36	3.66	24.3
3	60	3:2	36	3.65	34.2
4	30	5:0	24	2.55	29.2
5	30	4:1	24	2.78	23.1
6	30	3:2	24	2.75	19.9
7	30	2:3	24	2.81	18.3
8	30	1:4	24	3.45	20.5
9	30	0:5	24	2.64	20.1

• Cell growth follows the Contois kinetics [4, 6], which is often used to represent the characteristics of mycelial growth (T6, 7, 10).

• Considering the observed results mentioned earlier that EPS production is repressed at the beginning of the culture when cell concentration is low, and that a high carbon source concentration increases the lag time whereas the G/D has no effects, a repression term having G/X as a variable can be used (T7, 10).

• Dextrin hydrolysis is governed by the Michelis-Menten kinetics (T9).

The model parameters of a_1 through b_4 are the stoichiometric coefficients, and K's are appropriate constants to be specified.

Model Parameters Estimation

In order to reduce the number of parameters to be estimated, the enzyme activity related parameter, b_2, was fixed to be unity without losing generality.

The coefficients, a_1, a_2, and the kinetic parameters for r_1 and r_2 were determined by using the batch culture data (Fig. 1A) in the medium containing glucose as the sole carbon source. With these parameters estimated, the remaining twelve parameters were determined by using the batch culture data (Fig. 1F) in the medium containing dextrin as the sole carbon source. A genetic algorithm was used to estimate the parameters.

The procedure of model parameter estimation, using genetic algorithm [5], is as follows. Firstly, the initial

Table 2. Model structure for *A. blazei* batch culture with mixed carbon sources of glucose and dextrin.

Mass balance equations

Cell mass $$\frac{dX}{dt}=r_1+r_2+r_5 \quad \text{(T1)}$$

Glucose $$\frac{dG}{dt}=-a_1r_1-a_2r_2+b_3r_4-a_4r_5 \quad \text{(T2)}$$

Dextrin $$\frac{dD}{dt}=-a_3r_3-r_4 \quad \text{(T3)}$$

EPS $$\frac{dP}{dt}=b_1r_2+b_4r_5 \quad \text{(T4)}$$

Hydrolytic enzymes $$\frac{dE}{dt}=b_2r_3 \quad \text{(T5)}$$

Rate expressions

Cell growth $$r_1=\frac{k_1GX}{K_{G_1}X+G}\frac{K_{E_2}}{K_{E_5}+E} \quad \text{(T6)}$$

EPS production $$r_2=\frac{k_2GX}{K_{G_2}X+G}\frac{K_{I_1}X}{K_{I_1}X+G}\frac{K_E}{K_E+E} \quad \text{(T7)}$$

Hydrolytic enzyme induction $$r_3=\frac{k_3DX}{K_{D_1}X+D} \quad \text{(T8)}$$

Dextrin hydrolysis $$r_4=\frac{k_3DE}{K_{D_2}X+D} \quad \text{(T9)}$$

EPS production activated by enzymes $$r_5=\frac{k_5GX}{K_{G_3}X+G}\frac{K_{I_2}X}{K_{I_2}X+G}\frac{E}{K_E+E} \quad \text{(T10)}$$

Table 3. Model parameters estimated from batch culture data.

	Yield coefficients		
a_1	2.629×10^{0} [g-glc/g-DCW]	b_1	6.207×10^{-1} [g-EPS/g-DCW]
a_2	7.672×10^{0} [g-glc/g-DCW]	b_2	1.000×10^{0} [units /g-DCW]
a_3	2.464×10^{-2} [g-dex/g-DCW]	b_3	9.223×10^{-1} [g-glc/g-dex]
a_4	1.055×10^{0} [g-glc/g-DCW]	b_4	4.015×10^{0} [g-EPS/g-DCW]
	Kinetic parameters		
k_1	8.960×10^{-2} [1/h]	K_{D_2}	9.229×10^{0} [g-dex/g-DCW]
k_2	7.977×10^{-2} [1/h]	K_{G_3}	2.713×10^{0} [g-glc/g-DCW]
k_3	9.648×10^{-2} [1/h]	K_{I_1}	6.365×10^{0} [g-glc/g-DCW]
k_4	2.468×10^{0} [g-dex/(units·h)]	K_{I_2}	2.571×10^{0} [g-glc/g-DCW]
k_5	6.334×10^{-2} [1/h]	K_E	4.707×10^{0} [units]
K_{G_1}	9.885×10^{0} [g-glc/g-DCW]	K_{E_2}	2.639×10^{0} [units]
K_{G_2}	9.298×10^{0} [g-glc/g-DCW]	K_{E_5}	1.178×10^{0} [units]
K_{D_1}	4.527×10^{0} [g-dex/g-DCW]		

glc, glucose; dex, dextrin; DCW, dry cell weight.

guesses of the parameters to be estimated are generated by a random number generator and then coded as a binary unsigned integer of finite length to construct a virtual chromosome population. In reproduction of the population, the mating pool (population) of the next generation is selected by fitness. The parameter sets or chromosomes that have lower error values get more copies, the average ones stay even, and the worst group dies off. With an active pool of chromosomes looking for mates, crossover proceeds. The last operation is performed on a bit-by-bit basis. Every operation works with probability. The whole procedure is repeated until no further improvement occurs. More details on genetic algorithm can be found elsewhere [5, 11]. The estimates of the kinetic parameters are listed in Table 3.

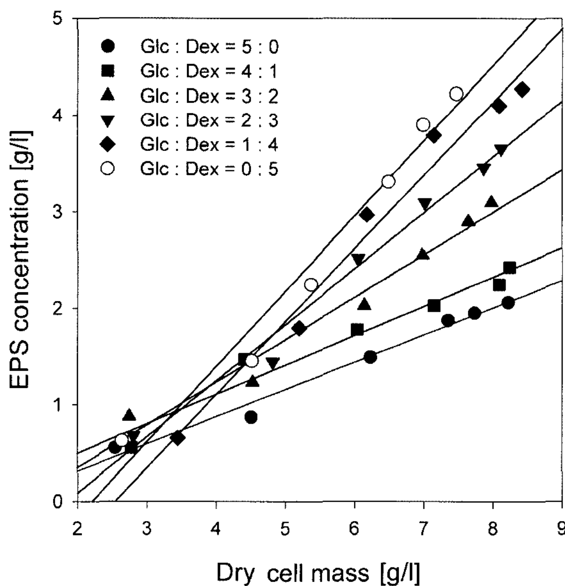

Fig. 2. Relationship between EPS and cell mass concentration at various G/D ratios.

Model Evaluation

The model developed for the *A. blazei* culture in this study is the first one of its kind. The accuracy of the model was evaluated by comparing the model prediction with the experimental data presented in Fig. 1. The model prediction showed an excellent overall goodness-of-fit with rather small mean normalized error squares values of 2.0×10^{-4}–2.5×10^{-3} (Fig. 3 and Table 4). Although the model parameters were estimated from the experimental data of two extreme cases with G/D ratios of 5:0 and 0:5, the model can well predict the data for the other cases also, implying that it has a good interpolation capability. However, the model shows significant errors in these cases, especially in fitting the glucose concentration profiles. Such inaccuracy can be attributed to the lack of dextrin measurement, and therefore, the lack of close assessment of dextrin hydrolysis. It should be noted that dextrin hydrolysis is represented by two extremely simplifying assumptions represented by Eqs. (4) and (T9). These assumptions were aimed at simplifying the very complex natures of dextrin itself and

Table 4. The goodness-of-fit of the model for various initial conditions.

G/D ratio	Mean normalized error squares*		
	Cell mass	Glucose	EPS
5:0	1.88×10^{-4}	1.79×10^{-3}	2.91×10^{-3}
4:1	1.81×10^{-3}	1.19×10^{-2}	1.34×10^{-2}
3:2	2.16×10^{-3}	1.34×10^{-2}	2.17×10^{-3}
2:3	1.46×10^{-3}	2.99×10^{-2}	1.40×10^{-3}
1:4	3.67×10^{-3}	2.69×10^{-3}	4.53×10^{-3}
0:5	2.47×10^{-3}	4.94×10^{-3}	8.69×10^{-4}

$* \bar{e} = \dfrac{\sum_{i=1,N}\left[\dfrac{A_{i,mod} - A_{i,meas}}{A_{max,meas}}\right]}{N}$, where $A_{i,mod}$, $A_{i,meas}$, and $A_{max,meas}$ are model estimates, measured values, and maximum measured values, respectively.

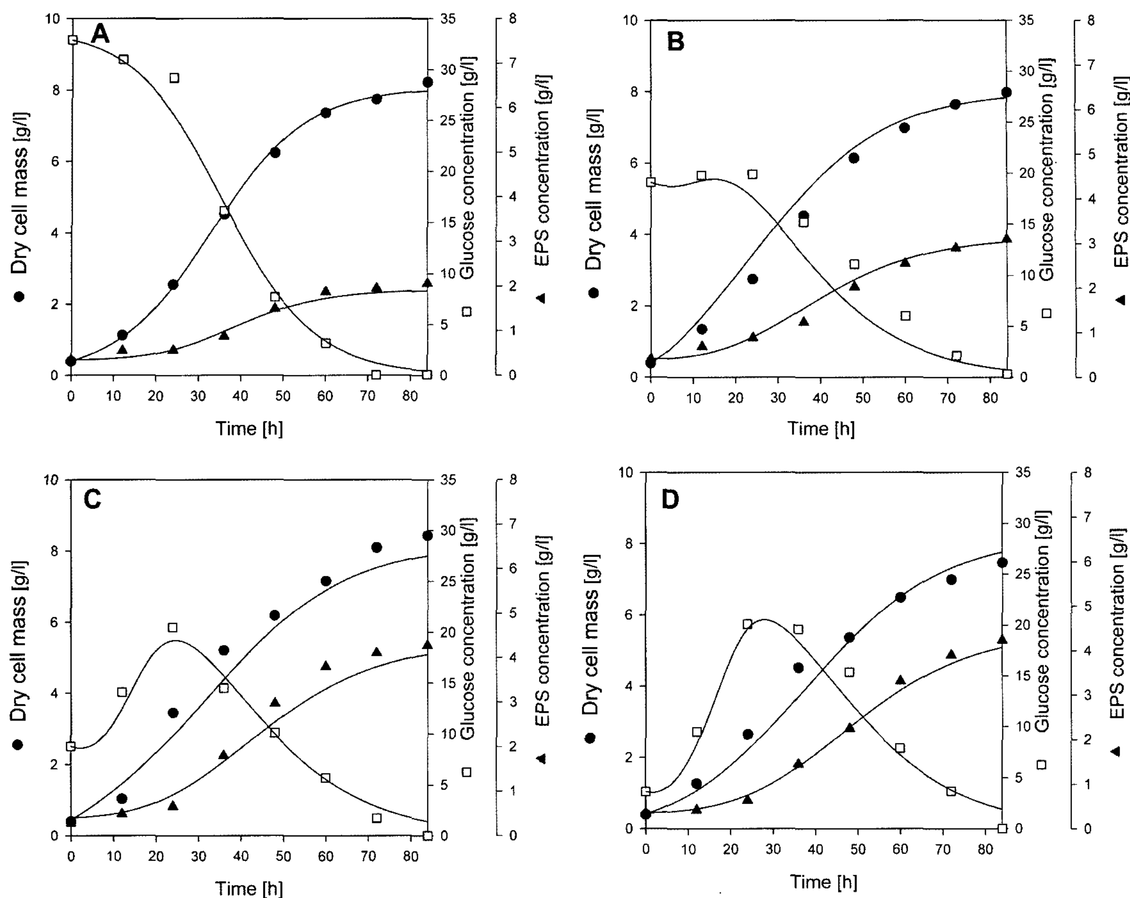

Fig. 3. Model fits of cell mass concentration (●), residual glucose concentration (□), and exopolysaccharide concentration (▲) profiles in batch culture with 30 g/l of carbon source. Symbols are for experimental data and lines for model prediction.
A. G/D ratio=5:0, **B**. G/D ratio=2:3, **C**. G/D ratio=1:4, and **D**. G/D ratio=0:5.

its hydrolysis, while retaining the basic mechanistic feature of dextrin hydrolysis. Furthermore, the model presents the predicted profiles of dextrin concentration, which is not easily measured. Although the model proposed has room for further improvement, it can predict the cell growth and EPS formation very well, and therefore, it can be a practically useful tool for the optimization of EPS production.

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