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Kinetic Model of Cell Growth and Secondary Metabolite Synthesis in Plant Cell Culture of *Thalictrum rugosum*

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A structured kinetic model was proposed to describe cell growth and synthesis of a secondary metabolite, berberine, in batch suspension culture of *Thalictrum rugosum*. The model was developed by representing the physiological state of the cell in terms of the activity and the viability, which can be estimated using the culture fluorescence measurement. In the proposed model, the cells were divided into three types; active-viable, nonactive-viable, and dead cells. The model was formulated in terms of cell growth (dry/fresh weight, activity, and viability), carbon source utilization (sucrose, glucose and fructose), and product formation (intracellular and extracellular berberine). The concept of cell expansion and the death phase were also included in this model to describe the sugar accumulation and the release of intracellular berberine into medium by cell lysis, respectively. The parameters used in this model were estimated based on the experimental results in conjunction with numerical optimization techniques. Satisfactory agreement between the model and experimental data was obtained. The proposed model could accurately predict cell growth and product synthesis as well as the distribution of the secondary metabolite between the cell and the medium. It is suggested that the proposed model could be extended as a useful framework for quantitative analysis of physiological characteristics in the other plant cell culture systems.

Key words: structured kinetic model, *Thalictrum rugosum*, plant cell culture, activity, viability, secondary metabolite, berberine

INTRODUCTION

Plant cell and tissue culture has been developed as an attractive alternative to agricultural (farming) or forestry technique for the production of commercially important biochemicals. The major valuable chemicals from plant cell culture are the secondary metabolites, which are biosynthetically derived from the primary metabolites. Although plant tissue culture techniques have been developed since 1940, significant advances for the commercial production using large scale bioreactor operation have been made only in the last decade [1,2].

In order to enhance the production of secondary metabolites in plant cell culture, rational and systematic strategies for bioreactor operation should be developed, and process optimization should also be carried out from a bioprocess engineering perspective. Mathematical model of the biological process is often considered as an invaluable tool to obtain and predict a lot of information about the physiological characteristics of the objective culture processes. It is also used to develop a rational approach to test the operating strategy and process optimization. Many of the unstructured kinetic models that have been applied to plant cell cultures hitherto have not been able to accurately predict the cell growth and the secondary metabolite production [3-5]. Structured kinetic models have therefore been developed since they have the capability to more accurately describe the physiological characteristics in plant cell culture based upon the information on a particular subset of the multiple cellular components and their interactions [6-11]. Although structured kinetic models have improved the predictable capability, there still remains a lack of detailed understanding on the kinetics for growth, secondary metabolite biosynthesis, and the distribution of the secondary metabolites between the intra- and the extracellular environment.

In this study, a structured kinetic model was proposed to describe the substrate utilization, the cell growth, and the secondary metabolite synthesis in *Thalictrum rugosum* plant cell culture. The model was formulated on the physiological state of the cell that account for the activity and the viability, linked with substrate consumption and secondary metabolite synthesis. Also the model includes cell expansion due to sugar concentration, and accounts for dead cells which can release intracellular secondary metabolite into the medium. Based on this approach, the proposed model can predict culture growth and

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secondary metabolite synthesis in a rigorous and quantitative manner.

MATERIALS AND METHODS

Cultures and Media

Thalictrum rugosum cells were provided by Dr. Peter Brodelius (Institute of Plant Biology, University of Lund, Sweden). Cell suspension cultures have been maintained on Murashige and Skoog (MS) medium prepared from MS salt mixture (GIBCO Laboratories, Grand Island, NY, U.S.A.) with 2 μM of 2,4-dichlorophenoxy acetic acid (2,4-D), vitamin stock solution, and 30 g/L of sucrose as the carbon source. The pH of the medium was adjusted to 6.0 before autoclaving. After autoclaving, the pH of the medium was about 5.8. For batch suspension culture, cells in the late exponential growth phase, which are usually 5-6 days old, have been used. In order to avoid heterogeneity of the inoculum, all the cells from different flasks were collected in a preautoclaved large flask, and mixed well by shaking. The cells were filtered through Whatman No. 1 filter paper on a Buchner funnel under slight vacuum and washed with the fresh medium. Approximately 5 g of cells by fresh weight was inoculated into a 125 mL Erlenmeyer flask containing 50 mL of the medium. The suspension cells were incubated on a rotary shaker at 180 rpm and at 25°C. Two or three replicas of the flasks were used for analysis. After filtration, the cells were collected for cell mass measurement and intracellular product determination. The filtrates were usually stored in a refrigerator for extracellular product and sugar assays.

Analytical Procedures

For dry cell weight (DCW) measurement, suspension cells were filtered with dried and pre-weighted Whatman No. 1 filter paper under slight vacuum. Filtered cells were washed with distilled water and dried in an oven at 60°C until it maintained to be a constant weight. For fresh cell weight measurement, suspension cells were filtered with wetted and pre-weighted Whatman No. 1 filter paper under slight vacuum.

For alkaloid analysis, intracellular berberine was extracted with HPLC-grade methanol. A sample containing 0.5 g of cells, by fresh weight, in 20 mL of methanol was sonicated at 125 W in a water bath for 1 hr. 10 μL of the filtered sample from the extract or the medium was injected into a HPLC system. Quantitative berberine analysis was carried out with an isocratic HPLC system using a Spectroflow 400 (Kratos Corp., Ramsey, NJ, U.S.A.), under the following conditions; SUPERCOSIL LC-18-DB column (15 cm × 4.6 mm, Supelco Inc., Bellefonte, PA, U.S.A.); flow rate, 2 mL/min; Mobile Phase, 1 mM tetrabutyl ammonium phosphate in water, adjusted to pH 2 with phosphoric acid (60%) and acetonitrile (40%); detection at 271 nm with a UV detector (Kratos Corp, Ramsey NJ, U.S.A.). The HPLC system was also used for the simultaneous analysis of sucrose and its hydrolyzed products, glucose and fructose under the following conditions; SUPELCOSIL LC-NH_2 column (25 cm × 4.6 mm, Supelco Inc., Bellefonte, PA, U.S.A.); flow rate, 2 mL/min; Mobile phase, 75% acetonitrile and 25% water; detection with a refractive index (RI) detector (Perkin-Elmer Corp., Wilton, CT, U.S.A.).

Fluorescence measurement for cell mass detection of *T. rugosum* was carried out with an *in situ* Fluorosensor™ (Ingold Electrodes Inc., Wilmington, MA, U.S.A.). This device was designed for detection of NADH. The light source was filtered at 350 nm to excite the fluorophore, NADH, in the cells [12]. The emitting fluorescence was filtered at 450 nm and can be measured by a detector. After amplification, the converted analog signal was typically in the range from 0 to 10 V. The culture fluorescence of the suspension cells was monitored by the Fluorosensor™ in a black box system to minimize the external light influences on the fluorescence signal.

MODEL DEVELOPMENT

Model Variables

It is assumed that cells are divided into two types, viable cells and nonviable (dead) cells, where viability is determined by interpretation of the NADH-dependent culture fluorescence data. Viable cells are further divided into two types, active-viable (dividable) cells and nonactive-viable (resting) cells. Since the observed characteristic of berberine synthesis in *T. rugosum* cell culture is mixed-growth associated, berberine is produced by both active-viable and nonactive-viable cells with a different production rate. Active-viable cells may become nonactive viable cells which then may further degenerate into dead cells when the carbon source is completely depleted. Finally, dead cells are broken up and the intracellular berberine is released into the medium by cell membrane disruption. This classification of the cells is schematically summarized in Fig. 1.

Variables used in the proposed model are expressed as following; X_{ad}, dry weight of active-viable cell; X_{nd}, dry weight of nonactive-viable cell; X_{vd}, dry weight of viable cell; X_{dd}, dry weight of nonviable cell; X_d, dry weight of total cell; S_S, sucrose concentration; S_G, glucose concentration; S_F, fructose concentration; P_I, intracellular product concentration; P_E, extracellular product concentration; X_f, fresh cell weight.

The viability, V, is defined as

$$V = \frac{X_{vd}}{X_d}\ ;\ \text{fraction of the total cells that are viable} \quad (1)$$

Also, the activity, A, is defined as

$$V = \frac{X_{ad}}{X_{vd}}\ ;\ \text{fraction of the viable cells that are biosynthetically active to be divided} \quad (2)$$

Dry Weight Equation of Active-Viable Cell

In the cell culture of *T. rugosum*, lag-time, t_L, was about one day. When sucrose is used as the carbon source in *T. rugosum* cell culture, it is rapidly broken down into glucose and fructose by extracellular or membrane bound invertases, and it has been found that only the hexoses are taken up into the

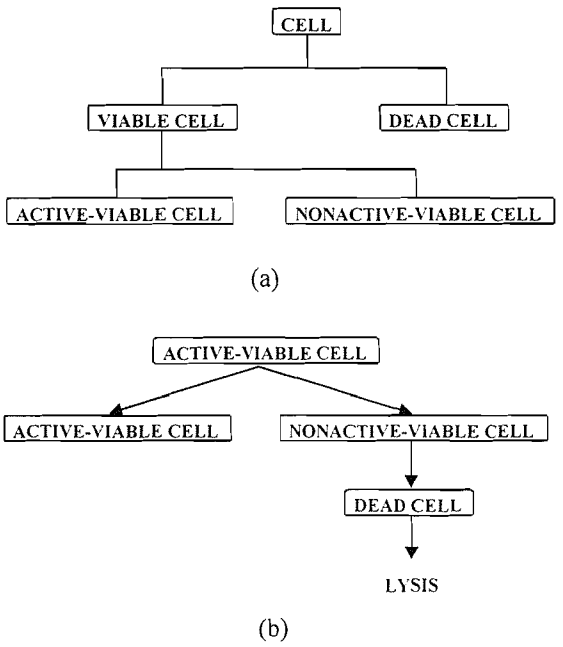

Fig. 1. A conceptual diagram of cell classification for the structured model: (a) cell classification based on viability; (b) cell differentiation based on activity.

cells [13,14]. Competitive-inhibition was observed in the uptake of the hexose [15]. Hence, the following equation was suggested for μ by other researcher [12];

$$\mu = \frac{\mu_{mG} S_G}{S_G + K_G(1 + S_F/K_F)} + \frac{\mu_{mF} S_F}{S_F + K_F(1 + S_G/K_G)} \quad (3)$$

where K is a Monod type constant, μ_m is the maximum growth rate and the subscript G and F designate glucose and fructose, respectively. It is assumed that the rate of loss of cell activity is proportional to dry weight of viable cell, because the factors of activity loss (susceptibility to shear, osmotic and sugar depletion effects) are affected by all viable cell. Also, it is assumed that the nonactive-viable cells do not consume substrates for growth. The quantity of active-viable cell can thereby be determined based on the substrate consumption rate.

A mass balance for dry weight of active-viable cell is given by,

$$\frac{dX_{ad}}{dt} = \left[1 - exp\left(-\frac{t}{t_L}\right)\right] \mu X_{ad} - k\,\phi X_{vd} \quad (4)$$

where

$$\phi = \frac{1}{A}\frac{X_f}{X_{vd}}\frac{X_{vd}}{X_d + S_t} \quad (5)$$

In Eq. (4), the first term represents the growth due to glucose and fructose utilization and the second term represents the activity loss. In the first term, $[1\text{-}exp(-t/t_L)]$ represents the time lag of the transient state until exponential growth begin. The variable, ϕ, represents the loss of activity due to susceptibility to shear, osmotic and sugar depletion effects. The term of $X_{vd}/(X_d+S_t)$ reflects sugar depletion effect (where S_t is total sugar concentration), and the ratio of X_f/X_{vd} is proportional to cell expansion that causes the susceptibility to shear and osmotic effect.

Dry Weight Equation of Nonactive-Viable Cell

It is assumed that decay of dry weight of nonactive-viable cell is proportional to the cell expansion (X_f/X_{vd}) and is proportional to dry cell weight (X_d). A mass balance for nonactive-viable cell dry weight is given by,

$$\frac{dX_{nd}}{dt} = k\,\phi X_{vd} - k_d \frac{X_f}{V} \quad (6)$$

The first term represents the loss of cell activity from Eq. (4), generating nonactive-viable cells, and the second term represents the loss of cell viability. Substrate is not consumed by nonactive-viable cell for growth.

Dry Weight Equation of Viable Cell

The dry weight of viable cell is the sum of that of the active- and nonactive-viable cell:

$$X_{vd} = X_{ad} + X_{nd} \quad (7)$$

Differentiating Eq. (7), and substituting Eq. (4) and (6) into two terms on the right-hand side, respectively, yields

$$\frac{dX_{vd}}{dt} = \left(1 - exp\left(-\frac{t}{t_L}\right)\right) \left[\frac{\mu_{mG} S_G}{S_G + k_G(1 + S_F/k_F)} + \frac{\mu_{mF} S_F}{S_F + k_F(1 + S_G/k_G)}\right] X_{ad} - \frac{k_d X_f}{V} \quad (8)$$

Dry Weight Equation of Nonviable Cell

The change in dry weight of nonviable (dead) cell is given by

$$\frac{dX_{dd}}{dt} = k_d \frac{X_f}{V} - k_L X_{dd} \quad (9)$$

where the first term represents the change of viable cell to dead cell, and the second term represents the decay of cell mass due to lysis.

Dry Weight Equation of Total Cell

Dry weight of the total cell is the sum of that of viable and nonviable cell as follow

$$X_d = X_{vd} + X_{dd} \quad (10)$$

Differentiating Eq. (10), and substituting Eq. (8) and (9) into two terms on the right-hand side, respectively, yields

$$\frac{dX_d}{dt} = \left(1 - exp\left(-\frac{t}{t_L}\right)\right) \left[\frac{\mu_{mG} S_G}{S_G + k_G(1 + S_F/k_F)} + \frac{\mu_{mF} S_F}{S_F + k_F(1 + S_G/k_G)}\right] X_{ad} - k_L X_{dd} \quad (11)$$

Activity Equation

Differentiating Eq. (2) yields

$$\frac{dA}{dt} = \frac{\frac{dX_{ad}}{dt}X_{vd} - X_{ad}\frac{dX_{vd}}{dt}}{X_{vd}^2} \tag{12}$$

Substituting Eq. (4) and (8) into Eq. (12), it can be rearranged as

$$\frac{dA}{dt} = \left(1 - exp\left(-\frac{t}{t_L}\right)\right) \left[\frac{\mu_{mG}S_G}{S_G + k_G(1 + S_F/k_F)} + \frac{\mu_{mF}S_F}{S_F + k_F(1 + S_G/k_G)}\right] (1 - A)A - k\,\phi + k_d\frac{AX_f}{V^2X_d} \tag{13}$$

Viability Equation

Differentiating Eq. (1) yields

$$\frac{dV}{dt} = \frac{\frac{dX_{vd}}{dt}X_d - X_{vd}\frac{dX_d}{dt}}{X_d^2} \tag{14}$$

Substituting Eq. (8) and (11) into Eq. (14), it can also be rearranged as

$$\frac{dV}{dt} = \left(1 - exp\left(-\frac{t}{t_L}\right)\right) \left[\frac{\mu_{mG}S_G}{S_G + k_G(1 + S_F/k_F)} + \frac{\mu_{mF}S_F}{S_F + k_F(1 + S_G/k_G)}\right] (1 - V)V - k_d\frac{X_f}{VX_d} + k_L(1 - V)V \tag{15}$$

Viability can be estimated based on normalized value of culture fluorescence. Eq. (16) is used for the quantitative representation of viability from the experimental results.

$$V = \frac{\frac{FI/FI_m}{X_d}}{\left[\frac{FI/FI_m}{X_d}\right]^o} \tag{16}$$

where FI is the relative fluorescence intensity and FI_m is the maximum relative fluorescence intensity during the cell cultivation.

Substrate Uptake Rate Equation

Sucrose is hydrolyzed to glucose and fructose by an invertase [13,14].

$$\frac{dS_S}{dt} = -k_c S_S \tag{17}$$

Glucose and fructose is consumed for the growth of cells and the synthesis of secondary metabolites, berberine, with different yield coefficients. Substrate consumption for cell maintenance is neglected.

$$\frac{dS_G}{dt} = 0.526k_cS_S - \frac{1}{Y_{X/S_G}}\left(1 - exp\left(-\frac{t}{t_L}\right)\right) \frac{\mu_{mG}S_GX_{ad}}{S_G + k_G(1 + S_F/k_F)} - \frac{\alpha X_{ad} + \beta X_{nd}}{Y_{P/S_G}} \tag{18}$$

$$\frac{dS_F}{dt} = 0.526k_cS_S - \frac{1}{Y_{X/S_F}}\left(1 - exp\left(-\frac{t}{t_L}\right)\right) \frac{\mu_{mF}S_FX_{ad}}{S_F + k_F(1 + S_G/k_G)} - \frac{\alpha X_{ad} + \beta X_{nd}}{Y_{P/S_F}} \tag{19}$$

The first term represents the hydrolysis of sucrose by invertase, the second term represents the substrate consumption for cell growth, and the third term represents the substrate consumption for product synthesis. The numerical modifier 0.526 reflects the fact that the mass of hexose are obtained from sucrose and water.

Intracellular Product Equation

Since *T. rugosum* stores the secondary metabolite, berberine, in the vacuole, berberine is mostly found as the intracellular product. As mentioned previously, it was assumed that berberine is synthesized by the active and nonactive-viable cells with different rates because the pattern of berberine production is mixed growth-associated. It was also assumed that only the destruction of cells cause the release of berberine from cells to the medium because extracellular berberine concentration increases dramatically after death phase. Since the rate of product release is proportional to that of cell lysis, the intracellular product, P_I, is expressed as

$$\frac{dP_I}{dt} = \alpha X_{ad} + \beta X_{nd} - \gamma k_L X_{dd} \tag{20}$$

The first and second terms represent the berberine synthesis by active and nonactive-viable cells, respectively. In particular, the first term represents growth-associated production and the second term represents nongrowth-associated production. The third term represents the release of berberine by cell destruction.

Extracellular Product Equation

It was assumed that extracellular berberine can be degraded and is described as

$$\frac{dP_E}{dt} = \gamma k_L X_{dd} - \lambda X_{dd} \tag{21}$$

The first term represents release of berberine by cell destruction and the second term represents berberine degradation by enzyme excreted from the disrupted cells, which is similar to the results reported by others [16]. In the second term, berberine degradation rate is dependent not on the extracellular berberine concentration but on the dry weight of dead cell because berberine degradation is only accomplished by the enzymes released from lysis of dead cell.

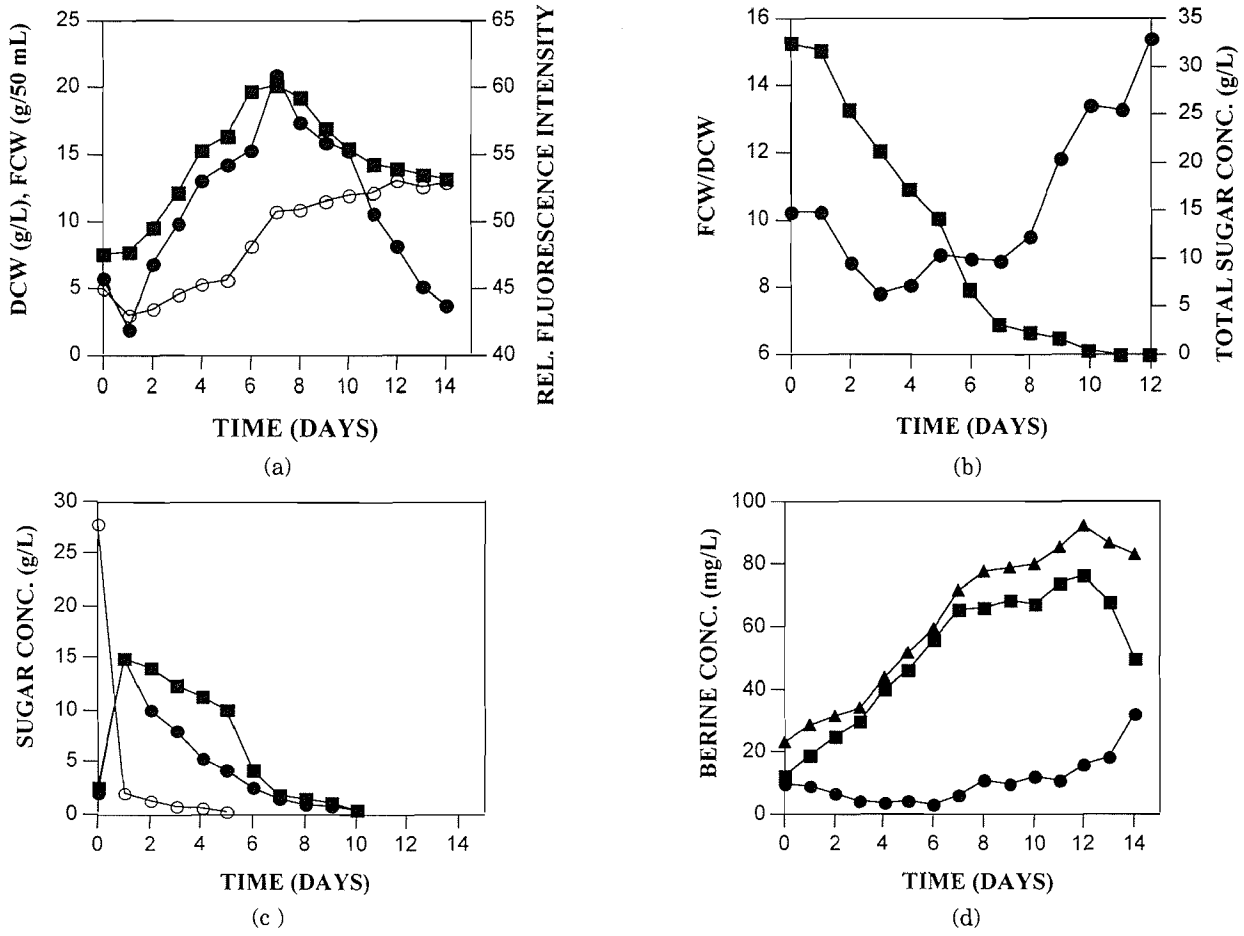

Fig. 2. Time course behavior of experimental results in batch culture; (a) dry cell weight (■), fresh weight (○) and culture fluorescence (●); (b) the ratio of fresh weight to dry cell weight (●) and total sugar concentration (■); (c) carbohydrate depletion (sucrose (○), glucose (●), fructose (■)); (d) the distribution of berberine (total (▲), intracellular (■), extracellular (●)).

Total Product Equation

The total berberine concentration is the sum of intracellular and extracellular berberine concentration:

$$P_t = P_I + P_E \tag{22}$$

Differentiating Eq. (22), and substituting Eq. (20) and (21) into two terms on the right-hand side, respectively, yields

$$\frac{dP_t}{dt} = \alpha X_{ad} + \beta X_{nd} - \lambda X_{dd} \tag{23}$$

Fresh Cell Weight Equation

Fresh cell weight is dependent on the dry cell weight as well as the sugar concentration. Fresh cell weight increases as dry cell weight increases, and decreases as cells rupture. The ratio of X_f/X_d increases as the sucrose concentration decreases in *T. rugosum* cell culture as shown in Fig. 2(b). This phenomenon might be due to an osmotic effect, wherein external sugar concentration governs the degree of cell expansion. The increase of water content in the cell leads to an increased value of fresh weight. This result suggests that the fresh weight increases with cell expansion due to the increase of osmotic pressure as sugar is consumed. This can be approximated as

$$\frac{dX_f}{dt} = \left(1 - exp\left(-\frac{t}{t_L}\right)\right) k_1 X_{ad} - k_2 k_L X_{dd} - \theta(s) X_d \tag{24}$$

where,

$$\theta(s) = \varkappa exp\left(1 - \frac{S_t}{S_t^0}\right) \tag{25}$$

$$S_t = S_S + S_G + S_F \tag{26}$$

In Eq. (24), the first term represents the effect of cell growth, the second term represents the cell disruption, and the third term represents cell expansion due to osmotic pressure. Eq. (25) is used for the quantitative measurement of cell expansion based on the experimental results.

RESULTS AND DISCUSSION

Experimental

Growth and Sugar Uptake

For the analysis of the kinetic parameters for the

cell growth and the substrate consumption in the suspension culture *T. rugosum* cell, batch experiments were carried out in shake flasks and samples were taken everyday. Fig. 2(a) shows the time course change of the cell growth rate. A lag phase in the cell growth was observed during the early stage of the cultivation. The exponential growth occurred after about 1 day, when most amount of added sucrose was depleted. Dry cell weight reached a maximum at 7 days of cultivation, and then subsequently decreased. In the stationary and death phase after 7 days, dry cell weight decreased rapidly, but fresh cell weight was nearly constant. In Fig. 2(b), the fresh cell weight/dry cell weight ratio was plotted, and it seems to increase as total sugar concentration decreased. As mentioned previously, it might be due to an osmotic effect, wherein external sugar concentration governs the degree of cell expansion [7]. The increase of water content in the cell leads to an increased value of fresh cell weight. This result suggests that the fresh weight increases with cell expansion due to the increase of osmotic pressure as sugar is consumed.

From the pattern of extracellular sugar consumption in Fig. 2(c), it could be considered that sucrose, the only carbon source added, was hydrolyzed quickly to glucose and fructose. Only the hexose sugars were taken up into plant cells [17]. As soon as 2 hrs after inoculation, the conversion of sucrose to its monomeric constituents was significant. Furthermore, it was found that glucose is more preferable to fructose in *T. rugosum* cell culture [10,18].

Fluorescence Measurement

The culture fluorescence has been used as a noninvasive measurement for *in situ* monitoring the metabolic information in bioreactors [12,19]. In order to clarify the estimation of cell mass by culture fluorescence, the time course change of fluorescence, dry cell weight and fresh cell weight in *T. rugosum* cell culture were monitored as shown in Fig. 2(a). When only the exponential growth phase during the culture is considered, relative fluorescence intensity could be correlated linearly with dry cell weight with a correlation coefficient of 0.905. The culture fluorescence had a more complicated functional dependence with dry cell weight after about 9 days, when the death phase began. The reason for such shift might be that dry cell weight includes viable cells as well as cell debris. Since only viable cells have an intact NAD-NADH conversion mechanism, NADH-dependent fluorescence data can not be reliable in the stationary phase and the death phase in the culture.

Secondary Metabolite Synthesis

The typical secondary metabolite production in plant cell cultures is nongrowth-associated [19], but the berberine production is mixed growth-associated as shown in Fig. 2(d). It was observed that the small amount of berberine existed in the medium until 12 days of the culture and then increased at the late stage of the culture due to cell lysis. Before cell lysis occurred, extracellular berberine was less than 15% of total berberine. In the death phase, berberine was released from the cells due to cell lysis and decreased, which might be due to the degradation by enzymes excreted during cell lysis. It was reported that *T. rugosum* cell can store berberine in the vacuole space by an intracellular product accumulation system [20]. But, if the metabolite is released, it can apparently undergo further metabolism.

Model

Parameter Estimation

The model was composed of the set of differential equations shown in Eqs. (11), (13), (15), (17)-(21) and (24). The parameters for the model were estimated by using a nonlinear parameter estimation technique [15,21]. While the parameter estimation was performed, model equations were solved simultaneously with numerical integration using standard Runge-Kutta method. The experimental data were compared to the model predictions by choosing parameters that give a best fit of the model to the data. The resulting estimated values of the set of parameters were shown in Table 1.

Growth and Sugar Uptake

Figs. 3 and 4 represent the experimental and model-predicted sugar consumption and cell growth behavior, respectively. It can be concluded that the experimental data were fairly accurately consistent with the solution of the model equations with the given initial condition for cell mass and sugar concentrations. Within 1 day, sucrose was rapidly depleted and mostly converted to glucose and fructose in stoichiometric amounts. Glucose was consumed first and then fructose was consumed during the further cultivation. The large value of k_c estimated shows the rapid depletion of sucrose, which is similar to the results obtained from an independent analysis of the initial time course data. Although both metabolic pathways for the carbohydrates (fructose and glucose) are probably dependent on hexokinase, the glucose in preference to fructose was observed, which was in accordance with that the order of magnitude of the estimated K_F was larger than that of K_G. The experimental results of carbon source consumption could be accurately predicted by the proposed model.

The lag time in the *T. rugosum* culture was approximately 1 day. When the carbohydrates began to be limited, exponential growth was ceased and dry cell weight decreased. However, the fresh weight was maintained during the death phase, which might be due to cell expansion by osmotic pressure. In the late death phase, cells were ruptured rapidly. Macromolecules and membrane constituents of the cells might be included in the observed value of dry cell weight. Hence, the dry weight based-cell mass would not be accurately predicted after twelve days. This phenomenon includes a discrepancy between experimental and model-predicted dry cell weight in the late death phase. On the other hand, model-predicted fresh cell weight in the late death phase accurately followed the experimental values. Maintenance of fresh weight in the late death phase can be explained as osmotic effect as mentioned previ-

Table 1. List of the structured model parameters for *T. rugosum* system in batch culture

Parameter	Dimension	Value	Parameter	Dimension	Value
μ_G	day^{-1}	0.3620	Y_{P/S_G}	g/g	0.038
μ_F	day^{-1}	0.3343	Y_{P/S_F}	g/g	0.038
K_G	g/L	3.9999	k_1	day^{-1}	1.200
K_F	g/L	7.6517	k_2	dimensionless	5.360
K	day^{-1}	0.016500	κ	day^{-1}	0.365
k_d	day^{-1}	0.007249	α	mgg^{-1} day^{-1}	0.733
k_L	day^{-1}	0.78490	β	mgg^{-1} day^{-1}	1.300
k_C	day^{-1}	2.55797	λ	mgg^{-1} day^{-1}	4.110
Y_{X/S_G}	g/g	0.58903	γ	mg/g	6.365
Y_{X/S_F}	g/g	0.55216	t_L	day	1.0

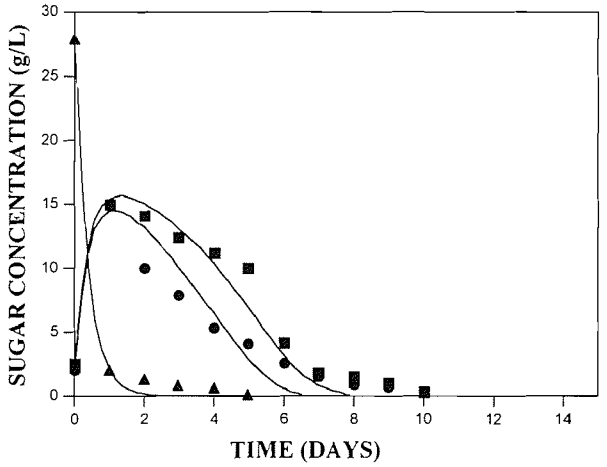

Fig. 3. The kinetic model results of time course change of carbohydrate depletion in batch culture. The symbols are experimental results (sucrose (▲), glucose (●), fructose (■)) and the lines are model prediction results.

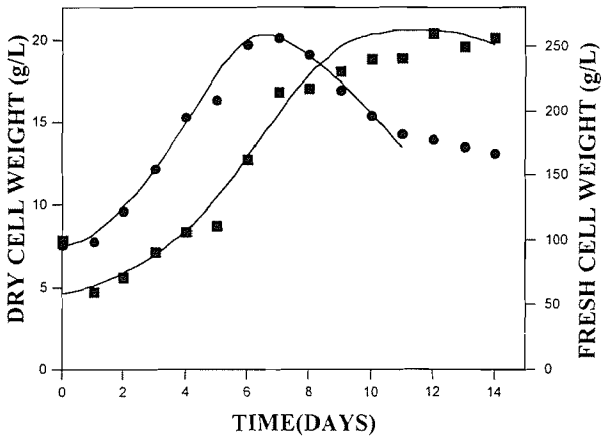

Fig. 4. The kinetic model results of the overall growth time course in batch culture. The symbols are experimental results (dry cell weight (●), fresh weight (■)) and the lines are model prediction results.

ously. The specific growth rate for glucose, μ_G, and the yield of dry cell weight on glucose, Y_{X/S_G} were larger than those for fructose, μ_F and Y_{X/S_F} as expected since glucose is preferred to fructose for cell growth.

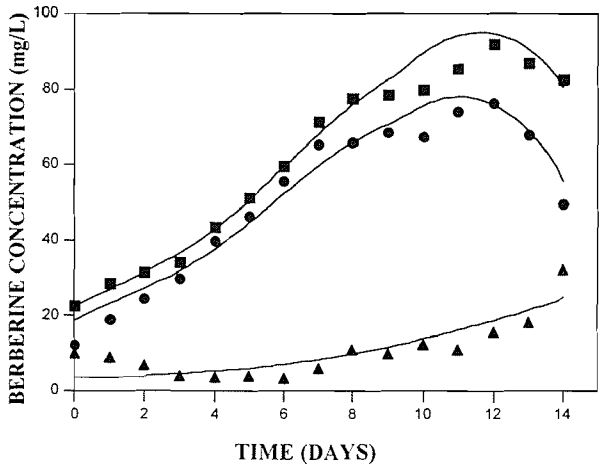

Fig. 5. The kinetic model results of time course change in the distribution of berberine in batch culture. The symbols are experimental results (total (■), intracellular (●), extracellular (▲)) and the lines are model prediction results.

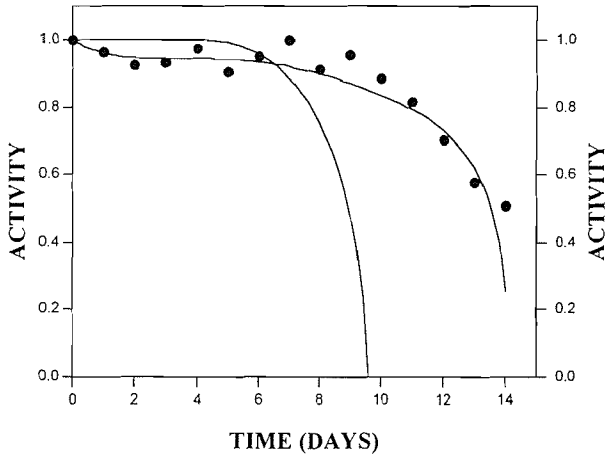

Fig. 6. The kinetic model results of time course change of the viability and activity in batch culture. The symbol is experimental viability result (●) and the solid line is model prediction result. The dotted line is model prediction activity result.

Secondary Metabolite Synthesis

Fig. 5 represents the experimental and model-predicted berberine synthesis, and the distribution of berberine in cells and in medium. Model prediction and experimental data for corresponding activity and viability in culture were shown in Fig. 6. The model accurately followed the experimental data and successfully described both growth-associated berberine production and nongrowth-associated production during the stationary phase. The estimated value of the nongrowth-associated product constant, β, was about two times larger than that of the growth-associated product constant, α. Model prediction in Fig. 6 for activity and viability shows that active-viable cell was related with growth-associated berberine production and nonactive-viable cell was related with nongrowth-associated berberine production. Also the model describes that most of berberine initially existed as intracellular form followed by a

rapid increase in extracellular berberine concentration during the death phase. Model prediction in Fig. 6 shows that this increase might be due to the increase of nonviable cell after the exponential growth was ceased. One discrepancy existing between model predictions and experimental results could be observed during the early stage of culture, where the release of intracellular berberine might be due to the "shock" by environmental change after inoculation. It is considered that the accuracy of the model to predict the product synthesis and its distribution between the cell and the medium could be enhanced by using the concept of viability and activity in the different classifications of cells.

CONCLUSION

A structured kinetic model was proposed to predict culture growth, secondary metabolism, and, substrate uptake in the suspension culture of *T. rugosum*. The activity and viability could be described by the proposed model. Substrate uptake rate was represented by competitive inhibition in uptake rates of hexose, glucose and fructose, which were converted from sucrose. Formation rate of secondary metabolites was governed by active-viable and nonactive-viable cell, and release rate of intracellular secondary metabolites into medium was determined by accumulation of nonviable cells. Cell expansion was described as an osmotic effect due to sugar concentration based on fresh weight to dry cell weight ratio. Utilizing nonlinear parameter estimation techniques, the parameters in the reaction rates were determined based upon available experimental data in *T. rugosum* batch culture. Using those coefficients, the proposed model could accurately predict the cell growth and secondary metabolite synthesis as well as the distribution of secondary metabolite between in the cell and in the medium. Eventually the model can be used to develop an operating strategy and control scheme for maximizing the production of secondary metabolites, in plant cell culture.

Based on the proposed model, the operating strategies for bioreactor operation such as periodic fed-batch culture that can provide to maintain cell activity, viable cell mass, and specific production rate, and perfusion culture will be investigated to maximize secondary metabolite synthesis in *T. rugosum* plant cell culture.

NOMENCLATURE

A	activity (g/g)
FI	relative fluorescence intensity
k	rate constant (day^{-1})
K	Monod constant (g/L)
P	product concentration (g/L)
S	substrate concentration (g/L)
t	time (days)
V	viability (g/g)
X	biomass concentration (g/L)
Y	yield coefficient (g/g)

Greek symbols

α	growth-associated production constant (g/g/day)
β	nongrowth-associated production constant (g/g/day)
κ	cell expansion coefficient (day^{-1})
μ	specific growth rate (day^{-1})
λ	product degradation constant (g/g/day)
γ	product release coefficient by cell lysis (g/g)
θ	function for cell expansion
ϕ	function for activity loss

Subscripts

c	conversion from sucrose
d	dry weight or death
E	extracellular
f	fresh weight
F	fructose
G	glucose
I	intracellular
L	lag phase
m	maximum
o	initial
S	sucrose
t	total
ad	active-viable cell
dd	dead cell
nd	nonactive-viable cell
vd	viable cell
P/S_F	product from fructose
P/S_G	product from glucose
X/S_F	biomass from fructose
X/S_G	biomass from glucose

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