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APPLICATION OF GENETIC ALGORITHM TO SELF-ORGANIZING FUZZY CONTROLLER IN FED-BATCH CULTURE OF *Scutellaria baicalensis G.* PLANT CELL

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Abstract – A self-organizing fuzzy controller is constructed for control of substrate concentration in fed-batch operation of a cell culture process. A genetic algorithm is used to generate fuzzy rules of the self-organizing fuzzy controller and to modify the universe of discourse automatically. The fuzzy controller is designed for the application of *Scutellaria baicalensis G.* plant cell culture process as a model system. A substrate feeding strategy of a two stage culture method to maximize flavone glycoside production in fed-batch culture of *Scutellaria baicalensis G.* plant cell is proposed based on structured model of growth and product synthesis. As a two stage culture, the feeding strategy consists of the first period at 22 g/L of glucose concentration to promote cell growth and the second period at 10 g/L of glucose concentration to promote flavone glycoside synthesis. The designed self-organizing fuzzy controller is applied to regulate the glucose concentration at a given set-point to increase flavone glycoside synthesis. The simulation results show that the proposed feeding strategy in a fed-batch culture enhances flavone glycoside production and the self-organizing fuzzy logic controller generated by genetic algorithm improves controller performance.

Key words : *Scutellaria baicalensis G.*, Flavone Glycosides, Two Stage Culture, Self-Organizing Fuzzy Controller, Genetic Algorithm

INTRODUCTION

Instead of agricultural techniques, a plant cell and tissue culture technique has been developed as an attractive alternative for the production of valuable compounds. To enhance product synthesis in plant cell culture, a strategy for bioreactor operation and process optimization should be developed from a bioprocess engineering perspective [Prenosil and Pedersen, 1983]. Also, since relationships between state variables and control variables cannot be determined by a simple function and an unpredictable probability factor exists in the cell culture process, bioreactor operation usually depends on an expert's experience and imagination. From these points of view, the control of the plant cell culture process by using classical controllers is very difficult due to the need for complicated quantitative knowledge for the processes under the influence of the control variables. The cell culture process seems to be the most appropriate kind of process for application of fuzzy control theory instead of traditional control techniques [Postlethwaite, 1989; Choi et al., 1998a]. However, the application of fuzzy controllers in bioreactor operation has been limited. First, a reliable linguistic model of the operator's control strategy may not always be available and some significant process changes may be beyond the operator's experience. Second, biochemical engineers are generally unfamiliar with the design of a fuzzy control algorithm. In this study, a genetic algorithm [Dejong, 1988; Hwang and Woo, 1989; Hideo et al., 1993; Choi et al., 1994; Lee et al., 1995] is applied to construct a self-organizing fuzzy logic controller automatically for the control of substrate concentration in a fed-batch culture process.

As a model system, the *Scutellaria baicalensis G.* plant cell culture process is chosen to construct the fuzzy controller. *Scutellaria baicalensis G.* can be used as a liver detoxicant, and its ether extract shows an augmentation of the cytotoxic effect of anticancer drugs [Ahn et al., 1991]. Roots of *Scutellaria baicalensis G.* contain valuable flavonoids [baicalin, wogonin-7-0-glucuronic acid (GA)]. A cell culture of *Scutellaria baicalensis G.* has been done to produce flavonoids economically [Seo et al., 1993; Choi et al., 1998b].

In this study, a bioreactor operating strategy to maximize flavone glycoside synthesis in fed-batch culture of *Scutellaria baicalensis G.* plant cell is proposed based on the structured kinetic model of growth and product synthesis. And a self-organizing fuzzy controller using a genetic algorithm is constructed for the control of substrate concentration in a fed-batch culture of *Scutellaria baicalensis G.* plant cell.

SUBSTRATE FEEDING STRATEGY

1. Kinetic Model

To construct a feeding strategy to increase the flavone gly-

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coside production in *Scutellaria baicalensis G.* plant cell culture, the kinetic model is formulated as follows. Based on the living state of cells, cells are divided into two types, viable cells and dead cells, and viability is defined as the proportion of dry weight of viable cells. Viability is determined by interpretation of the NADH-dependent culture fluorescence data on a basis of dry cell weight since only a living cell has intracellular NADH. Based on the division ability of cells, viable cells are divided into two types, active-viable (dividable) cells and nonactive-viable (resting) cells, and activity is defined as the ratio of active-viable cell dry weight to viable cell dry weight. The active-viable cell is divided into active-viable or becomes nonactive viable cell. A nonactive viable cell becomes a dead cell as time goes by. The details of the model have been explained previously by the authors as shown in Table 1 [Choi et al., 1998b].

With a nonlinear parameter estimation technique [Metzler et al., 1974], model equations are solved simultaneously with numerical integration. The prediction by the proposed model is well consistent with experimental data [Choi et al., 1998b].

2. Feeding Strategy in Fed-Batch Cultivation

Typical characteristics of plant cells in a suspension culture include slow cell growth rate and substrate uptake rate compared to those of the microbes. In batch suspension culture, *Scutellaria baicalensis G.* cell shows slow cell growth rate and substrate uptake rate [Choe et al., 1993; Choi et al., 1997]. Continuous feeding of substrate in fed-batch operation or continuous operation is not appropriate due to the slow uptake rate of substrate and growth rate, and formation of aggregates of plant cells, which results in even larger volumes and effluence of unconsumed substrate. An approach to overcoming difficulties associated with continuous culture is semicontinuous culture, fed-batch culture. Since most of the secondary metabolites synthesis in plant cell culture are stimulated in

Table 1. List of kinetic model equations

Viability, V Activity, A	$V=\frac{X_{vd}}{X_d}\qquad A=\frac{X_{ad}}{X_{vd}}$
Active-viable Dry Weight Equation	$\frac{dX_{ad}}{dt}=\left[1-\exp\left(-\frac{t}{t_L}\right)\right]\frac{\mu_{max}S}{K_s+S}X_{ad}-k\phi X_{vd},\quad \text{where } \phi=\frac{1}{A}\frac{X_f}{X_d}\frac{1}{1+S/X_d}$
Nonactive-viable Dry Weight Equation	$\frac{dX_{nd}}{dt}=k\phi X_{vd}-k_d\frac{X_f}{V}$
Viable Dry Weight Equation	$X_{vd}=X_{ad}+X_{nd}\qquad \frac{dX_{vd}}{dt}=\left[1-\exp\left(\frac{t}{t_L}\right)\right]\frac{\mu_{max}S}{K_s+S}X_{ad}-\frac{k_dX_f}{V}$
Nonviable Dry Weight Equation	$\frac{dX_{dd}}{dt}=k_d\frac{X_f}{V}-k_LX_{dd}$
Dry Weight Equation	$X_d=X_{vd}+X_{dd}\qquad \frac{dX_d}{dt}=\left[1-\exp\left(-\frac{t}{t_L}\right)\right]\frac{\mu_{max}S}{K_s+S}X_{ad}-k_LX_{dd}$
Activity Equation	$\frac{dA}{dt}=\left[1-\exp\left(-\frac{t}{t_L}\right)\right]\frac{\mu_{max}S}{K_s+S}(1-A)A-k\phi+k_d\frac{AX_f}{V^2X_d}$
Viability Equation	$\frac{dV}{dt}=\left[1-\exp\left(-\frac{t}{t_L}\right)\right]\frac{\mu_{max}S}{K_s+S}(1-V)AV-k_d\frac{X_f}{VX_d}+k_L(1-V)V$ $V=\frac{\frac{FI/FI_m}{X_d}}{\left[\frac{FI/FI_m}{X_d}\right]^0}$
Substrate Uptake Rate Equation	$\frac{dS}{dt}=-\frac{1}{Y_{X/S}}\left[1-\exp\left(-\frac{t}{t_L}\right)\right]\frac{\mu_{max}S}{K_s+S}X_{ad}-\frac{1}{Y_{A/S_G}}(\alpha X_{ad}+\beta X_{nd})$
Product Equation	$\frac{da}{dt}=\alpha X_{ad}+\beta X_{nd}-Y_{a/b}k_3\frac{a}{K_{3b+a}}X_{vd}-Y_{a/w}k_4\frac{a}{K_{4w+a}}X_{vd}$ $\frac{db}{dt}=k_3\frac{a}{K_{3b+a}}X_{vd}-\lambda_3X_{dd}$ $\frac{dw}{dt}=k_4\frac{a}{K_{4w}+a}X_{vd}-\lambda_4X_{dd}$
Fresh Weight Equation	$\frac{dX_f}{dt}=\left[1-\exp\left(1-\frac{t}{t_L}\right)\right]k_1X_{ad}-k_2k_LX_{dd}-\theta(s)X_d,\quad \text{where } \theta(s)=\kappa\exp\left(1-\frac{S}{S^0}\right)$

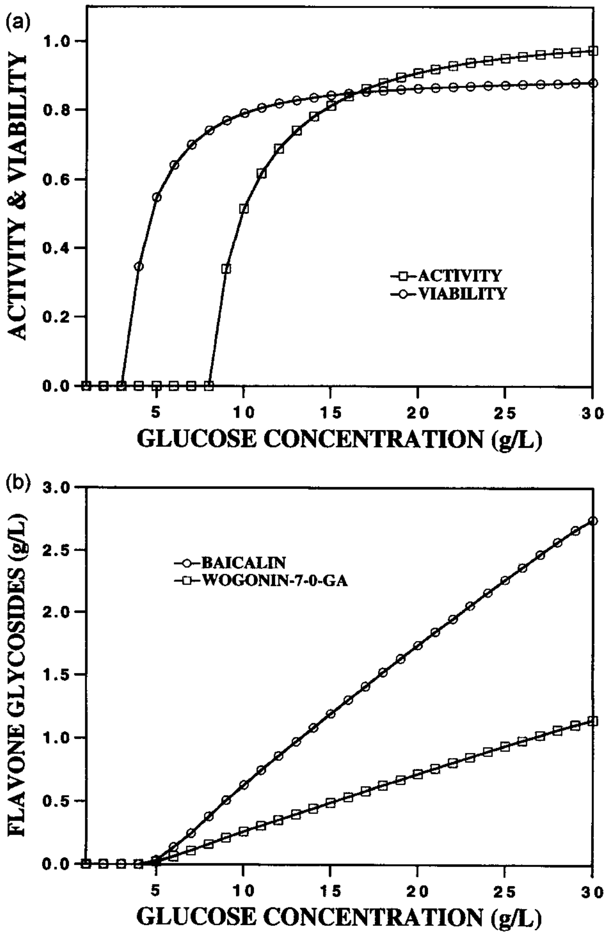

Fig. 1. Effect of glucose concentration on (a) activity and viability (b) baicalin and wogonin-7-0-GA concentration.

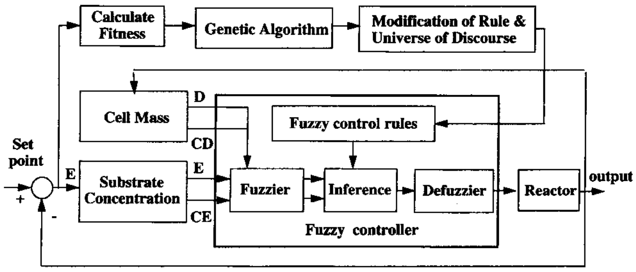

Fig. 2. Schematic diagram of self-organizing fuzzy logic controller.

the transition state, the fed-batch culture could be an attractive method. As a strategy for the fed-batch culture, a two-stage culture has been used to produce secondary metabolites in a plant cell culture [Buitelaar and Tramper, 1993; Choi et al., 1994]. The first stage is directed towards rapid cell growth and the second stage is geared to product synthesis where growth and the division are both very low. In order to construct a feeding strategy, effects of initial glucose concentration on the flavonoid production, activity and viability are simulated with a glucose concentration range of 1-30 g/L at 17 days of culture period as shown in Fig. 1.

As the two stage culture, the substrate feeding strategy for the fed-batch cultivation of *Scutellaria baicalensis G.* plant cell was constructed as a single switch between two constant glucose concentrations. In the first period, 22 g/L of glucose concentration is maintained to promote cell growth as well as flavone glycoside production. In the second period, 10 g/L of glucose concentration is maintained to promote flavone glycoside synthesis while viability is kept for flavone glycoside synthesis and activity is kept low to prevent cell growth as shown in Fig. 1.

SELF-ORGANIZING FUZZY CONTROLLER

1. Fuzzy Controller

The fuzzy logic controller is designed to regulate the glucose concentration at the given set-points to maximize the synthesis of flavone glycosides in *Scutellaria baicalensis G.* plant cell culture. The fuzzy control algorithm [Sugeno and Nishida, 1985; Zadeh and Lotfi, 1993] proceeds as follows: (1) fuzzification of fuzzy variables, glucose concentration and total cell dry weight, using the membership function, which includes error of substrate concentration, change of error of substrate concentration, total dry cell weight, and change of total cell dry weight; (2) inference using fuzzified variables and control rules by the min-max composition to obtain a fuzzy output, glucose feeding rate; and (3) defuzzification of glucose feeding rate by the center of gravity method to obtain a numerical value.

Since a reliable linguistic model of the operator's control strategy may not always be available and some significant process changes may be beyond the operator's experience, it is difficult to construct the appropriate fuzzy rules and universe of discourse in a fuzzy controller. In this study, a genetic algorithm is applied to construct the fuzzy rules by the modification of the membership function and modification of the universe of discourse. By application of the genetic algorithm, a self-organizing fuzzy logic controller is constructed for the control of glucose concentration to maximize the flavone glycoside synthesis in a fed-batch culture of *Scutellaria baicalensis G.* plant cell as shown in Fig. 2.

2. Fuzzy Rules Generation

While it is intented to apply fuzzy control to a bioprocess and industrial process, one of the key problems to be solved is to find fuzzy control rules. For the fed-batch operation of *Scutellaria baicalensis G.* plant cell, the fuzzy controller consists of a four input, single output process. The input variables are the set point error of glucose concentration (E), change in error of glucose concentration (CE), total cell dry weight (D), and change in total cell dry weight (CD). Control action (DU) is the change in glucose flow rate to be applied to the process. Consequently, by expressing such subjective evaluations represented by natural language as membership functions, control knowledge can be described as production rules having the following five fuzzy variables:

Rule A if E is E_i and CE is CE_i then DU is DU_i (A=1···N) (1)

Rule B if D is D_i and CD is CD_i then DU is DU_i (B=1···M) (2)

where, E_i, CE_i, D_i, CD_i and DU_i represent the linguistic labels

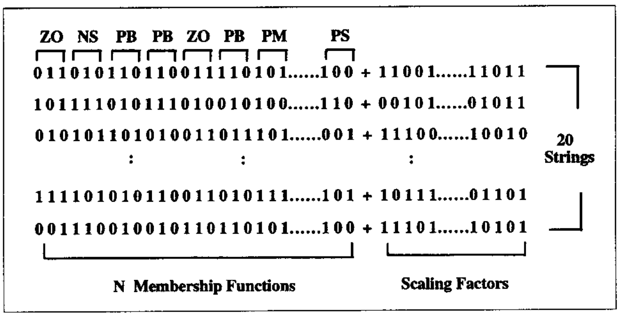

Fig. 3. Strings for the scaling factors and N+M membership functions.

of fuzzy variables E, CE, D, CD and DU, respectively. These labels consist of the following: Positive Big, Positive Medium, Positive Small, Zero, Negative Small, Negative Medium, Negative Big, Small, Small Medium, Medium, Medium Large and Large, abbreviated as PB, PM, PS, Z, NS, NM, NB, SS, SM, MM, ML, and LL, respectively.

The adjustment of fuzzy rules consisting of N+M rules in Eqs. (1) and (2) suitable for a control system is done by modification of the membership function of antecedent and consequent in fuzzy conditional statements or modification of the universe of discourse. The membership function of the consequent in fuzzy rules and the universe of discourse are modified for optimization of fuzzy rules, which are automatically generated by the genetic algorithm. Fig. 3 shows the scaling factor of the universe of discourse expressed by a binary string and the example of a string composed of N+M membership functions. The optimization process is based on the genetic algorithm in cooperation with the represented string.

3. Genetic Algorithm

The genetic algorithm is a powerful optimization technique inspired by Darwinian evolution theory, a natural evolution of selection by fitness [Dejong, 1988; Hwang and Woo, 1989; Hideo et al., 1993; Lee et al., 1995]. It represents the variables in the search into a binary coded string, which is referred to as a chromosome. In this study, fuzzy rules are converted to the binary coded string to be used as a chromosome. The performance measure is a real number which is referred to as a fitness value. The fitness function is shown in Eq. (3).

$$\text{Fitness} = \frac{K}{\sum_{i=1}^{n} |y_{set} - y_i| + \sum_{i=1}^{n} (y_{set} - y_i)^2} \qquad (3)$$

Where, y_{set} is set point of substrate concentration and y_i is the actual substrate concentration. K is a fitness constant and n is the number of generation. After evaluation of each fitness value, all the chromosomes are combined with each other through genetic operators to reproduce a new set of chromosomes to be evaluated. Genetic operators are prepared as the following three variations: reproduction, crossover, and mutation.

In reproduction, each individual's probability of being reproduced is proportional to the string's fitness. One way to arrange for such proportional reproduction is to create a roulette wheel in which the circumference is divided into as many segments existed in the population. The length of each segment is made to be proportional to the fitness of the corresponding string. Reproduction proceeds by spinning the wheel many times, and each time selecting a string to carry forward into next generation. In this way the reproduction step generates a list of copies of a subset of the starting population. The fittest individuals tend to produce the most copies.

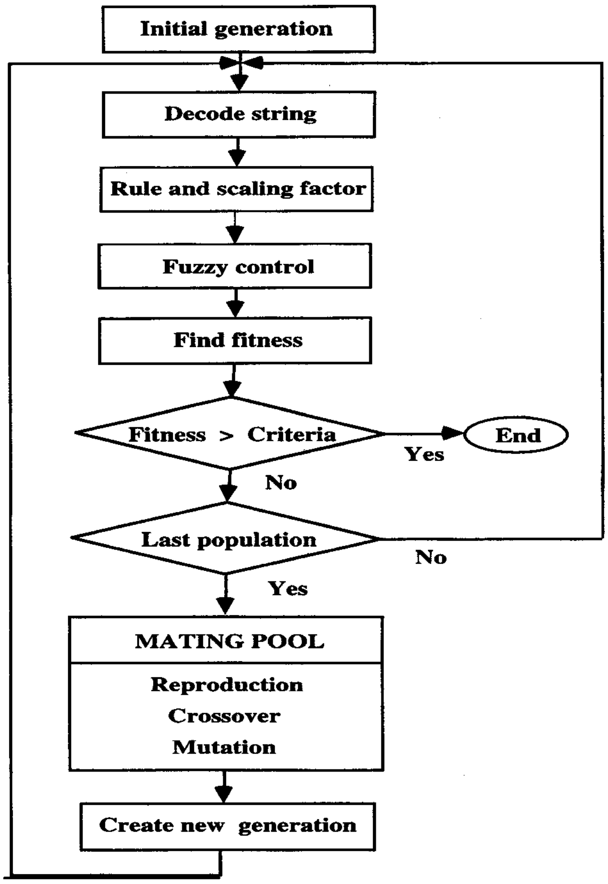

Fig. 4. Flow chart of genetic algorithm.

The crossover operator simulates the recombination of genetic elements to be possible by sexual modes of reproduction. Crossover begins with the selection of a random integer larger than zero and less than string length, defining thereby a crossover point. Two strings are mated by joining the prefix of one string with the suffix of the other string relative to the crossover point. With any sparse search through a large parameter space there is the danger of converging on a solution that is only locally rather than globally optimal. To avoid such traps, mutations are introduced after crossover. The overall process of a genetic algorithm for fuzzy rule generation and modification of universe of discourse is shown in Fig. 4.

RESULTS AND DISCUSSION

1. Fuzzy Rules

A fuzzy rule and its parameters can be optimized by the genetic algorithm. Initial parameters for the genetic algorithm are as follows: population size, 20; length of individual string, 252; crossover rate, 1.0; and mutation rate, 0.03. Fig. 5 shows the fitness value changes as generation proceeds. Its

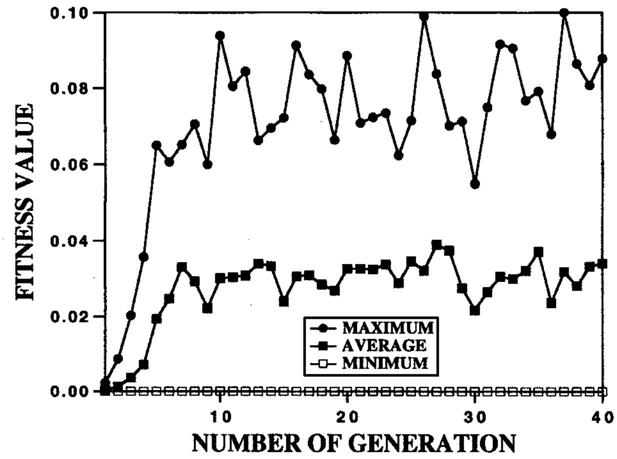

Fig. 5. Fitness value of individual generation.

vertical axis is the inverse of the sum of error, deviation from glucose set-points. The maximum, average, and minimum fitness are almost monotonically increasing in accordance with the generation. Maximum fitness is achieved after the 37th generation. Although few differences exist among parameters for maximum, average, and minimum fitness, the populations become saturated at their maximum values. So the differences should be considered as the drift caused by characteristics of the proposed genetic algorithm. It can be concluded that the genetic algorithm can optimize the fuzzy rule base and its parameters in a short period of trials. The membership function and fuzzy control rules generated in the 37th generation are used for the control of the fed-batch process. Fuzzy rules generated by genetic algorithm consist of 45 relations between the error of glucose concentration, the change in error of glucose concentration and glucose feeding rate as well as 20 relations between total dry cell weight, the change in total dry cell weight and glucose feeding rate. Membership functions of glucose concentration and membership functions of dry cell weight are shown in Fig. 6. And fuzzy rule lookup tables of glucose concentration and fuzzy rule lookup tables of dry cell weight are shown in Fig. 7.

2. Fed-Batch Culture Controlled by Self-Organizing Fuzzy Controller

The simulation results represent the time course profile of glucose concentration, cell activity, cell viability, dry cell weight, baicalin production and wogonin-7-0-GA production. The maximum dry cell weight, specific production yield and productivity were also calculated. The simulation result for the control performance by the self-organizing fuzzy logic controller is shown in Fig. 8(a). The self-organizing fuzzy logic controller shows the good control performance to keep the trajectory of glucose concentration. As shown in Fig. 8(b), the activity was reduced to zero at the 12th day, but viability was maintained above 0.3 until the 17th day. Flavone glycosides, baicalin and wogonin-7-0-GA synthesis are carried out by viable cell. Since cell viability is maintained, a specific production rate for flavone glycosides can be maintained. The simulation result shows that maximum cell dry weight was about 23.2 g/L at the 12th day as shown in Fig. 8(c). Practically 75 g/L of plant cell mass has been obtained in a stirred tank bioreactor with a hollow-paddle type stirring wing to achieve sufficient mixing [Matsuhara et al., 1989]. Thus, the suggested operation is possible by the development of an aeration-agitation method in practical operation. The amounts of baicalin and wogonin-7-0-GA produced are 3.25 g/L and 1.29 g/L, respectively, as shown in Fig. 8(d). Flavone glycoside pro-

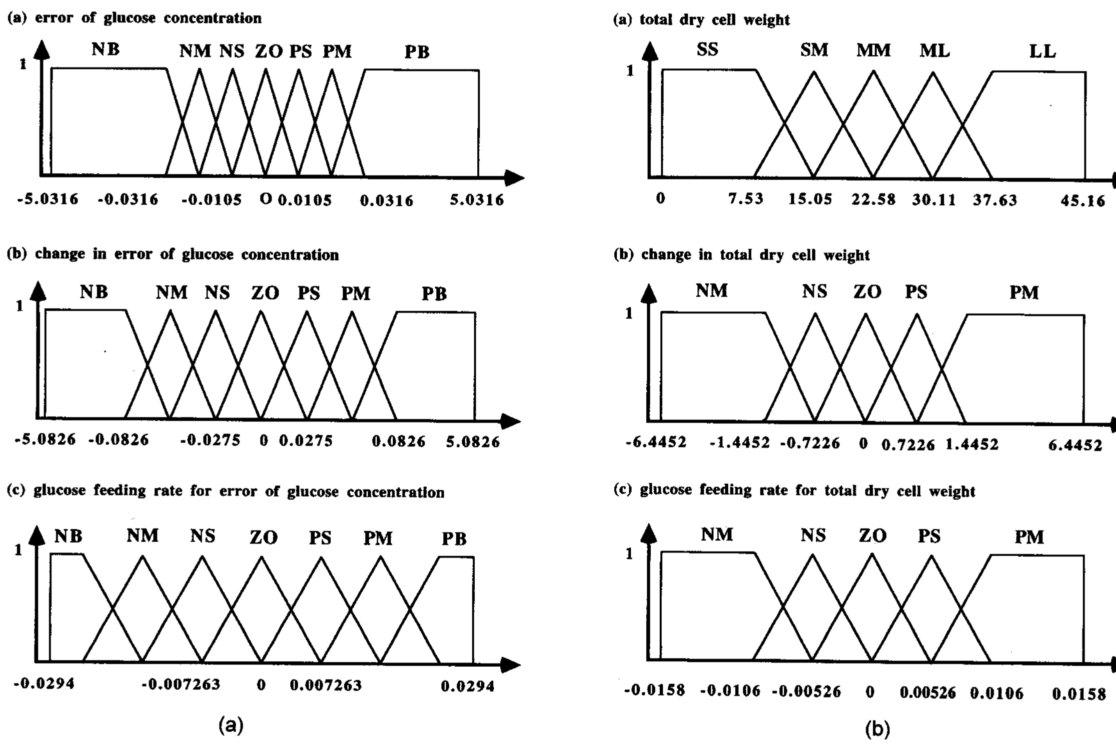

Fig. 6. Membership functions of (a) glucose concentration, (b) dry cell weight.

CD \ D	SS	SM	MM	ML	LL
NM	ZO	ZO	PS	NS	NS
NS	PS	NB	ZO	ZO	PB
ZO	PB	ZO	NB		ZO
PS	ZO				ZO
PM	PB		ZO	ZO	PS

(a)

E \ CE	NB	NM	NS	ZO	PS	PM	PB
NB	NB		NM	NB	PS		PM
NM	ZO	ZO	NB	PS	PS	PS	PS
NS	NM	NB	ZO	NM	NB	ZO	NS
ZO	NS	PM	NS	PS	NS	ZO	NS
PS	NS		NB	NB	NM	ZO	NM
PM	NS	PB	NM	ZO	PS		ZO
PB	NM	NM	PS	PS	PB	PM	PM

(b)

Fig. 7. Fuzzy rule lookup table of (a) glucose concentration, (b) dry cell weight.

Table 2. Experimental and simulation result of batch and two stage fed-batch culture

	Batch experiment	Two stage fed-batch culture
Maximum		
dry cell weight (g/L)	17.50	23.20
Product concentration (g/L)		
baicalin	2.72	3.25
wogonin-7-0-GA	1.09	1.29
Specific production yield (g/g glucose)		
baicalin	0.09	0.11
wogonin-7-0-GA	0.03	0.04
Specific production yield (g/g DCW)		
baicalin	0.15	0.14
wogonin-7-0-GA	0.06	0.05
Productivity (g/LDay)		
baicalin	0.19	0.21
wogonin-7-0-GA	0.07	0.08

duction in two stage culture is larger than that of batch culture. Also the production yield and productivity of flavone glycosides are increased as shown in Table 2; two stage culture with control of glucose concentration by self-organizing fuzzy controller improves the flavone glycosides production compared with that in the batch operation.

CONCLUSIONS

The proposed substrate feeding strategy in fed-batch culture, the two stage culture with a switching set-point of glu-

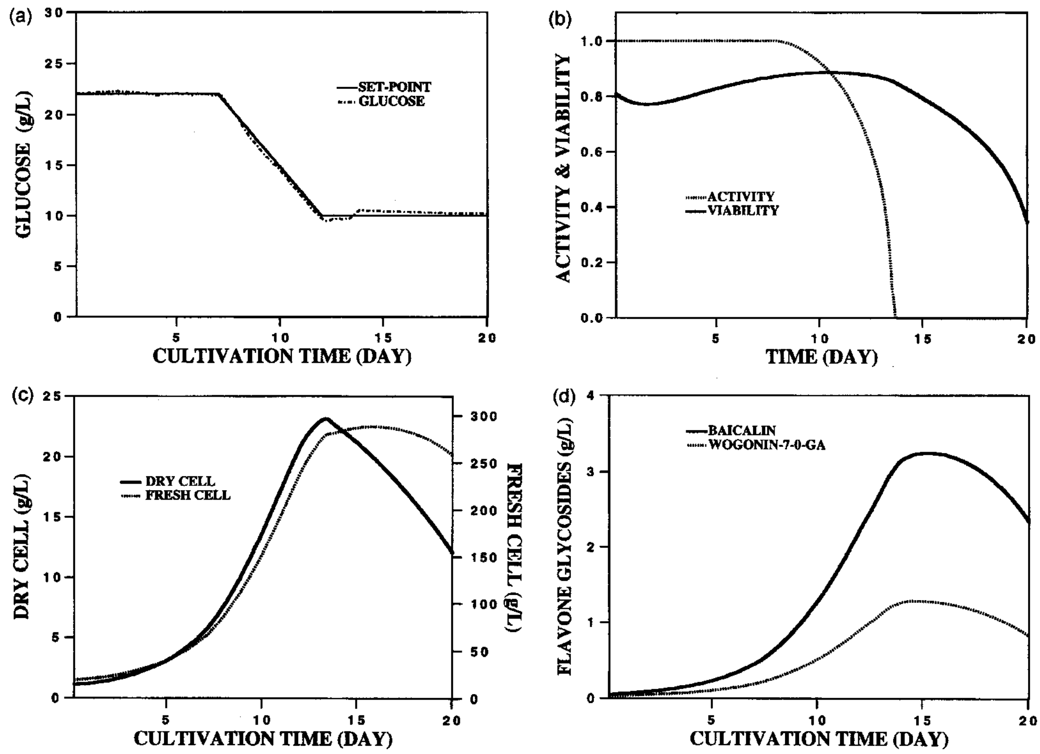

Fig. 8. Simulation result of fed-batch operation controlled by self-organizing fuzzy logic controller (a) glucose concentration, (b) activity and viability, (c) dry cell and fresh cell weight, (d) baicalin and wogonin-7-0-GA concentration.

cose concentration enhances flavone glycoside production in *Scutellaria baicalensis G.* plant cell. A substrate feeding strategy to consider physiological parameters related to growth and product synthesis could be applied to the plant cell culture process. For control of substrate concentration, a self-organizing fuzzy controller is developed using a genetic algorithm. In the simulation results, substrate concentration trajectory to increase flavone glycoside production in a two stage culture could be successfully maintained by a self-organizing fuzzy logic controller. The self-organizing fuzzy logic controller generated by the genetic algorithm improves the controller performance since the convergence of the desired trajectory enhanced with a decrease of error sum of process variable to set value. Thus, the genetic algorithm could be applied to generate the fuzzy control rules and modification of the universe of discourse to control substrate feeding rate in a fed-batch culture of *Scutellaria baicalensis G.* plant cell. The designed self-organizing fuzzy controller can generate the fuzzy rules and modify the universe of discourse automatically, which just needs the cell kinetics without any information and experience in fed-batch operation in bioprocessing.

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 Letters

α : growth-associated production constant [day^{-1}]
β : nongrowth-associated production constant [day^{-1}]
k : cell expansion coefficient [day^{-1}]
μ : specific growth rate [day^{-1}]
l : product degradation constant [gg^{-1} day^{-1}]
g : product release coefficient by cell lysis [g/g]
q : function for cell expansion
f : function for activity loss

Subscripts

d : dry weight or death
S : glucose
L : lag phase
max : maximum
ad : active-viable cell
dd : dead cell
nd : nonactive-viable cell
vd : viable cell
a/S : apigenin from glucose
X/S : biomass from glucose
a/w : apigenin to wogonin-7-0-GA
a/b : apigenin to baicalin

Superscript

o : initial

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