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Control of Feed Rate Using Neurocontroller Incorporated with Genetic Algorithm in Fed-Batch Cultivation of *Scutellaria baicalensis* Georgi

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Abstract To enhance the production of flavonoids [baicalin, wogonin-7-*O*-glucuronic acid (GA)], which are secondary metabolites of *Scutellaria baicalensis* Georgi(G.) plant cells, a multilayer perceptron control system was applied to regulate the substrate feeding in a fed-batch cultivation. The optimal profile for the substrate feeding rate in a fed-batch culture of *S. baicalensis* G. was determined by simulating a kinetic model using a genetic algorithm. Process variable profiles were then prepared for the construction of a multilayer perceptron controller that included massive parallelism, trainability, and fault tolerance. An error back-propagation algorithm was applied to train the multiplayer perceptron. The experimental results showed that neurocontrol incorporated with a genetic algorithm improved the flavonoid production compared with a simple fuzzy logic control system. Furthermore, the specific production yield and flavonoid productivity also increased.

Key words: *Scutellaria baicalensis G.*, flavonoids, fed-batch culture, multilayer perceptron, genetic algorithm

Higher plants represent a valuable resource for a great variety of special chemicals, including pharmaceuticals (ajamalcine, berbine, codeine, digoxin, reserpine, and vincristine), flavors and fragrances (strawberry, vanilla, and rose), pigments (anthocyanins, betacyanins, shikonin, and saffron), and fine chemicals (pyrethrin, salannin, and protease) which are mostly typical secondary metabolites that are biosynthesized from primary metabolites. Recent improvements in plant cell and tissue culture techniques have been proposed as an alternative for the production of plant-derived chemicals [3, 13, 18, 19].

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Scutellaria baicalensis Georgi(G.) is an important medicinal herb that is widely used for the treatment of various inflammatory diseases, hepatitis, tumors, and diarrhea in East Asian countries such as China, Korea, and Japan [9]. Its ether extract is reported to have a potent cytotoxic principle against L1210 cells, and exhibit antitumor activity to Sarcoma-180 in mice [1]. The roots of *S. baicalensis G.* are known to contain a large number (over 40) of flavonoids, frequently identified as glucuronides. The major products are baicalin, baicalein, wogonin, and wogonin-7-*O*-glucuronic acid(GA) [23, 24].

One important goal in a plant cell culture is to enhance the yield of the product. Therefore, to enhance the production of secondary metabolites in a plant cell culture, operating and control strategies that consider the different stages related to the cellular differentiation and secondary metabolite production of the plant cells are required [16, 27]. Accordingly, the current study investigated the time-course profile of the optimal substrate feeding rate as an optimal bioreactor operating strategy using a genetic algorithm, mathematical kinetic model, and determined initial substrate concentration for the construction of a bioreactor operating strategy [6].

Since the relationship between the state variables and the control variables can not be clearly defined by a simple function and unpredictable probability factors existing in a bioprocess, the bioreactor operation usually depends on expert experience and imagination [12]. As such, the control of a plant cell culture process using a classical controller is very difficult because complicated quantitative knowledge of the processes under the influence of the control variables is required [5, 6]. To overcome these difficulties, a fuzzy control system has been proposed [6]. In addition, artificial neural network is well-known to be suitable for monitoring and controlling knowledge-poor processes, since it can perform rational reasoning with a

precise mathematical model of the process, work with incomplete process information, and handle a large amount of information quickly through parallel processing [21, 25]. In the current study, a multiplayer perceptron is applied to control the substrate feeding in the fed-batch cultivation of *S. baicalensis* G. plant cells. Profiles of the optimal glucose feeding rate and process variables, prepared by a genetic algorithm based on the simulation of a kinetic model, are used as the learning input-output data set for the multiplayer perceptron. The trained neural network is then successfully applied to regulate the substrate feeding rate using the model reference adaptive control method. The performance of the neurocontrol system is evaluated through the production of flavonoids and compared with that of a simple fuzzy control system [6].

The callus and suspension cultures, cell dry weight, glucose concentration, and flavonoid analysis of the *S. baicalensis* G. plant cells were all performed in the same way as in a simple fuzzy control system [6, 15, 22]. Because controlling the substrate feeding can promote the productivity of the target metabolites, a fed-batch culture system is the most effective bioreactor operating system [8, 11, 26]. The bioreactor operating system was also set up in the same way as in a simple fuzzy control system [6]. The bioreactor control logic was coded with C language.

Artificial neural networks (ANNs) have recently received considerable attention in the control community, mainly due to their powerful mapping capacity, trainability property, real-time adaptation without instability, handling of severe nonlinearity and noise, and parallel implementation paradigm, thereby leading to the emergence of a new term, neurocontrol [4, 10]. On the basis of the above merits, the ANNs are now widely used in many nonlinear control applications [17, 20].

To enhance the production of flavonoids in a fed-batch cultivation of *S. baicalensis* G., a multilayer perceptron controller was applied using the model-reference adaptive control method, where the goal is to generate a control input signal so that the system follows a desired trajectory determined by the reference model. In a back-propagation neural network, the function is commonly in the form of a sigmoid function in order to obtain a signal between 0 and 1 [2].

$$y_j=g(x_i)=\left[1+\exp\left(\sum_{i=1}^{n} x_i w_{ij}+\theta_j\right)\right]^{-1} \quad (1)$$

where x_i is the input signal, y_j is the fired output signal, w_{ij} is the weight associated with the input signal x_i, and θ_j is the threshold value of neuron j.

In order to make the multilayer perceptron an actual controller, a supervised learning method was adopted, whereby the neural network was trained to map the input sensor signals onto the desired outputs - the glucose feeding rates. The actual adjustment of the connection weights for a bioprocess application was performed using an error back-propagation (BP) algorithm, which is basically a form of the gradient descent method, meaning that the output-layer errors are successfully propagated backwards through the network. The error for training the neural network was defined by the following equation (2):

$$E=\frac{1}{2}\sum_{i=1}(y_i-d_i)^2 \quad (2)$$

where y_i is the fired output and d_i is the desired output from neuron i. A schematic description of the artificial neural network training is shown in Fig. 1.

Based on the constructed kinetic model for the batch culture and proposed bioreactor operating strategy [6, 14], the optimal glucose feeding profile was heuristically investigated, and the input-output data set for training the artificial neural network was prepared through a fed-batch kinetic model simulation using a genetic algorithm. A genetic algorithm is a type of stochastic search method based on the principles of three operators; reproduction,

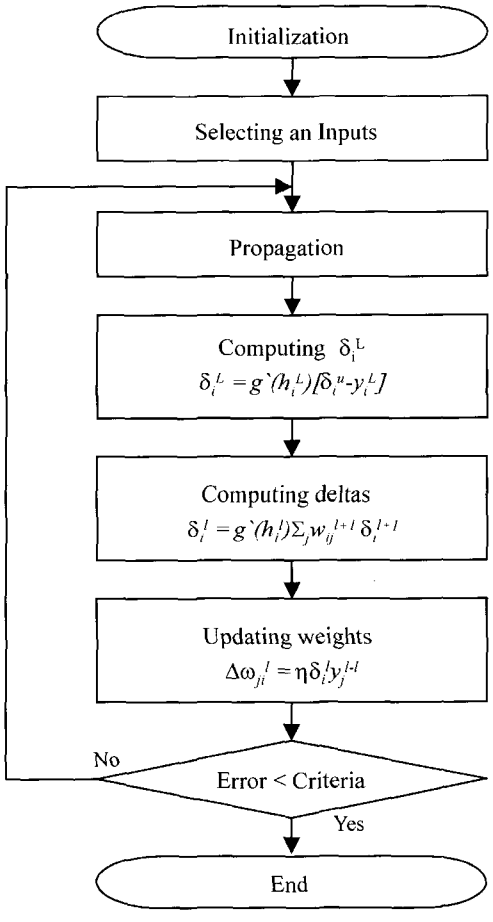

Fig. 1. Schematic diagram of artificial neural network training

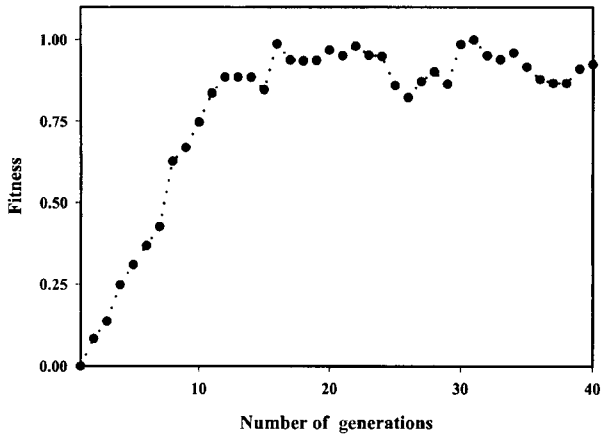

Fig. 2. Fitness value with each generation.

crossover, and mutation, as inspired by Darwin's evolution theory; natural evolution of selection by fitness [7]. The algorithm represents the search variables as a binary coded string, referred to as a chromosome. A population of chromosomes is then prepared and their performances are evaluated by actually applying these binary encoded parameters to the objective system. The performance measure is a real number, referred to as a fitness value. The initial parameters for the implementation of the genetic algorithm were as follows; number of population, 20; length of individual, 280; crossover rate, 1.0; mutation rate, 0.03. The chromosome consisted of 40 fragments of substrate feed rate. The substrate feed rate was determined between 0 and 0.1 ml/min. The fitness value was defined, as shown in equation (3).

$$\text{Fitness}=\lambda \times B \quad (3)$$

where B is the concentration of baicalin and λ is the normalization factor.

Figure 2 shows the fitness value changes with various generations. The vertical axis is the normalization value of the maximum baicalin production in each generation at the end of the fed-batch cultivation, and the fitness increased almost monotonically in accordance with each generation. The maximum fitness was achieved after the 31st generation. Although slight differences existed among the fitness parameters, the population became saturated at the maximum values. The differences were considered to be drift due to the characteristics of the proposed genetic algorithm. Accordingly, it was concluded that the genetic algorithm could optimize the profile of the substrate feeding rate within a short period of trials, and it could be used to calculate the process variable profiles (glucose concentration, cell dry weight, and flavonoids production) based on a simulated fed-batch cultivation of *S. baicalensis* G.

Using the genetic algorithm, the optimal profiles of the glucose feeding rate and process variables were heuristically decided based on a simulated fed-batch cultivation using a kinetic model. Figure 3 shows the time-course profiles of the process variables in the fed-batch culture using the fittest substrate feeding rate profile. The optimal glucose feeding rate and simulated process variable profiles were utilized to train the artificial neural network controller.

The multilayer perceptron was then applied to a fed-batch cultivation of *S. baicalensis* G. The user-defined parameters for the construction of the multilayer perceptron were as follows; number of inputs, 4 (cell dry weight, glucose concentration, baicalin, and wogonin-7-*O*-GA concentrations); number of outputs, 1; number of neurons in hidden layers, 30 (1st) and 15 (2nd); respectively; learning rate, 0.7; and momentum coefficient, 0.5. Figure 4 represents the change in the cell dry weight in the neurocontrol experimental results. The maximum cell dry weight reached 7.835 g l^{-1}, 17 days after inoculation. The glucose concentration varied

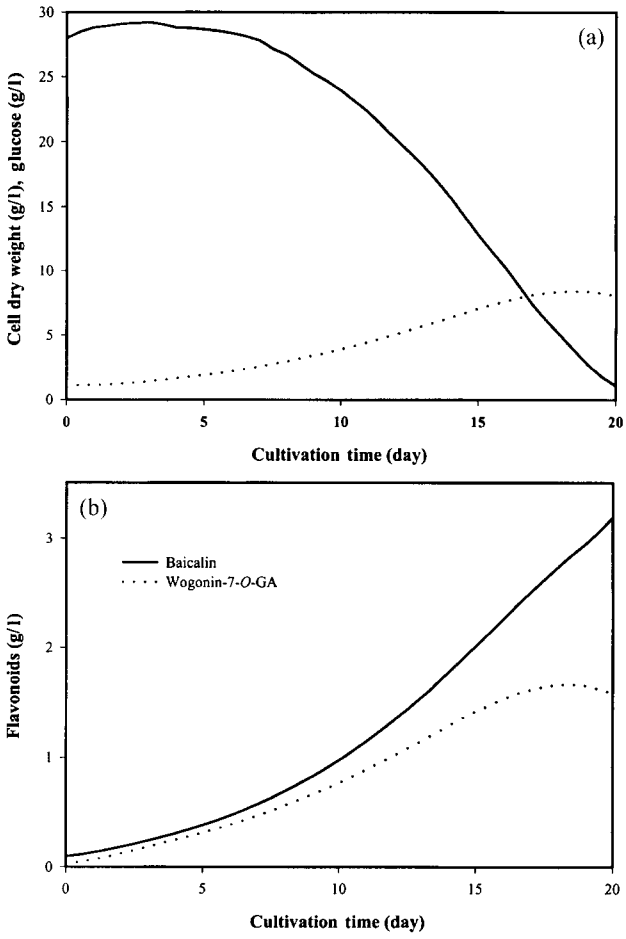

Fig. 3. Profile of glucose concentration maximizing the flavonoid production, identified by genetic algorithm and process variable profiles, in fed-batch cultivation of *S. baicalensis* G.
Lines: (a) glucose, (b) cell dry weight (——); baicalin wogonin-7-*O*-GA (·····).

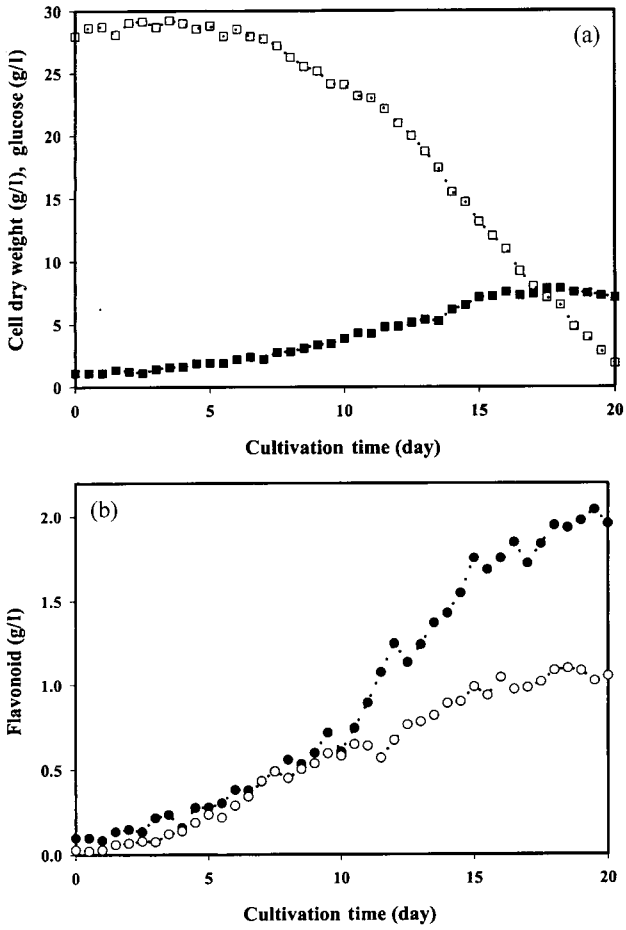

Fig. 4. Experimental results of fed-batch culture using a neurocontroller. (a) Cell dry weight and glucose, (b) flavonoid production.
Symbols: cell dry weight (■); glucose (□); baicalin (●); wogonin-7-*O*-GA (○).

smoothly for the entire period of the fed-batch culture. The maximum production of baicalin and wogonin-7-*O*-GA was 2.042 g l^{-1} and 1.098 g l^{-1}, respectively. The flavonoid production in a two-stage culture using the neurocontrol was higher than that in the batch culture, and the specific production yield and flavonoid productivity were increased. When compared with simple fuzzy logic control, the actuation speed with respect to errors in the state variables was slow. Therefore, this suggested that a rapid response to a variation in the glucose concentration resulted in an unstable change in the overall process or disturbance. Because the characteristics of a plant cell culture process include sensitivity to environmental changes, a rapid change in the glucose feeding rate had apparently a negative effect on both the growth and flavonoid production of *S. baicalensis* G. Table 1 summarizes the experimental results of the batch culture, fed-batch culture using a simple fuzzy logic controller, and fed-batch culture using a neurocontroller. The specific production yield and flavonoid productivity were both enhanced in the fed-batch culture with incorporated neurocontroller with a genetic algorithm. This result suggested that a set-point regulating controller that did not consider the overall process state was ineffective. Instead, due to the biological sensitivity of a plant cell culture process, a safe operation is required that considers the overall process. Accordingly, an artificial neural network appeared to be well suited, since it can consider many process variables, including the glucose concentration, before firing the output signal of the glucose feeding rate. In addition, the artificial neural network controller designed in the current study was found to enhance the specific production rate and productivity, as shown in Table 1. Therefore, it can be concluded that an artificial neural network is a more suitable controller than a fuzzy logic controller in the fed-batch cultivation of *S. baicalensis* G.

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Table 1. Experimental results of batch and fed-batch cultures of *S. baicalensis* G.

	Batch [6]	Fed-batch culture with simple fuzzy control [6]	Fed-batch culture with neurocontrol
Cell dry weight (g/l)	7.610	7.394	7.835
Product (g/l)			
Baicalin	1.200	1.372	2.042
Wogonin-7-*O*-GA	0.467	0.504	1.098
Specific production yield (g/g cell dry weight)			
Baicalin	0.157	0.186	0.261
Wogonin-7-*O*-GA	0.061	0.068	0.140
Productivity (g/l)			
Baicalin	0.060	0.069	0.102
Wogonin-7-*O*-GA	0.023	0.025	0.055

NOMENCLATURE

B : Baicalin concentration (g/l)
E : Error
d : Desired output (dimensionless)
x : Input signal (dimensionless)
w : Weight associated with input (dimensionless)
y : Fired output signal (dimensionless)

Greek Symbols

λ : Normalization factor
θ : Threshold for neuron

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