Software Sensing for Glucose Concentration in Industrial Antibiotic Fed-batch Culture Using Fuzzy Neural Network

Toshiaki Imanishi[1], Taizo Hanai[2], Ichiro Aoyagi[2], Jun Uemura[2], Katsuhiro Araki[2], Hiroshi Yoshimoto[2], Takeshi Harima[2], Hiroyuki Honda[1], and Takeshi Kobayashi[1]*

[1] Department of Biotechnology, School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan
[2] Pfizer Pharmaceuticals Inc., Aza 5-gochi 2-banchi, Taketoyo-cho, Chita-gun, Aichi 470-2393, Japan

Abstract In order to control glucose concentration during fed-batch culture for antibiotic production, we applied so called "software sensor" which estimates unmeasured variable of interest from measured process variables using software. All data for analysis were collected from industrial scale cultures in a pharmaceutical company. First, we constructed an estimation model for glucose feed rate to keep glucose concentration at target value. In actual fed-batch culture, glucose concentration was kept at relatively high and measured once a day, and the glucose feed rate until the next measurement time was determined by an expert worker based on the actual consumption rate. Fuzzy neural network (FNN) was applied to construct the estimation model. From the simulation results using this model, the average error for glucose concentration was 0.88 g/L. The FNN model was also applied for a special culture to keep glucose concentration at low level. Selecting the optimal input variables, it was possible to simulate the culture with a low glucose concentration from the data sets of relatively high glucose concentration. Next, a simulation model to estimate time course of glucose concentration during one day was constructed using the on-line measurable process variables, since glucose concentration was only measured off-line once a day. Here, the recursive fuzzy neural network (RFNN) was applied for the simulation model. As the result of the simulation, average error of RFNN model was 0.91 g/L and this model was found to be useful to supervise the fed-batch culture.

Keywords: process control, software sensor, fuzzy neural network, antibiotic production, fed-batch fermentation

INTRODUCTION

Production of antibiotics in industrial plant is conventionally carried out by fed-batch culture since the use of concentrated production media in batch culture usually reduces antibiotic productivity. In antibiotics production, one of the most important things is to keep concentration of substrate (*i.e.* glucose concentration) at low level. Generally, high glucose concentration in broth is not good for fermentative production. For example at high glucose concentration, catabolite repression occurs and the production of secondary metabolites such as antibiotics is significantly repressed [1]. In addition, Crabtree effect is also observed [2], in which *Escherichia coli* and *Saccharomyces cerevisiae* produce acetate and ethanol, respectively, even if they are in aerobic conditions, and accumulation of acetate or ethanol inhibits their growth and also reduces the expression of introduced genes. It is very important for antibiotic production to keep glucose concentration at relatively

low level [1]. However, if glucose concentration is too low, a risk of glucose starvation becomes high and it causes a significant reduction of production and resulted in a big economic damage.

At the laboratory level, an on-line glucose sensor is available but the reproducible response of this sensor is limited to short term [3]. Since industrial antibiotic production in fed-batch mode usually lasts about 1-3 weeks, the reliability as the process sensor is so low. There is no on-line glucose sensor available for such a long term. Therefore, at industrial fermentation plant, glucose concentration is not measured, or measured off-line once a day or two days and glucose feed rate is determined by an expert worker using the calculated consumption rate. Glucose concentration is normally controlled at a high level to avoid the depletion of glucose, but such a level is not optimal for the production of desired substances such as antibiotics in some cases.

Recently, a method so called "software sensor" has been studied by some researchers [4,5]. By this method, unmeasurable process variables of interest are estimated from measurable ones in computer using software. Artificial neural network (ANN) and expert systems are often used as the software. The models to estimate the

* **Corresponding author**
Tel: +81-52-789-3213 Fax: +81-52-789-3214
e-mail: takeshi@nubio.nagoya-u.ac.jp

enzyme concentration were constructed using ANN in lipase, glucoamylase and xylanase production processes [4].

In the present study, we applied fuzzy neural network (FNN) as software to estimate glucose feed rate and glucose concentration in an industrial antibiotic fermentation. FNN modeling is one of the knowledge information processing methods, which has a structure of neural network and enables to fuzzy reasoning. The neural network is possible to learn automatically a complex relation of inputs and outputs. Fuzzy reasoning can treat the human's ambiguous reasoning as the linguistic expression. High estimation ability of an FNN model has been proven in our previous studies such as Japanese *sake* mashing process [6,7]. We also constructed an FNN model so as to simulate correctly effluent chemical oxygen demand (COD) value [8]. To improve simulation accuracy further, the FNN model with recursive learning data-renewing method, called as the RFNN model [9], was newly proposed.

In the present study, the data measured in industrial antibiotic production plant at a pharmaceutical company were used to construct an estimation model for glucose feed rate using the FNN model. Next, a simulation model for time course of glucose concentration was constructed using the RFNN model.

MATERIALS AND METHODS

Measured Variables

In the present study, we used the data of 15 fed-batch cultures for industrial antibiotic production using *Actinomyces* strain. Table 1 shows the process variables measured in the antibiotic production. Glucose concentration, packed cell volume (PCV) and viscosity of broth were measured off-line, and the others were on-line. Glucose concentration was measured by a colorimetric analysis using glucose oxidase-peroxidase system. PCV was determined by measuring volume of solid component after centrifuged the broth at 3,000 rpm (1,700 × g) for 10 min. Viscosity of broth was measured by a rotating viscometer (B-type, Tokimec Inc., Tokyo). Air flow rate was measured by a corn-type flowmeter (Tokyo Keiso Co., Tokyo). Total weight of broth was determined by measuring the head using a differential pressure transmitter (Hitachi Co., Tokyo). Temperature of broth was measured by a resistance thermometer (Okazaki Manufacturing Co., Tokyo). Oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER) were calculated by percentage of O_2 and CO_2 at inlet and outlet of the fermentor measured by a mass spectrometer (Thermo Onix Inc., North Velasco Angleton, TX, USA; Prima 600S). Glucose feed rate was measured by an electromagnetic flowmeter (Yokogawa Electric Co., Tokyo). Dissolved oxygen concentration (DO) and pH were measured by each probe sensor (Toa DKK Co., Tokyo).
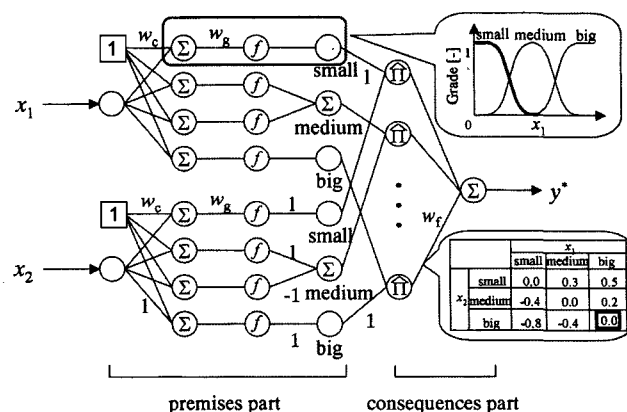

Fig. 1. Structure of fuzzy neural network. The example for two input and one output is shown.

Fuzzy Neural Network (FNN)

In the present study, "Type I" FNN [6,10] as shown in Fig. 1 was used. An FNN combines a simplified fuzzy reasoning with an ANN. In the premises part, the numerical values of input variables are altered to the grade of fuzzy variables by membership functions. Each membership function consists of sigmoid functions. The consequences part represents the fuzzy rules. The connection weights of the network correspond to the fuzzy reasoning parameters. The relationships between input and output are automatically identified by learning through an error backpropagation algorithm [11], and are represented as linguistic IF-THEN rules.

If unnecessary variables are used as the input for the FNN model, the fuzzy rules of the model would become difficult to be understood and the accuracy of this model would be lower than that using only necessary variables. Therefore, the parameter increasing method (PIM) [12] was used to select input variables and optimize the FNN model. The first step in PIM selection is to choose the most useful single input variable for accurate prediction. The number of membership functions of the input variable is set at two. In the next step, the second most useful variable is selected. If the variable is the same as the one selected in the previous step, the number of membership functions for the variable is increased from two to three. By repeating this operation, the most useful combination of input variables and membership functions is determined as the prediction model. This FNN was applied to construct the model to estimate glucose feed rate.

Recursive Fuzzy Neural Network (RFNN)

We also constructed a simulation model for time course of glucose concentration during one day. To simulate time course of glucose concentration, recursive learning data-renewing method based on a recursive parameter estimation method [13] was also applied to the FNN model, called as the recursive FNN (RFNN)

Table 1. Candidates of input variables for construction of model and intervals of measurements

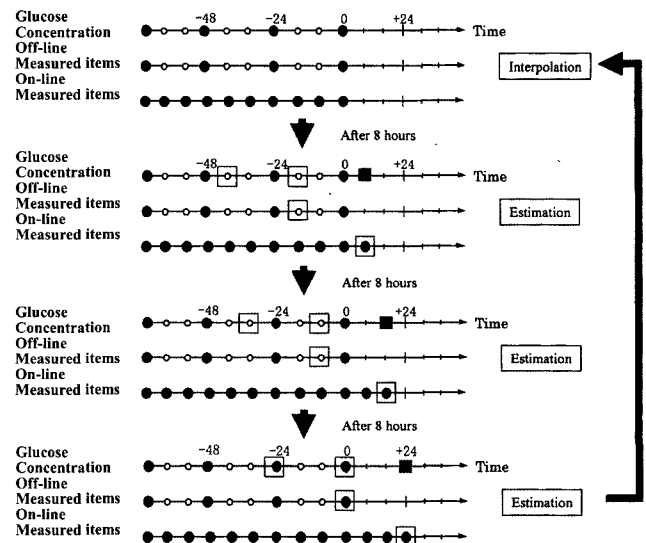| Interval of measurement*	Candidate of input variables
1	Glucose concentration at the latest sampling time (g/L)
1	Glucose concentration before 24 h from the latest sampling time (g/L)
1	Glucose consumption during 24 h before the latest sampling time (kg)
1	Glucose feed during 24 h before the latest sampling time (kg)
1	Differences of glucose concentration between the objective value and the actual value at the latest sampling time (g/L)
1	Packed cell volume(PCV) at the latest sampling time (mL-cell/15 mL-broth)
1	Viscosity at the latest sampling time (cp)
2	Air flow rate (m³/min)
2	Total weight of broth (ton)
2	Oxygen uptake rate(OUR) (mmol O_2/kg-broth/h)
2	pH (-)
2	Dissolved oxygen concentration(DO) (ppm)
2	Cultivation time (h)
2	Integrated value of OUR during pre-culture (mmol O_2/kg-broth/h)

* 1:24 h (off-line), 2:30 sec (on-line)

Fig. 2. Estimation procedure of simulation model for glucose concentration using RFNN. □: data used for estimation as input variable, ■: estimated value, ●: measured value, O: interpolated value.

model. In the RFNN model, the learning data sets are renewed and the FNN model is reconstructed in order to fit to the renewed data sets [9]. The reconstructed model reflects the last situation of fermentor and the high accuracy of estimation model is achieved. The detail of the theoretical background for RFNN has been discussed in our previous paper [9]. The PIM was also used to select input variables.

Estimation Model for Glucose Feed Rate

In the actual fermentation plant, glucose concentration was measured off-line once per day, and the glucose feed rate was adjusted by calculated consumption rate. The estimation model for glucose feed rate consists of 2 steps. First, total amount of glucose consumption was estimated using FNN. Next, the glucose feed rate was calculated based on the material balance. The variables measured on-line were averaged during the past 24 h. The candidates for input variables of glucose feed estimation models are shown in Table 1. Temperature at the broth was controlled well, and this was not contained in the candidate of the input variables for estimation model. CER was removed from input variables, since the correlation coefficient between CER and OUR was 0.95 and this is too high to contain in the input variables.

To construct the estimation model for glucose feed rate, the data sets from 6 fed-batch cultures were used for the learning data for FNN. In order to avoid over learning for FNN, other data sets from 6 fed-batch cultures were used for the evaluation data. The remaining data sets from 2 fed-batch cultures were used for the

simulation data to check the estimation ability. The estimation ability of the model was evaluated by the differences between the actual glucose concentration and the calculated one by the material balance with the estimated amount of glucose feed rate. As the result, the number of learning, evaluation and simulation data sets were 128, 125 and 42, respectively.

A special cultivation was carried out, *i.e.* the fed-batch culture keeping glucose concentration at low level. The data sets for this culture were used for the simulation at low glucose concentration.

Estimation Model for Time Course of Glucose Concentration

As discussed in the above section, the glucose feed rate on daily basis is estimated from the model constructed. Glucose concentration is also estimated from the glucose feed rate and the material balance. However, it is an average glucose concentration on daily basis, and we cannot supervise the actual fermentation situation until the next measurement time on next day. Many on-line measuring process variables are available as shown in Table 1, and it will be possible to estimate more reliable glucose concentration until the next measurement time if we use these on-line variables. Therefore, the RFNN model was constructed to estimate time course of glucose concentration using the on-line and off-line measuring variables. Here, off-line measuring variables, such as PCV and viscosity of broth were interpolated to continuous values by the cubic spline function [14,15].

The same learning, evaluation and simulation data sets with the estimation model of glucose feed rate

Table 2. Estimation errors

|	Average error (g/L)	Maximum error (g/L)
Learning data (128)*	0.97	3.30
Evaluation data (125)*	1.02	4.40
Simulation data (42)*	0.95	3.47

* Number of data sets

were used to construct the RFNN models. However, the time interval of the data was 1 h (theoretically, it is possible to shorten until 30 sec). For recursive learning, the learning data sets were renewed and the RFNN model was reconstructed in order to fit to the renewed data sets. Estimation procedure is shown in Fig. 2. Here, the time interval for estimation is set to 8 h for simplicity. Off-line data were interpolated to 2 data points during 24 h by the cubic spline function. Simulation was started after 4 days to collect sufficient number of data. As shown in Fig. 2, the glucose concentration (closed square) at 8 h after the start point was estimated using the interpolated values of off-line variables and the present values of on-line ones (open square). At 16 h and 24 h after the start point, the glucose concentration was estimated in the same way. When the present glucose concentration after 24 h was estimated, glucose concentration, PCV and viscosity were measured off-line. Using these actual measured values, recursive learning was done. After this learning, interpolation was carried out by 5 data sets to get interpolated values of off-line variables and those were used for reconstruction of each model. Thus, the simulation continued until the end of the fermentation.

The estimation ability of the model was evaluated by the differences between the actual glucose concentration and the estimated glucose concentration every 24 h.

RESULTS AND DISCUSSION

Estimation Result of FNN Model for Glucose Feed Rate

"OUR" was selected as the first input variable in the FNN model. It is reasonable for this variable to be selected as the first input variable, since "OUR" strongly related to activity of the microorganism. "Cultivation time" was selected as the second input variable. Selection of this variable is also reasonable since condition of microorganism varied by the cultivation time. "Air flow rate" was selected as the third input variable. "Air flow rate" was high at early cultivation time, and became low at middle and late cultivation time. Since the microorganism grows at early cultivation time and produces the antibiotics at middle and late cultivation time, this input variable would discriminate the growth phase and production phase. Only these three variables were selected by PIM and using these variables as the input variables of FNN model was thought as enough for estimation. Each variable was selected one time by PIM.
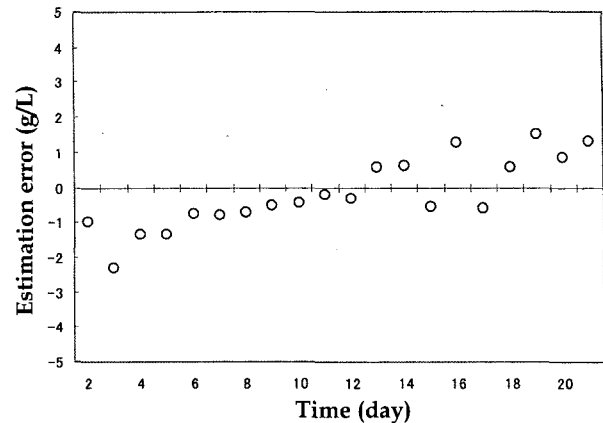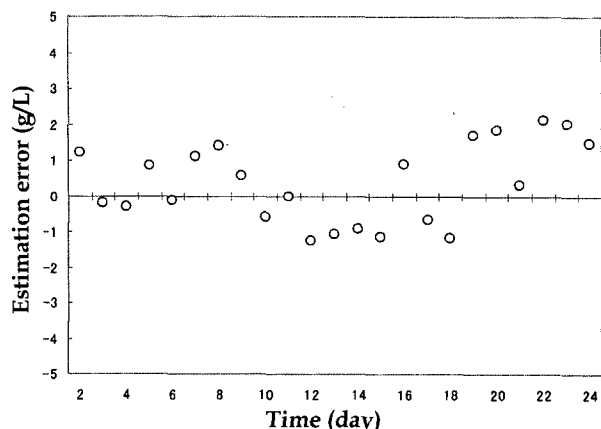

Fig. 3. Simulation results of glucose feed rate using FNN model. Estimation errors for glucose concentration are plotted. O: estimated value.

The estimation results for the learning, evaluation and simulation data are shown in Table 2 for this model. As shown in Table 2, estimation errors of the FNN model were low and almost the same as the measuring errors mainly caused by non-homogeneous culture conditions. Fig. 3 shows one of the simulation results. High estimation accuracy was maintained during the cultivation in the FNN model.

Application for Cultivation with Low Glucose Concentration Using FNN Model

It was shown above that the FNN model could estimate glucose concentration with high accuracy. In actual plant operation, glucose concentration is normally set at relatively high level, since starvation of glucose causes very severe damage to microorganism, although low glucose concentration is better for antibiotic production. Therefore, we applied the FNN model to keep glucose concentration at low level. Considering the maximum error for glucose concentration shown in Table 2, the objective glucose concentration was set to about 60% of that in the previous batches.

A special cultivation was carried out to keep glucose concentration low. In this cultivation, measurement of glucose concentration was done more often than once per day, although possibility of contamination became high due to so often samplings of culture broth. However, only the data for glucose concentration once per day were used for the simulation. Using the input variables selected in the previous section, the simulation was done for this special cultivation, but the simulation error became high (average error: 1.70 g/L, maximum error: 4.03 g/L). This is reasonable since the FNN model was constructed using the data sets with relatively high glucose concentration. If we have many data sets with low glucose concentration, it will be possible to construct FNN model with high estimation accuracy. In this case, the input variables shown in the previous section may be changed.
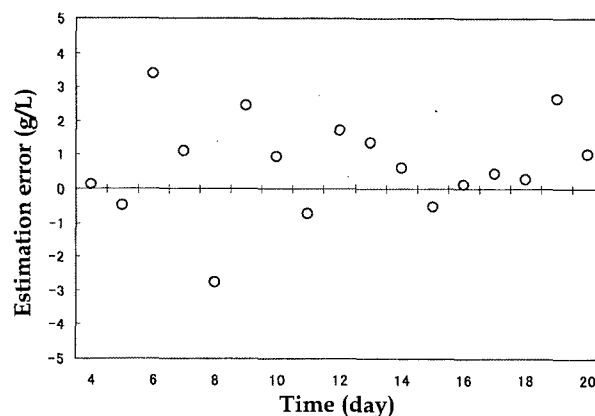
Fig. 4. Simulation results of glucose feed rate at the fermentation keeping low glucose concentration using FNN model. Estimation errors for glucose concentration are plotted. O: estimated value.

Fig. 5. Simulation results of time course for glucose concentration using RFNN model. Estimation errors for glucose concentration are plotted. O: estimated value.

Therefore, we selected the input variables from candidates in Table 1 by trial and error method. As the result, the FNN model with high accuracy was acquired. "OUR", "Glucose consumption during 24 h before the latest sampling time", "Viscosity" and "PCV" were selected as the input variables. Average and maximum errors using these input variables were 0.95 g/L and 2.13 g/L, respectively. Fig. 4 shows the simulation errors for the data at low glucose concentration, and the good simulation results were obtained during the whole cultivation. It is practically difficult to collect many data sets with low glucose concentration in industrial scale fermentation without good glucose control, since there is a high risk of glucose starvation and it causes a significant reduction of production and resulted in a big economic damage. If we have many data sets with relatively low glucose concentration, we can simulate the cultivation with low glucose concentration with high accuracy by selecting the optimal input variables, which was shown here. If this kind of cultivation with low glucose concentration is repeated, we can obtain many data sets with low glucose concentration and it will be possible to construct very safely a new FNN model for low glucose concentration. This is very attractive for operators in industrial antibiotic production plant.

Results of Simulation Model for Time Course of Glucose Concentration Using RFNN

As mentioned above, glucose concentration can be estimated from the FNN model. However, it is an average glucose concentration on daily basis, and we cannot expect the actual fermentation situation until the next measurement time at next day. Therefore, the simulation model to estimate time course of glucose concentration using off-line and on-line data by RFNN was constructed. "Differences of glucose concentration between the objective value and the actual value at the

latest sampling time", "Glucose feed during 24 h before the latest sampling time", "OUR" and "Total weight of broth" were selected as the input variables.

Average and maximum errors for simulation data using these input variables were 1.21 g/L and 3.37 g/L, respectively. Fig. 5 shows the simulation errors for one of simulation data, and shows high estimation accuracy by RFNN model during the culture although the errors were a little bit high at initial cultivation time.

Using this model, glucose concentration can be monitored on-line (it was estimated every hour for simplicity, but it is possible to estimate every 30 sec). Without the RFNN model, operator cannot recognize the possibility of extremely low glucose concentration until the next measurement time for glucose concentration. Therefore, the RFNN model is very useful as the software sensor for industrial antibiotic production plant.

In the present paper, glucose concentration was only discussed extensively. However, there is no doubt that other process variables such as antibiotic concentration can be estimated using the same models.

CONCLUSION

Estimation models for glucose feed rate were constructed by FNN. Estimation error of the FNN model was almost same as the measuring error. Using the RFNN model, simulation for time course of glucose concentration was possible with high accuracy. At industrial fermentation plant, using the models constructed in this paper as the "software sensor", glucose concentration of broth could be kept at target value and operator could monitor glucose concentration with high accuracy.

REFERENCES

[1] Aiba, S., A. E. Humphrey, and N. F. Millis (1973) *Bio-*

280

Biotechnol. Bioprocess Eng. 2002, Vol. 7, No. 5

chemical Engineering. pp. 85-86. University of Tokyo Press, Tokyo, Japan.

[2] Reed, G. (1981) *Prescott and Dunn's Industrial Microbiology.* p. 36. AVI Publishing Co., Westport, Conn., USA.

[3] Lin, H. K., S. Iijima, K. Shimizu, F. Hishinuma, and T. Kobayashi (1989) Control of gene expression from the *SUC2* promoter of *Saccharomyces cerevisiae* with the aid of a glucose analyser. *Appl. Microbiol. Biotechnol.* 32: 313-316

[4] Linko, S., Y. Zhu, and P. Linko (1999) Applying neural networks as software sensors for enzyme engineering. *TIBTECH* 17: 155-162

[5] Thibult, J., V. V. Breusegem, and A. Cheruy (1990) On-line prediction of fermentation variables using neural networks. *Biotechnol. Bioeng.* 36: 1041-1048.

[6] Hanai, T., A. Katayama, H. Honda, and T. Kobayashi (1997) Automatic fuzzy modeling for *Ginjo* sake brewing process using fuzzy neural networks. *J. Chem. Eng. Jpn.* 30: 94-100.

[7] Honda, H., T. Hanai, A. Katayama, H. Tohyama, and T. Kobayashi (1998) Temperature control of *Ginjo* sake mashing process by automatic fuzzy modeling using fuzzy neural networks. *J. Ferment. Bioeng.* 85: 107-112.

[8] Tomida, S., T. Hanai, N. Ueda, H. Honda, and T. Kobayashi (1999) Construction of COD simulation model for activated sludge process by fuzzy neural network. *J. Biosci. Bioeng.* 88: 215-220.

[9] Tomida, S., T. Hanai, H. Honda, and T. Kobayashi (2000) Construction of COD simulation model for activated sludge process by recursive fuzzy neural network. *J. Chem. Eng. Jpn.* 34: 369-375.

[10] Horikawa, S., T. Furuhashi, and Y. Uchikawa (1991) A study on fuzzy modeling using fuzzy neural networks. *Proc. of International Fuzzy Engineering Symp. '91.* November 13-15. Yokohama, Japan.

[11] Rumelhart, D. E., G. E. Hinton, and R. J. Williams (1986) Learning internal representations by error propagation. *Parallel Distributed Processing* 1: 318-362.

[12] Hanai, T., A. Kakamu, H. Honda, T. Furuhashi, Y. Uchikawa, and T. Kobayashi (1996) Modeling of total evaluation process of *Ginjo* sake using a fuzzy neural network. *Trans. Soc. Instrument Control Engineers* 32: 1113-1120.

[13] Adachi, S. (1996) *Identification of System Variables for Control.* (in Japanese), pp. 115-131. Tokyo Denki Daigaku Shuppan Kyoku, Tokyo, Japan.

[14] Kreyszig, E. (1988) *Advanced Engineering Mathematics.* (In Japanese) pp. 29-33. Baifuukan, Tokyo, Japan.

[15] Gen, M. and K. Ida (1988) *Library for Numerical Calculation Based on Turbo C.* (in Japanese), pp. 129-134. HBJ Shuppan Kyoku, Tokyo, Japan.