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On-line Monitoring and Control of Substrate Concentrations in Biological Processes by Flow Injection Analysis Systems

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Abstract Concentrations of substrates, glucose, and ammionia in biological processes have been on-line monitored by using glucose-flow injection (FIA) and ammonia-FIA systems. Based on the on-line monitored data the concentrations of substrates have been controlled by an on-off controller, a PID controller, and a neural network (NN) based controller. A simulation program has been developed to test the control quality of each controller and to estimate the control parameters. The on-off controller often produced high oscillations at the set point due to its low robustness. The control quality of a PID controller could have been improved by a high analysis frequency and by a short residence time of sample in a FIA system. A NN-based controller with 3 layers has been developed, and a 3(input)-2(hidden)-1(output) network structure has been found to be optimal for the NN-based controller. The performance of the three controllers has been tested in a simulated process as well as in a cultivation process of *Saccharomyces cerevisiae,* and the performance has also been compared to simulation results. The NN-based controller with the 3-2-1 network structure was robust and stable against some disturbances, such as a sudden injection of distilled water into a biological process.

Keywords: bioprocess monitoring and control, flow injection analysis, neural networks, controller

INTRODUCTION

The control of substrate concentrations in a biological process plays an important role for achieving a few process objectives, such as high cell density and product yield, low production of inhibitory by-products, *etc.* Many strategies have been studied to control the substrate concentrations in biotechnological processes [1-3]. Four basic control approaches have been employed for the control of substrate concentrations in a bioreactor; a) open-loop control schemes based on a predicted cell growth rate or measured data (*e.g.* [4]), b) control schemes based on non-feed based parameter such as pH, off-gas, and dissolved oxygen concentration (*e.g.* [5-7]), c) control schemes based on mass balance equations using data obtained by several sensors (*e.g.* [8]), and d) control schemes based on direct, on-line measurements of substrate concentrations (*e.g.* [9]). Recently, with the development of new on-line monitoring techniques for substrates, metabolites and products some control strategies using on-line monitored data have received much attention for the control of substrate concentrations in biological processes.

Many studies with a variety of modern analytical methods have been performed for the monitoring of substrate concentrations in fermentation processes [10-13,42]. Especially, a flow injection analysis (FIA) technique has been extensively studied due to its advantage of being fast, accurate, stable, and sensitive. The FIA technique has been used to monitor the medium components and products in biological processes on-line, and has helped to understand the metabolism of the microorganisms [14-17] and to operate the bioprocess optimally [18-21]. The principle of the FIA technique and its further applications on biological processes can be found in a few review papers [22-23].

Characteristics of the FIA system, such as the fast, reliable monitoring technique of substrate concentrations is good precondition for an effective control of a bioprocess. With a FIA system it is possible to on-line monitor the concentrations of substrates and then to control them in bioprocesses by a few control algorithms. In a production process of colominic acid the concentrations of ammonia have been on-line monitored using a FIA system, and the process has been controlled for a high yield of product with a control strategy based on a specific cell growth rate [24]. The concentrations of ammonia in a simulated bioprocess (not contained microorganisms) have also been monitored with a FIA system and controlled with a neural network based control algorithm [38].

However, no systematic studies have been performed for the on-line monitoring and control of substrate con-

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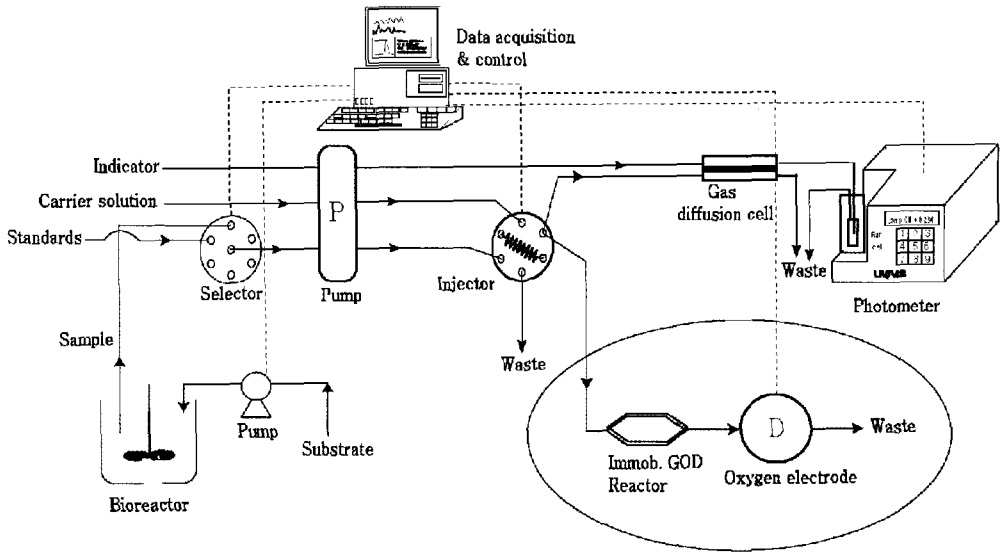

Fig. 1. Set-up of Glucose-FIA and Ammonia-FIA

centrations by FIA systems in biological processes by FIA systems. This work focused on a systematic study of the controller performance based on the on-line monitored of substrates with a FIA system. More concretely, the aim of this work is to investigate, by simulation and experiments, the performance of a few controllers such as on-off controller, PID (proportional integral and differential) controller and neural network (NN) based controller, when the concentrations of substrates such as glucose and ammonia are on-line monitored in a simulated process and in a real cultivation process of *Saccharomyces cerevisiae* by FIA systems, such as glucose-FIA and ammonia-FIA and controlled to set point.

MATERIALS AND METHODS

The FIA Systems

Two different flow-injection analysis (FIA) systems, glucose-FIA and ammonia-FIA, have been used to on-line monitor the concentrations of glucose, and ammonia (Fig. 1), respectively.

For the measurement of glucose concentrations some glucose oxidase (GOD, from *Aspergillus niger*, Sigma Co., Munich, Germany) were immobilized on VA-Epoxy-Biosynth E3-carrier (Riedel de Haen Co., Seelze, Germany) [25] and filled into a cartridge (MobiTec Co., Goettingen, Germany). The immobilized GOD-cartridge was integrated into the glucose-FIA system with a dissolved oxygen electrode (Yellow Springs Instruments Co., Ohio, USA). The glucose-FIA had employed carrier buffer solution (pH 6.75) containing 40 mM K_2HPO_4, 50 mM NaCl, and 25 mM EDTA at 1.2 mL/min. The injection volume of the samples or standards was 35 μL, which was pumped at 0.25 mL/min to the injector.

The ammonia-FIA system had a gas diffusion cell unit (0.1 cm^2) with a polypropylene membrane (Type 2 E-PP-HF, 0.2 μm pore size, Akzo-Nobel Co., Wuppertal, Germany). In the gas diffusion cell unit samples were mixed with a 0.1 N sodium hydroxide solution and gaseous ammonia, and then the samples were reacted with the bromcresol working indicator solution [26,38]. The resulting colour change was detected at 580 nm by a spectrophotometer (Skalar Co., Hannover, Germany) with 500 μL micro flow-through cell (Hellma Co., Muehlheim, Germany). In the ammonia-FIA system, both the 0.1 N NaOH carrier solution and the bromcresol indicator solution were pumped at 1.5 mL/min to the gas diffusion cell unit countercurrently. The sample volume injected was 20 μL, and the measurements were carried out at room temperature.

A peristaltic pump (IPNS 4, Ismatec Co., Glattbrugg, Switzerland) was used to feed the concentrated solutions of glucose or ammonia to a bioreactor. Electrical outputs of the pump were correlated to the substrate feeding rates, and the correlation was included in the control programs. All valves and peristaltic pumps in the FIA systems were controlled by a computer using the software CAFCA (Computer Assisted Flow and Control Analysis, University of Hannover, Hannover, Germany). This software has also been employed to measure and evaluate the FIA signals as well as to control the concentration of sucbstrate in a bioreactor with three different control algorithms.

The Control Algorithms Used

Three controllers *i.e.* an on-off controller, a PID (proportional integral and differential) controller, and a NN (neural network)-based controller have been used and developed in this work. The measured variable is the concentration of a substrate, *i.e.* glucose or ammonia, and the manipulated variable is the pumping rate of the con-

centrated substrate into a bioreactor.

The on-off controller is simple and cheap. It needs only two output signals, or in other words, it works through an on-off action. Therefore, it can be easily applied to a process where there is not a need for exact control.

The principle of a PID controller is well known. The controller is a combination of a proportional controller with integral and differential parts. The PID control scheme with output U at time t is given by the equation:

$$U(t) = k_P \times X_W + \frac{K_P}{T_1}\int X_W dt + k_P \times T_D \frac{dX_W}{dt} + U_0$$

where U_0, X_W, K_P, T_D, and T_I are the initial output value, control error (difference between a set point and a measured value at time t), proportional gain, differential time, and integral time, respectively.

Artificial neural network (NN) is an algorithm that computes a set of output data from a set of input data. It has the capacity of learning through the means of input/output sets of pattern data, and a well-trained neural network can predict output data from a set of input data never seen before. In recent years several models have been developed for artificial neural networks and were extensively used in the fields of biotechnology, *e.g.* modeling of a bioprocess [27] and control systems [28-29]. Among some NN-models, a backpropagation model is the most commonly used model for a neural network in control engineering. In this work, the backpropagation network with one hidden layer is, therfore, considered for the development of a NN-based controller. A number of neurons in three layers (input, hidden, and output) are interconnected. The connection weights in the network structure are estimated using a sigmoidal activation function. During the NN training, all input and output values have been transformed linearly into the interval of 0.1~0.9. The prediction errors were backpropagated by the multiplication of a derivative of the activation function to update the weights. After a training the output values were retransformed to give new predicted values [28-29]. The training of the neural network was programmed in Turbo Pascal by using a toolbox [30] and was run on a personal computer.

All three control algorithms used here were programmed in Turbo Pascal and were added to the software CAFCA in order to calculate the substrate feeding rate.

Computer Simulations

For the estimation of optimal parameter values in a control scheme, it is necessary to make computer simulations for a bioprocess with one of the three controllers mentioned above. A simulation program was written in Turbo Pascal, and optimal control parameter values were estimated as follows:

(1) Control parameters in a control algorithm were assumed randomly, and then a model is set up by using the mass balances of the substrate and cell. The concentrations of the substrate and cell in a bioreactor are given by the following transient state equation:

$$\frac{dC_{Sub}}{dt} = -qX + \frac{FC_{Sub}^{*}}{V} - \frac{C_{Sub}}{V}\frac{dV}{dt}$$

$$\frac{dX}{dt} = \mu X - \frac{X}{V}\frac{dV}{dt}$$

where C_{Sub}, C_{Sub}^{*}, X, V, and F are substrate concentrations in the bioreactor and in the feeding reservoir, cell mass, culture volume, and volumetric substrate flow rate, respectively. The specific cell growth rate (μ) and specific substrate consumption rate (q) can be estimated on the basis of the substrate concentration profile in a batch phase that was measured in experiments. The equations can be then simplified under the assumption of a constant culture volume and exponential cell growth rate, and can be integrated as follows:

$$C_{Sub} = C_{Sub}^{0} - P_1 \exp(\mu t) + P_2 t$$

$$P_1 = \frac{qX_0}{\mu}, \qquad P_2 = \frac{F C_{Sub}^{*}}{V}$$

The substrate concentration in a bioreactor can be calculated by using the estimated parameter values (P_1, P_2), when C_{Sub}^{0} is the initial concentration of the substrate. The substrate feeding rate (F) can also be calculated, when the on-line monitored data of substrate concentrations (C_{Sub}) were employed by a FIA system.

(2) Using the model and the estimated controller parameters, a simulation has been performed for a bioprocess with a controller, and simulation results have been compared with a set point of the controller.

(3a) If the control error, *i.e.* difference between the set point and the simulation results is over the convergence range (5%), new controller parameters could be evaluated by using a simplex algorithm [31].

(3b) If the control error fell within the convergence range, *e.g.* under a 5% error range, then the controller parameters would be used for the controller experiments.

Besides the estimation of the controller parameters the simulation program was also used for the following:

a) The comparison of theoretical control results of a process with experimental results by one of three control algorithms was done in consideration for residence time of samples, culture volume, analysis frequency and measurement error of a FIA system.

b) The graphical demonstration of simulation and experimental results was made for substrate feeding rate, theoretical control results, differences between the set point and experimental data *etc.*

Biological Processes

To investigate the robustness and quality of a controller, a few experiments have been carried out in a continuously stirred tank reactor (CSTR) of 500 mL working volume and in a fed-batch cultivation processes with *S. cerevisiae*. 0.1 M potassium phosphate solution at pH 7.0

was used as a model medium and was continuously pumped to the CSTR system with an influent and effluent rate of 0.3 mL/min. The concentrations of glucose or ammonia in the reactor have been monitored on-line by the glucose-FIA or the ammonia-FIA and then, controlled to a set point by one of the three controllers outlined above. For real cultivation of *S. cerevisiae* a stirred bioreactor with 5 L working volume has been operated at 25°C and pH 6.8. The cultivation medium consisted of the following (in gram per liter): glucose, 1~30; $(NH_4)_2SO_4$, 4.5; $(NH_4)_2HPO_4$, 1.9; KCl, 0.9; $MgSO_4.7H_2O$, 0.34; $CaCl_2.2H_2O$, 0.42; Na-citrate, 5.9; trace element solution ($FeCl_3.6H_2O$, 0.015; $MnSO_4.H_2O$, 0.0105; $ZnSO_4.7H_2O$, 0.009; $CuSO_4.5H_2O$, 0.0024; inositol, 0.06; Ca-pantothenate, 0.03; vitamine B_1, 0.006; vitamine B_6, 0.0015; biotin, 0.00003), 1 mL/L. During the cultivation, the glucose concentrations have been monitored on-line by the glucose-FIA and then controlled by one of the three control algorithms. Cell free samples were taken from the bioreactor by using a tubular *in situ* membrane system (Eppendorf-Netheler-Hinz Co. Eppendorf, Germany).

RESULTS AND DISCUSSION

Control of Glucose Concentration by an On-off Controller

Growth of microorganisms often depends on initial substrate concentrations in a batch mode of biological processes, such as in the example of the cultivation processes of *Escherichia coli* [32] and *Klebsiella oxytoca* [33-34]. But, the substrate concentrations that were controlled to a set value also play an important role for the high cell mass production in a fed-batch cultivation process [35-36]. The influence of the substrate concentrations on the cell mass production was investigated in a few fed-batch cultivations of *Saccharomyces cerevisiae*, when the concentration of glucose was controlled by an on-off controller.

After the substrate, or 2 g/L glucose, was completely consumed in the culture medium, the substrate concentration in the fed-batch mode was controlled to a set value of either 5 g/L or 10 g/L, with upper and lower limits of 0.5 g/L, using the data monitored on-line by the glucose-FIA system in Fig. 2. As expected, more cell mass was produced in the fed-batch fermentation during the control of the glucose concentration to 10 g/L than to 5 g/L. The on-off controller often produced oscillations at the set point due to little flexibility of the two parameters, "on" and "off". However, the on-off controller can be used to investigate biological characteristics of microorganisms, such as cell growth characteristics. Fig. 3 showed the time profiles of the oscillated glucose concentration, the cell mass production, and the glucose concentration that was fed in the cultivation process of *S. cerevisiae* during the control of the glucose concentration to 1.25 ± 0.75 g/L. During the oscillations, the glucose concentrations were the same at different times, but the cell mass concentration increased with time. These data could be used to develop a cell growth model [37].

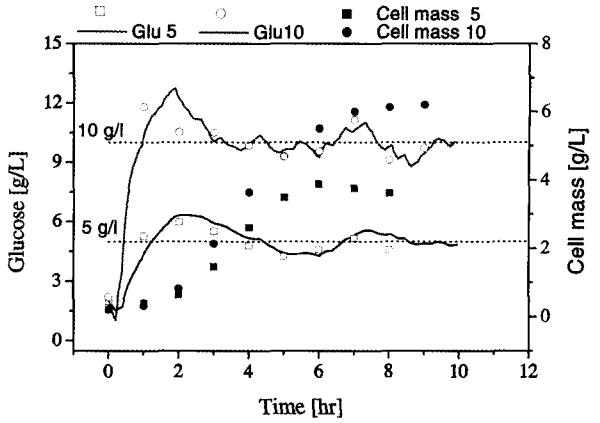

Fig. 2. Time courses of glucose and cell mass production in the cultivation of *S. cerevisiae* controlled to 5 g/L and 10 g/L by on-off controllers. Symbols (□, ○, ■, ●) represent off-line data for glucose and cell mass, while lines represent on-line FIA data (Analysis frequency of the gluocse-FIA: 24 1/hr).

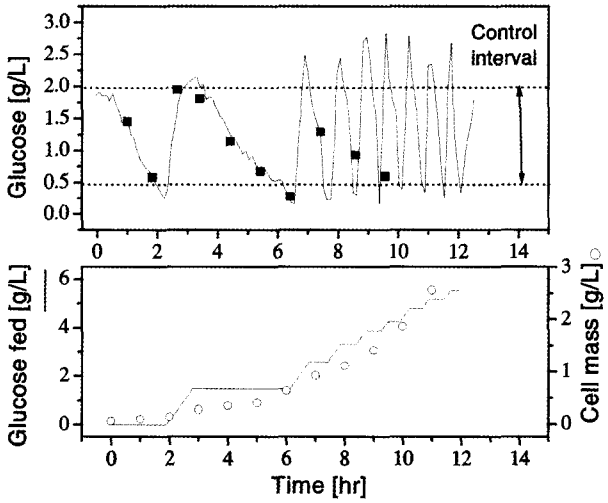

Fig. 3. Time profile of glucose concentrations oscillated and the correlation the cell mass production to the glucose concentrations fed in the cultivation of *S. cerevisiae* by an on-off controller (C_{Glu}^{upper}: 2.0 g/L, C_{Glu}^{lower}: 0.5 g/L). Symbols (■,○) represent off-line data for glucose and cell mass, while lines represent on-line data (Analysis frequency of the glucose-FIA : 24 1/h).

PID Control of Glucose Concentration

Analysis frequency of a FIA system, *i.e.* measured data per analysis time, often influences the accuracy of monitoring data and also the control quality of a controller. The higher the analysis frequency of a FIA system is, the more accurate are the on-line monitoring results. To investigate the control quality of the PID controller in a FIA system with different analysis frequencies (3~24 1/h) a

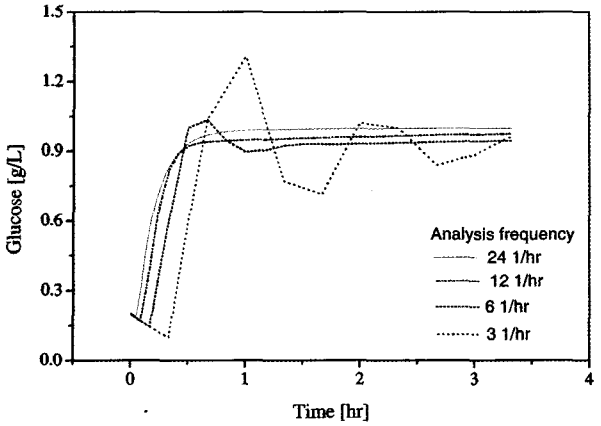

Fig. 4. Simulation results of a PID controller in a FIA system with different analysis frequencies. Lines represent analysis frequencies. For example, 24 1/h (solid line) means 24 times analysis per hour.

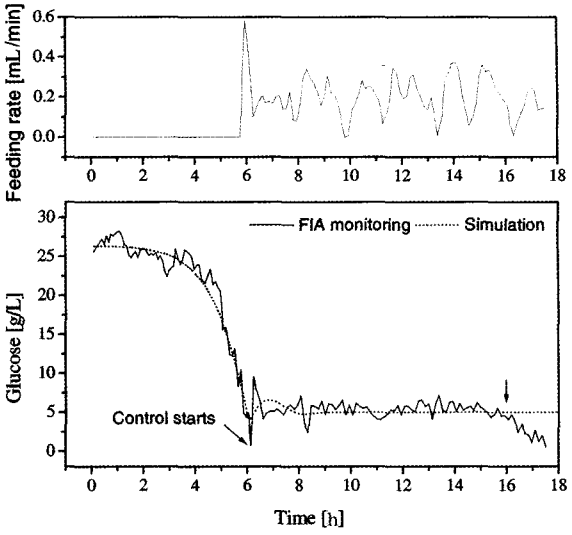

Fig. 5. Time courses of glucose concentration and feeding rate in the cultivation of *S. cerevisiae* that was controlled by a PID controller (set point: 5 g/L). Solid line represents on-line FIA data, while dotted line represents simulation results.

few simulations based on a CSTR experiment with the glucose consumption rate of 0.05 g/L/min were performed at the set point of 1 g/L glucose using the model described in computer simulations. The simulation results have been presented in Fig. 4, where some controller parameter values at 24 1/h were employed. Oscillations began to occur at the analysis frequency of 6 1/h, *i.e.* one FIA signal per 10 min and the overshoots became higher with decreasing analysis frequency. This shows that the analysis frequency of a FIA system should be as high as possible, *e.g.* at least 12 1/h for the control of glucose concentration in a microbial process with the substrate consumption rate of 0.05 g/L/min in a batch mode.

The analysis frequency of a FIA system also has a close relation to sampling procedures (*e.g.* use of a membrane sampling device), medium concentrations (*e.g.* dilution of samples) and measurement methods. A tubular membrane sampling device has been used in our work, so that a little time delay could not be avoided. Dilution of samples can also make the analysis frequency lower [39]. Thus, both sampling procedures and substrate concentrations that were measured should be carefully taken into account for the improvement of a bioprocess control.

During the simulation, the controller parameters have been estimated considering the residence time of samples in the tubing from the sampling device in the bioreactor to the glucose-FIA system. The residence time of samples to the glucose-FIA system could be measured by comparing the off-line data of the glucose concentration in the reactor with the on-line monitoring data of the glucose-FIA system. Fig. 5 showed the control results of glucose concentrations to 0.5 g/L by the PID controller with the residence time of samples being 17 min, when the initial concentration of glucose in the bioreactor was 27 g/L. The analysis frequency of the glucose-FIA system used was 20 1/h. During the control of the glucose concentrations, *i.e.* from 5.8 to 16 h, the mean errors between the set point and experiment results were about 22%, or 5 ± 1.1 g/L. After 16 h, the cell mass that was produced was high enough, that the sampling tube in the FIA system was often blocked, and it was often not possible to measure the glucose concentrations.

PID Control of Ammonium Concentration

Even though glucose is being used as a main carbon source in many biotechnological processes, ammonia also plays a crucial role for cell growth and for the high yield of bioproducts in a few bioprocesses [40-41]. For example, in the cultivation of *Pseudomonas putida* for the production of poly-β-hydroxy butyrate (PHB), ammonia has been used as an important nitrogen source for high cell mass production. However, high concentrations of ammonia often inhibit the growth of cells in the cultivation of *Mycobacterium chlorophenolicum*, so that the ammonia concentration should be kept low during the cultivation [42]. Therefore, it is very important to monitor and control the concentrations of ammonia in a biological process. When an ammonia concentration decreased linearly in the batch mode, the control quality of the PID controller was investigated for different set points (*i.e.* 0.75 g/L, 1.0 g/L, 1.25 g/L) with the analysis frequency of 20 1/h in the ammonia-FIA system. Table 1 presented the mean control errors in the experiments and simulation results when no measurement errors of the ammonia-FIA system were taken into consideration. The mean control errors in the experiments were higher than those in the simulations, although the controller parameters optimized by the simulations have been used for experiments. This large difference in the mean control errors between simulations and experiments showed that some measurement errors of FIA systems should be taken into consideration for a simulation. So, the measurement er-

Table 1. Mean control errors in experiments and simulations for three different set points controlled by a PID controller

Set point (g/L)	Errors in simulation (%)	Errors in experiment (%)
0.75	0.6	8.2
1.00	2.8	9.0
1.25	3.1	9.5

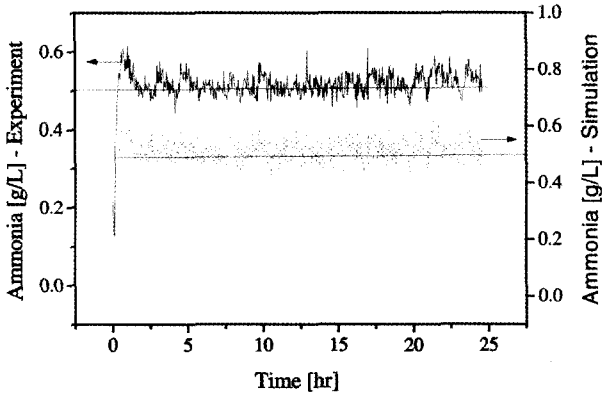

Fig. 6. Time course of ammonia concentrations in simulation and experiment by a PID controller (set point : 0.5 g/L; analysis frequency of the ammonia-FIA : 24 1/h). Solid line represents on-line FIA data, while dotted line represents simulation results.

ror of 5% in a FIA system has been used for further simulations.

Fig. 6 showed the simulation and experimental results for the ammonia concentration that was controlled to 0.5 g/L, when the controller parameters were estimated using the linear decrease rate of ammonia concentration in the batch mode. The ammonia concentration in the experiment increased up to 0.6 g/L within 10 min and was controlled to 0.5 g/L stable. The mean control error between the set point and the experimental data was 9.3%, while it was 9.6% between the set point and the simulation results. However, the difference in the mean control errors between the simulation and experimental results was small because a 5% measurement error of the FIA system and a 5 min residence time of samples in the sampling tubing have been used for the simulation.

A simulation has been performed for the set point of 0.25 g/L ammonia with a 5% measuremet error of the FIA system and compared with experiment results in Fig. 7. The time course of the ammonia feeding rate has also been shown. During the control the difference in the mean control errors between simulation and experimental results was very small (about 1.4%) although the mean control error between the set point and the measured values in the simulation and the experiment was about 10%. The ammonia feeding pump has been operated almost continuously from the beginning of the control. These two results in Figs. 6 and 7 showed that the mea-

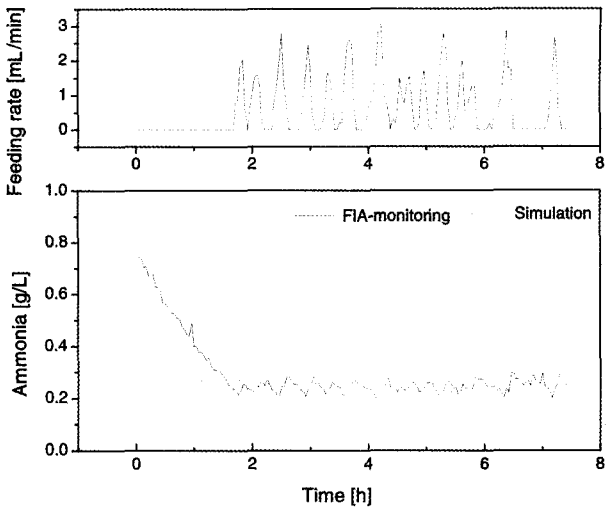

Fig. 7. Time courses of ammonia concentration and ammonia feeding rate in the CSTR controlled by a PID controller (set point : 0.25 g/L). Solid line represents on-line FIA data, while dotted line represents simulation results.

surement error of a FIA system should be taken into account for a simulation of a control scheme.

The large mean control errors between the set points and the experimental results in Figs. 6 and 7 showed the low robustness of the PID controller against oscillations caused by a long residence time of samples as well as the high measurement errors of a FIA system. The oscillations produced by an analysis and controlling system can be compensated by using some filters, such as the kalman filter. The robustness of a PID controller can also be improved by using a control algorithm based on three values at the time of t_{i-2}, t_{i-1}, and t_i.

Development of a NN-based Controller for the Control of Ammonia and Glucose Concentrations

To improve the control quality a neural network based controller was developed. In a neural network structure the number of layers and neurons, *i.e.* connection weight factors are closely related to the complexity of a NN-based controller. As the number of neurons are increased, the learning rate of the neural network is also increased, but the NN-based controller becomes more time-consuming. A neural network with three layers was used in this work. In the input layer, three neurons based on the substrate concentrations at time t_{i-1}, t_i, and t_{i+1} have been employed, whereas in an output layer, one neuron has been used to calculate the substrate feeding rate. The numbers of neurons in a hidden layer were varied to investigate the performance of the neural network structure. In a simulation, the weight factors of the network could be first introduced by a random procedure in the range of -0.5 and + 0.5. They have been then optimized using a simplex algorithm until an error between a set point and simulated values reaches a criteria, *i.e.* within 5 %. Simulation results with some numbers of neurons in a hidden

Table 2. Simulation results with different neural network structure

Network structure	Numbers of connection	Training cycles	Mean errors (without M.E.)	Mean errors (with 5% M.E.)
3 – 0 - 1	3	5000	3.0 %	8.3 %
3 – 1 - 1	4	1000	2.9 %	8.2 %
3 – 2 - 1	8	1000	0.9 %	6.2 %
3 – 3 – 1	12	5000	> 50 %	> 50 %
3 – 3 - 2	15	5000	> 50 %	> 50 %

M.E. represents the measurement error of an ammonia-FIA system

layer were presented in Table 2, when the ammonia concentration in the CSTR was controlled to 0.5 g/L.

The first network structure in Table 2 has no neurons in the hidden layer, and the controller with this network structure is the same as a PID controller. The last network structure contained two neurons in the output layer for the prediction of the ammonia concentration at time t_{i+1} as well as for the calculation of the ammonia feeding rate. The results in Table 2 showed that the network structure with 3 hidden neurons obtained large mean errors in 5,000 training cycles. The calculation time of a network structure usually increases with training cycles, so that a NN-based controller having the training cycles over 5,000 is not practical for the control of a bioprocess when using a simplex algorithm. The 3-2-1 structure with and without a 5% measurement error of the ammonia-FIA system had less mean control errors than the 3-1-1 structure in 1,000 training cycles, even though the former was a little more time-consuming. Therefore, in this work, a 3-2-1 based neural network structure has been used to control the substrate concentrations in biotechnological processes.

The NN-based controller (3-2-1 network structure) has been experimentally compared with the PID controller (3-0-1 structure) for the control of ammonia concentration in a CSTR system. The controller parameters in the NN-based controller and the PID controller have been estimated by using a linear decrease of the ammonia concentration in batch mode. Fig. 8 presented the control results of the ammonia concentration as 0.7 g/L by a NN-based controller and a PID controller with a 5% measurement error of the FIA system and 150 training cycles. The mean control error of the NN-based controller (17.5%) was lower than that of the PID controller (30.3%) in the experimental range. These experimental results showed that the 3-2-1 NN-based controller has an advantage over the PID controller for the control of substrate concentrations in biological processes.

The control quality of a NN-based controller will also be influenced by the residence time of samples transported from a bioreactor to a FIA system. Two simulations with a NN-based controller were performed at 5 min and 30 min residence times of the samples and were compared with a simulation result with a PID controller at 30 min residence time. In Fig. 9, the NN-based controller at 5 min residence time produced no oscilla-

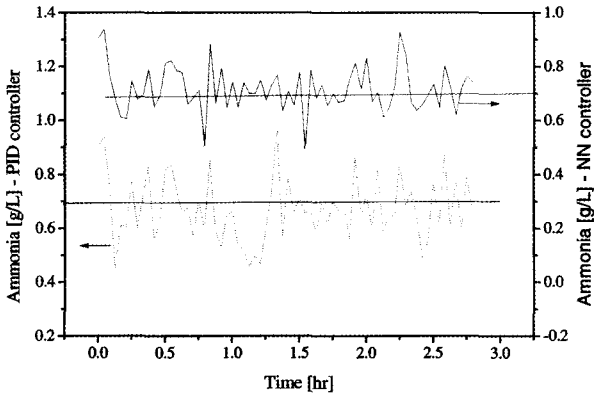

Fig. 8. Experimental comparison of a 3-2-1 NN-based controller with a PID controller for the control of ammonia concentration to 0.7 g/L (5% measurement error, 150 training cycles). Solid line represents on-line FIA data controlled by the NN controller, while dotted line represents on-line FIA data controlled by the PID controller.

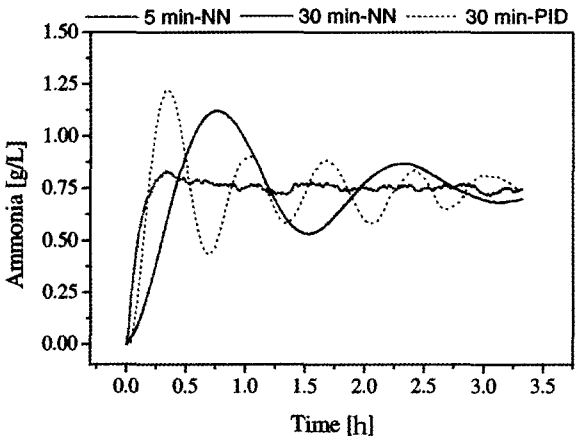

Fig. 9. Time courses of simulations with different residence times of samples by a NN-based controller and a PID controller (set point: 0.75 g/L). 5 min-NN (30 min-NN) means that the NN controller has 5 min (30 min) of residence time of samples. 30 min-PID means that the PID controller has 30 min of residence time of samples.

tions, whereas the controller at 30 min residence time

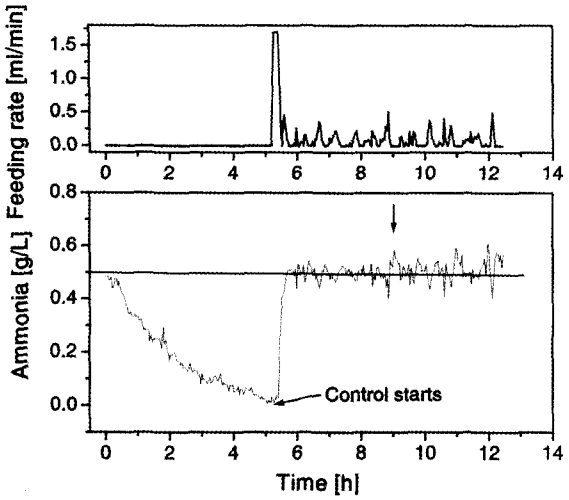

Fig. 10. Time course of ammonia concentration and feeding rate in a CSTR controlled by a NN-based controller (set point : 0.5 g/L). Solid line represents on-line FIA data.

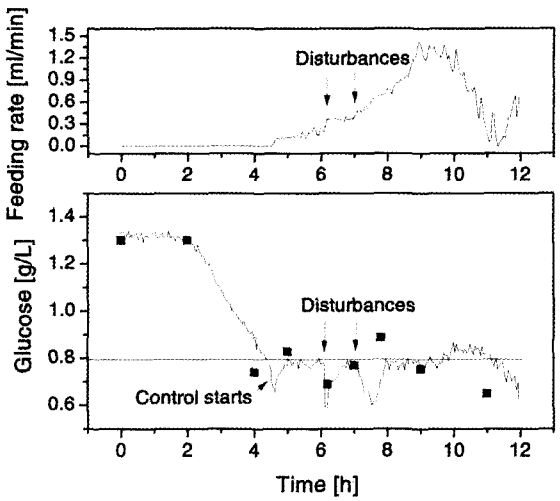

Fig. 11. Time course of glucose concentration and feeding rate in the cultivation of *S.cerevisiae* controlled by a NN-based controller (set point : 0.8 g/L). Symbol (■) represents off-line data, while solid line represents on-line FIA data.

produced two oscillations when the same controller parameters were employed. However, the overshoot produced by the NN-based controller at 30 min residence time (0.37 g/L) was lower than that produced by the PID controller at 30 min residence time (0.45 g/L).

The concentrations of ammonia often exponentially decrease in a batch experiment exponentially. The NN-based controller has been used to control the ammonia concentration at 0.5 g/L, when the substrate concentration in a CSTR system decreased exponentially. The time course of the ammonia concentration and feeding rate is shown in Fig. 10. During the control the ammonia concentration agreed to the set point (mean control error : 10.2%), but oscillations began to produce after 9 h. As shown in Fig. 10 the ammonia feeding pump operated at its maximal rate (1.8 mL/min) in the beginning of the control, but afterwards it worked almost like an on-off controller. This on-off action of the ammonia feeding pump made the robustness of the NN-based controller low. Therefore, in order to make a NN-based controller more robust, the substrate should be fed into a bioreactor continuously as much as possible.

The glucose concentration in a real cultivation of *S. cerevisiae* with 5 L working volume has been controlled by the NN-based controller as shown in Fig. 11. The controller parameters were estimated by a simulation with a 20 min residence time of samples and a 5% measurement error of the glucose-FIA. The initial glucose concentration in the batch experiment was 1.3 g/L, and the glucose concentration in the bioreactor was monitored by the glucose-FIA. After the cultivation of 4.2 h, the concentrated glucose in the feed reservoir was fed into the bioreactor and its concentration was controlled at 0.8 g/L. To investigate the robustness of the NN-based controller, one liter of distilled water was introduced into the bioreactor at 6 and 8 h. The glucose concentration in the reactor decreased very quickly after injecting the distilled water. However, the glucose concentration in the reactor reached to the set point within 40 min, so that the NN-based controller showed its highest robustness against disturbances. As shown in Fig. 11, the glucose feeding pump operated continuously and fed the concentrated glucose in the feed reservoir into the bioreactor even though two disturbances occured during the control. After 10.5 h the glucose concentration decreased slowly due to the introduction of air bubbles into the in-line membrane sampling device of the reactor. However, the control quality during the control experiment was relatively good to some disturbances and the mean control error was about 12.0%. The on-line data of the glucose-FIA also agreed with the off-line data measured by the YSI analyser (Yellow Springs Instrument Inc., USA). The control results of glucose concentrations in the real cultivation process showed the high robustness of the NN-based controller and showed its stability against disturbances, when the glucose feeding pump operated continuously with optimally concentrated substrate.

CONCLUSION

The on-off controller, the PID controller, and the NN-based controller were used and developed to control the concentrations of substrates in various biotechnological processes, which were monitored on-line by FIA systems. The control quality, or accuracy and robustness, of the on-off controller depended largely on the analysis frequency of the FIA system and dynamics of the bioprocess. Control stability of the PID controller was influenced by the analysis system, largely depending on measurement errors of the FIA systems and residence time of samples. But, a high control quality for the PID controller could not be achieved without an improvement of measurement

methods. The NN-based controller was developed using a feedforward backpropagation network structure with three layers. The weight factors of the network structure were estimated by using a simulation with a simplex algorithm. Although the NN-based controller was more time consuming than the PID controller, it was more robust and stabler than the PID controller. The NN-based controller has showed its high robustness and good stability against artificial disturbances in the cultivation process of *S. cerevisiae*. Therefore, some biological processes in industries can be on-line monitored and controlled by using a FIA system with a NN-based controller.

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