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APPLICATION OF ADAPTIVE CONTROL WITH RULE-BASES TO CELL RECYCLED CONTINUOUS BIOREACTOR FOR ETHANOL PRODUCTION

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Abstract—An adaptive control algorithm with rule-bases was developed for cell recycled continuous bioreactor. Ethanol fermentation by *Saccharomyces cerevisiae* was used as a model system. Since the developed algorithm employed the fermentation model, it was possible to estimate the physical state of the system. Five rule-bases were obtained from the experiments and used to adjust the control input. Good control performance was observed using a rule-based STR (self-tuning regulator) compared to the result using conventional STR. When disturbances were given, rule-based adaptive control scheme showed faster recovery of setpoint. The rule-based STR developed in this study was stable, robust and showed good tracking performance.

INTRODUCTION

With the development of fermentation technology, high density culture of microorganism is very important to enhance product concentration and productivity. To obtain a high cell density, it is necessary to feed the medium continuously, especially when high substrate level inhibits the growth of microorganism and the biosynthesis of the product. Therefore, fed-batch culture and cell-recycled continuous culture have been studied extensively [1-22]. Especially, cell recycled continuous culture has many advantages. Since the dilution rate can be maintained much higher than the maximum specific growth rate, high productivity can be thus obtained. Inhibitory substances including products or by-products can be removed and high cell density can be obtained, and *in situ* product separation is possible, which makes down-stream processing easier and more economical. In wastewater treatment, activated sludge process employing cell recycle has been used successfully. The growing prospects of ethanol as a fuel and future chemical feedstock have prompted considerable efforts to increase the efficiency of production in cell recycled continuous bioreactor [3-19].

Cysewski and Wilke [3-5] and Ghose and Tyagi [6, 7] used a settling tank to recycle yeast cells, where increase of the productivity about four times was reported compared to the result obtained using a conventional continuous bioreactor. The major constraint of this system is in the requirement of a large settler to allow enough residence time for the cells to be settled. Margaritis and Wilke [8, 9] designed a "rotor-fermentor" which combined the function of fermentor and filter in one unit. It was claimed that the ethanol productivity could be increased by ten times compared with an ordinary continuous bioreactor. Frequent failure of membrane filters is the limitation in real applications. Productivity in ethanol fermentation can also be increased by decreasing the inhibition effect of ethanol. Cysewski and Wilke [7, 10] demonstrated that alcohol can be removed continously during fermentation by operation under vacuum conditions. The main disadvantage in vacuum fermentation is high power consumption for maintaining vacuum conditions and high capital cost [5] which is reported to be 30 times higher than conventional process [5]. In the 1980's, with the development of membrane technology, the reasonably short residence time of cell and the high efficiency of separation have been available [11]. Rogers et al. [12] and Hoffman et al. [13] employed flat plate membrane, Nishizawa et al. [14], Cheryan and Mehaia [15] and Lee and Chang [16] used hollow fiber, and Lafforgue et al. [17] and Jarzebski et al. [18] chose tubular type membrane. Lee and Chang [16] and Jarzebski et al. [18] adopted cell bleeding to keep the cell concentration at a desired value. As the cell population grows highly dense, the

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fermentation broth has the characteristics of pseudoplastic [23], and the permeability of the membrane is deteriorated. Therefore, it is of significance to control the cell bleeding rate which has a direct effect on cell concentration.

The fermentation process has time-varying and nonlinear nature during cell growth. In addition, since the reliable on-line sensors which can detect the state variables such as the concentrations of cells and the metabolites are still lacking, there is always time-delay in the off-line measurement of state variables. Thus the controller having constant gain has not been applied effectively to the fermentation process [24, 25]. It is therefore essential to adopt adaptive control algorithm which identifies the changes of the fermentation process with time and generates control input to get a good control performance. Even though there are many schemes in adaptive control algorithm, MRAS (model-reference adaptive system) [26] and STR (self-tuning regulator) [27-29] have widely been used.

Ko et al. [29] demonstrated the usefulness of the adaptive control in activated sludge process. Shi et al. [24, 30] used adaptive control algorithm for lactic acid production. The control inputs were cell bleeding rate and permeation rate, and the control outputs were cell concentration and lactic acid concentration. Decoupling strategy was employed to minimize the interaction of the control loop in MIMO (multi-input multi-output) system.

The studies on the adaptive control of ethanol production in a cell recycled continuous bioreactor have not been performed much. Lee and Yoo [31] employed adaptive control algorithm for ethanol production in a recycled cell system. Cell bleeding rate influencing the cell concentration was selected as a control input and cell concentration as a control ouput. In the present algorithm, rule-bases were included in the control scheme. The advantages of the incorporation of rule-base into adaptive control are: (1) the control actions considering the physical meaning of bioprocess can be given, so the great change in the control input computed from the adaptive algorithm can be adjusted to attain the desired control objective. (2) as rule-bases are obtained from the experiences, adaptive control scheme containing rule-bases can be more stable and robust than adaptive algorithm only.

In this study, rule-bases obtained from experiments were combined into the adaptive algorithm and applied to ethanol fermentation in a cell recycled system.

STRUCTURE OF CONTROL MODEL

Mass balance equations describing the operation of the cell recycled fermentation system are

$$\frac{d(VX)}{dt} = \mu(VX) - BX \tag{1}$$

$$\frac{d(VS)}{dt} = \xi(VX) + F(S_0 - S) \tag{2}$$

$$\frac{d(VE)}{dt} = \pi(VX) - FE \tag{3}$$

, where μ, ξ and π denote specific growth rate, specific consumption rate and specific production rate, respectively. The above equations are rearranged as a vector form.

$$\dot{\mathbf{X}} = \mathbf{A} \cdot \mathbf{X} \tag{4}$$

, where

$$\dot{\mathbf{X}} = (dX/dt,\ dS/dt,\ dE/dt)^T \tag{5}$$

$$\mathbf{X} = (X,\ S,\ E)^T \tag{6}$$

$$\mathbf{A} = (A_X,\ A_G,\ A_E)^T \tag{7}$$

The components of Eq. (7) are

$$A_X = (\mu - F/V \quad 0 \quad 0)$$
$$A_G = (\xi \quad F(S - S_0)/V/S \quad 0)$$
$$A_E = (\pi \quad 0 \quad -F/V)$$

X is the vector of state variables which describe cell, glucose and ethanol concentration, respectively. The vector **A** includes the specific growth rate of cell, the specific consumption rate of substrate and the specific production rate of ethanol. Depending on state variables such as cell concentration, substrate concentration, ethanol concentration, temperature, pH, dissolved oxygen (DO) and so forth, these parameters are assumed to be constant during the fermentation. The control objective in this article is to manipulate cell bleeding rate for tracking the setpoint of cell concentration.

Eq. (4) is discretized by a first-order Euler approximation.

$$X_{i+1} = X_i + \Delta t(\mu_i X_i - B_i X_i) \tag{8}$$

$$S_{i+1} = S_i + \Delta t[\xi_i X_i + (F_i/V_i)(S_0 - S_i)] \tag{9}$$

$$E_{i+1} = E_i + \Delta t[\pi_i X_i - (F_i/V_i)E_i] \tag{10}$$

From the above discretized equations, parameters can be expressed by recursive least-squares method as follows:

$$\mu_{i+1} = \mu_i + \Delta t P_i X_i [X_{i+1} - X_i - \Delta t \mu_{i-1} X_i + \Delta t (F_i/V_i) X_i] \tag{11}$$

Table 1. The compositions of the media used throughout the study

Growth medium		Production medium			
		Batch culture		Continuous culture	
Glucose	20 g/L	Glucose	100 g/L	Glucose	150 g/L
Yeast extract	3 g/L	Yeast extract	5 g/L	Yeast extract	7.5 g/L
Bactopeptone	5 g/L	KH_2PO_4	5 g/L	KH_2PO_4	7.5 g/L
Malt extract	3 g/L	$(NH_4)_2SO_4$	5 g/L	$(NH_4)_2SO_4$	7.5 g/L
		$MgSO_4 \cdot 7H_2O$	1 g/L	$MgSO_4 \cdot 7H_2O$	1.5 g/L

$$\xi_{i+1} = \xi_i + \Delta t P_i X_i [G_{i+1} - G_i - \Delta t \xi_{i+1} X_i - \Delta t (F_i/V_i)(G_o - G_i)] \quad (12)$$

$$\pi_{i+1} = \pi_i + \Delta t P_i X_i [E_{i+1} - E_i - \Delta t \xi_{i+1} X_i + \Delta t (F_i/V_i) E_i] \quad (13)$$

, where

$$P_{i+1} = \frac{P_i}{\lambda_i} \left(1 - \frac{(\Delta t\ X_{i+1})^2\ P_i}{\lambda_i + (\Delta t\ X_{i+1})^2\ P_i}\right)$$

P is an information matrix which is given initially by users. Parameters of step i are updated from the variables of step i−1 and step i, and the estimated parameters of step i−1. Updated parameters of step i are used in the generation of control input.

Bleeding rate was selected as a control input, cell concentration as a control output in the present study. Let X^* be the setpoint cell concentration, X_{i+1} is to be X^* at each step. Therefore, bleeding rate is determined as

$$B_i = \frac{-X^* + X_i + \mu_i X_i \Delta t}{(\Delta t X_i)/V_i} \quad (14)$$

From the measurements of variables at step i and the estimated parameter values at step i, control input is generated and maintained from step i to step i+1.

MATERIALS AND METHODS

1. Microorganism and Medium

The microorganism used in the ethanol fermentation studies was *Saccharomyces cerevisiae* (ATCC 24858). The compositions of media used are summarized in Table 1. The glucose used in the growth medium and in the production medium was of reagent grade (Junsei Co. Japan). When glucose concentration was varied, all other components were also varied in proportion to the glucose concentration.

2. Reactor System

The experimental setup is shown in Fig. 1. To a 2-L jar fermentor (Bioflo model C30, New Brunswick Scientific Co.) a flat-plate membrane module (Millipore, Lab Cassete XX42 YLC KO) was attached. The effective area of a sheet of flat membrane was 60 cm^2. The material of the sheet membrane was nitrocellulose. Operation conditions were chosen to keep the membrane fouling to a minimum, i.e. the flow rate of broth at the entrance of the membrane module corresponding to 200 ml/min, control of the pressure across the filtration unit below 1.5 atm. A part of the permeate stream was removed from the system at a desired rate to balance with medium stream, excess of permeate being recirculated to the fermentor. Cell bleeding rate was manipulated by peristaltic pump (Cole-Parmer Co.) interfaced to the computer.

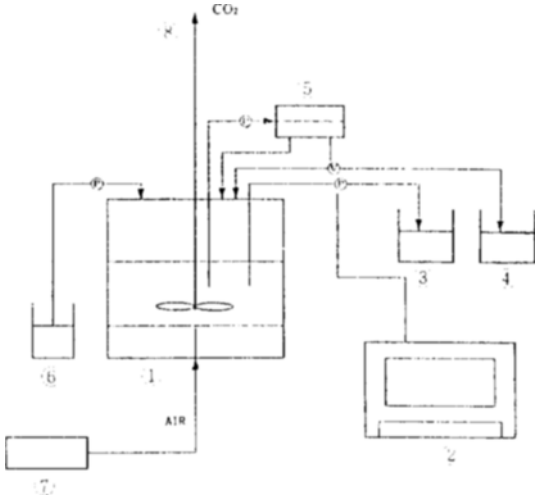

Fig. 1. Schematic diagram of experimental setup.
① Fermentor, ② Computer, ③ Cell bleeding reservoir, ④ Permeate reservoir, ⑤ Membrane module, ⑥ Medium reservoir, ⑦ Compressor, ⑧ Condenser, Ⓟ Peristaltic pump, Ⓥ Valve.

Yeast cells for the innoculum were grown in a 250 ml flask containing 50 mL of growth medium in a rotary shaking incubator for 16 h at 30℃ and transferred to the 2-L jar fermentor. The culture was grown aerobically batchwise followed by feeding of production

medium for continuous operation. The batch culture time was 9 h for initial glucose concentration of 30 g/L. The working volume was 600 mL including the amount of holdup in the filter. The initial was pH 4.5, the air flow rate 0.5 vvm, the temperature 30℃, and the agitation rate 400 rpm.

3. Control Procedure

The setpoint of cell concentration was determined as 130 g/L considering the rheological properties of the fermentation broth, the membrane characteristics and the cell activity for ethanol production [32]. The control procedure was as follows: The control actions were not given until the cell concentration reached 130 g/L. The dilution rate was changed from 0.3 h^{-1} to 1.3 h^{-1} when the cell concentration was 130 g/L. The steady state at which the glucose concentration and the ethanol concentration were constant was obtained for 10 h or so. The cell concentration was measured every 30 min. Other state variables such as ethanol concentration and glucose concentration were measured every 2 h. The cell bleeding rate as a control input was calculated every 30 min. The upper limit of the control input was 0.15 h^{-1} to prevent the excessive cell bleeding.

4. Assay

Biomass was estimated after diluting the sample and measuring its absorbance at 525 nm using spectrophotometer (Kontron, model Uvikon 930). Dry cell weight was determined by centrifuging the cell suspension, resuspending in distilled water and drying at 90℃ for 24 h after a second centrifugation. Glucose concentration was determined by DNS (dinitrosalicylic acid) method. 1 mL of the supernatant of the centrifugated broth was reacted with 1 mL of DNS reagent at 100 ℃ for 5 min. 10 mL of distilled water was added after the reaction and optical density was measured at 546 nm. Ethanol was measured by gas chromatography (Yanaco G-1800) with a thermal conductivity detector.

RESULTS AND DISCUSSION

1. Control Using a Conventional STR

The control results using a conventional STR (self-tuning regulator) are shown in Fig. 2. The cell concentration was deviated approximately ±20% from the setpoint. The setpoint tracking was not desirable because of the large changes in the control input computed from the STR algorithm. Especially, the value of the control input was above 0.1 h^{-1} around 76 h and the cell mass was thus almost below the setpoint after 76 h. The decrease of the cell mass influenced the

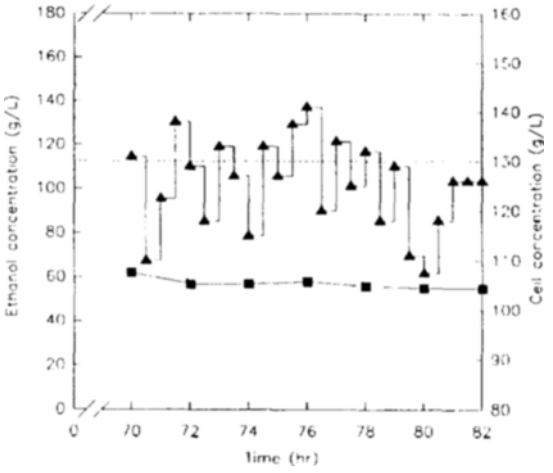

Fig. 2. Control of cell recycled continuous bioreactor using a conventional STR.
−▲− Cell concentration (g/L), −■− Ethanol concentration (g/L)

ethanol productivity and glucose conversion. The ethanol productivity was initially high as 80 g/L/h but decreased to 68 g/L/h when the cell mass became below the setpoint. It was therefore necessary to improve the control performance for the ethanol productivity.

2. Rule-bases

It was important to adjust the control input from the STR algorithm to attain the desired control objective. Thus, rule-bases were built from experiments to get good control performance.

If the cell concentration at step i in the reactor is higher than the setpoint by 5% and the bleeding rate at step i is also higher than the estimated specific growth rate at step i, it is better for the control input computed from the adaptive algorithm to be maintained as it is in Eq. (15).

$$\text{If}(((x_i - x_{st})/x_{st} > .05) \text{ and } (B_i > U_i)) \text{ then } B_i = B_i \quad (15)$$

, where x_i, x_{st}, B_i and U_i are the cell concentration at step i, the cell concentration of the setpoint, the bleeding rate from step i to step i+1, and the specific growth rate from step i to step i+1, respectively. The comparisons between the bleeding rate and the specific growth rate in Eq. (15) were made to 10^{-2} order. Eq. (16) could also be explained vice versa.

$$\text{If}(((x_i - x_{st})/x_{st} < -.05) \text{ and } (B_i < U_i)) \text{ then } B_i = B_i; \quad (16)$$

If the cell concentration at step i in the reactor is smaller than the setpoint by 5% and the bleeding rate

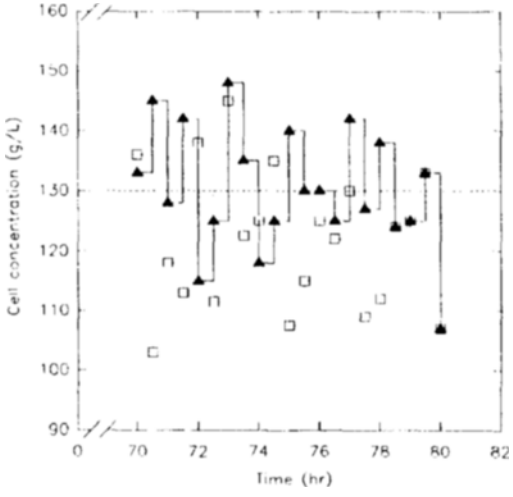

Fig. 3. Control of cell recycled continuous bioreactor with rule-based STR. The rule-bases were Eqs. (15), (16), (17) and (18).
−▲− multiplying factor of 1.15 in Eq. (18), −□− multiplying factor of 1.35 in Eq. (18)

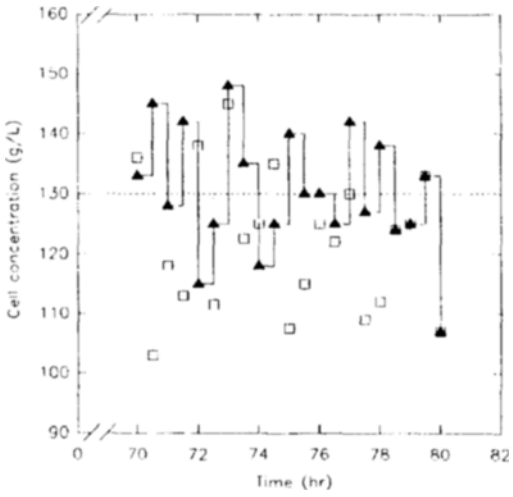

Fig. 4. Control of cell recycled continuous bioreactor with rule-based STR. The rule-bases were Eqs. (15), (16), (17) and (18).
−▲− multiplying factor of 0.35 in Eq. (18), −□− multiplying factor of 0.25 in Eq. (18)

at step i is also smaller than the estimated specific growth rate at step i, it is desirable for the control input computed from the adaptive algorithm to zero.

$$\text{If}(((x_i - x_{si})/x_{si} < -.05) \text{ and } (B_i > U_i)) \text{ then } B_i = 0; \quad (17)$$

In other words, on-off control was implemented in Eq. (17). Shi et al. [30] also used on-off control to get good control performance in the adaptive control of lactic acid fermentation system.

When the cell concentration at present stage is greater than the setpoint and the control input calculated from the adaptive algorithm is smaller than the specific growth rate at step i, the bleeding rate at step i is multiplied by 1.25 to avoid the excessive accumulation of the cell.

$$\text{If}(((x_i - x_{si})/x_{si} > .05) \text{ and } (B_i < U_i)) \text{ then } B_i = B_i{}^{*}1.25; \quad (18)$$

The multiplying factor of 1.25 in Eq. (18) was obtained from the experiments. When multiplying factor of 1.15 was given to Eq. (18), the cell mass was mainly above the setpoint as shown in Fig. 3. Because the bleeding rate had mainly the limiting value, the cell concentration was very fluctuating around the setpoint and the ethanol productivity decreased. For larger multiplying factor of 1.35, the cell concentration was almost below the setpoint as shown in Fig. 3, and glucose was not consumed much with the surplus glucose concentration of 30 g/L. Thus ethanol productivity was low. Since the cell concentration was to be neither low nor high contrary to the two previous cases, the multiplying factor of 1.25 was selected in determining the bleeding rate in Eq. (18).

When the cell mass is smaller than the setpoint and the bleeding rate has the same value of the specific growth rate, the bleeding rate is reduced to a fourth of the value computed from the adaptive algorithm to prevent the excessive bleeding of the cell as shown in Eq. (19).

$$\text{If}(((x_i - x_{si})/x_{si} < -.05) \text{ and } (B_i = U_i)) \text{ then } B_i = B_i{}^{*}0.25; \quad (19)$$

When the multiplying factor in Eq. (19) was 0.35, the cell mass was below the setpoint as denoted in Fig. 4, so setpoint tracking performance was not good. The ethanol productivity was almost the same as in the case of the conventional STR. It was therefore necessary to reduce the multiplying factor in Eq. (19). Fig. 4 shows that the setpoint tracking was very good compared to the case of the conventional STR, when the multiplying factor of 0.25 in Eq. (19) was employed. The cell mass was deviated the setpoint about ± 10%. The ethanol productivity was improved as 79 g/L/h and glucose conversion ratio was almost above 90%.

3. Control Using Rule-based STR

The Eqs. of (15), (16), (17), (18) and (19) were incorporated for the control of the cell recycled continuous bioreactor. When the rule-bases were used along with

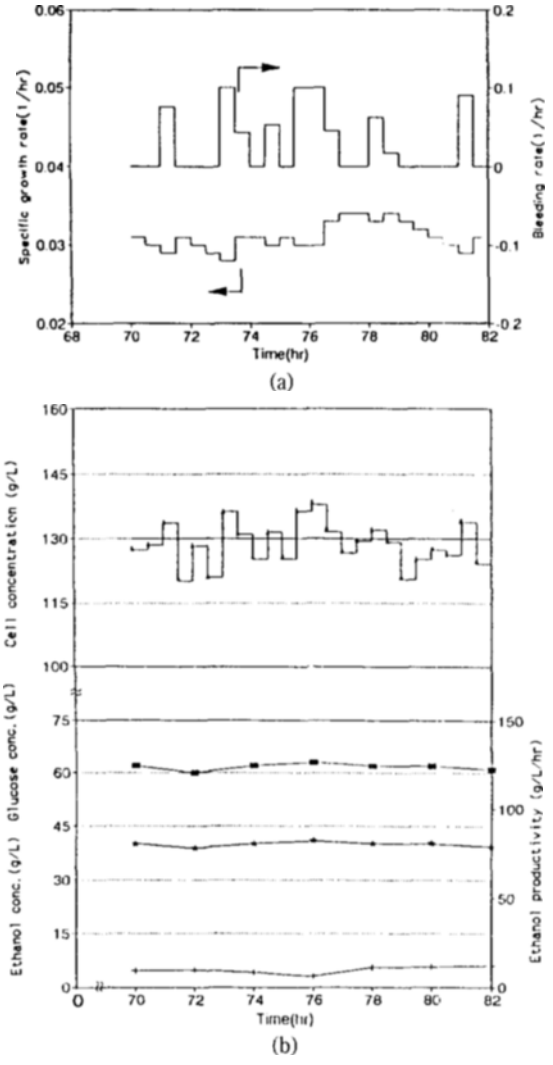

Fig. 5. Control of cell recycled continuous bioreactor with rule-bases.

(a) parameter estimation and control input, (b) setpoint tracking and other state variables.
−▲− Cell concentration (g/L), −■− Ethanol concentration (g/L), −+− Glucose concentration (g/L), −★− Ethanol productivity (g/L)

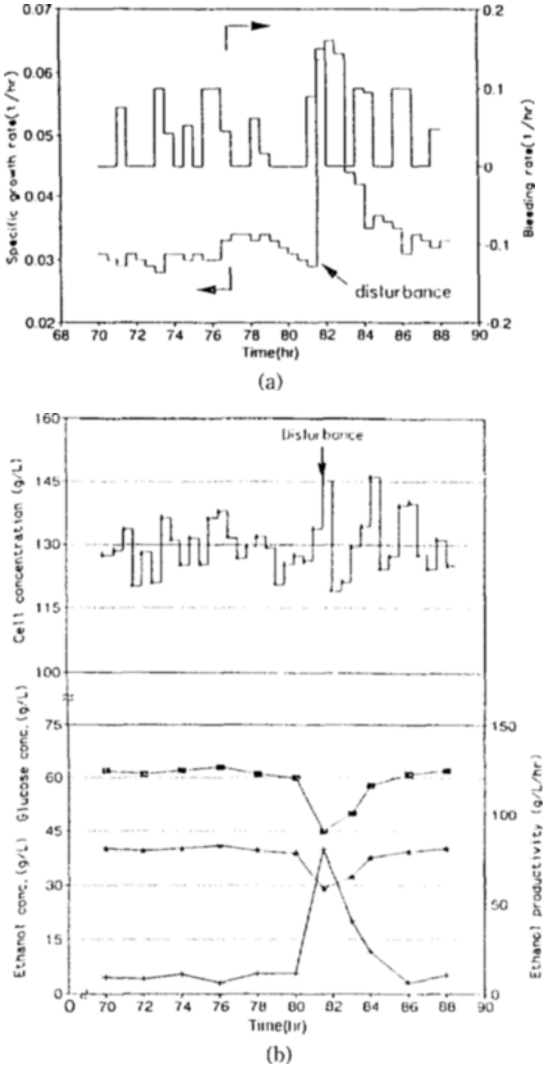

Fig. 6. Control of cell recycled continuous bioreactor with rule-bases when disturbance was given.

(a) parameter estimation and control input, (b) setpoint tracking and other state variables.
−▲− Cell concentration (g/L), −■− Ethanol concentration (g/L), −+− Glucose concentration (g/L), −★− Ethanol productivity (g/L)

the STR. The performance of the setpoint tracking was very good, deviating from the setpoint approximately ± 8% as represented in Fig. 5. Compared to the results using the conventional STR (Fig. 2), the control input with rule-bases did not have upper value. Fig. 5 shows that the ethanol productivity was 80 g/L/h and maintained the value to the end of control. The glucose conversion ratio was as high as 94%.

It is necessary to test to the developed control algorithm whether the algorithm shows the fast recovery of the setpoint. The following scenario was made for this purpose : the media supply was suddenly stopped at 81 h and the working volume decreased to half in 30 min because of the trouble of the feeding pump. The operator perceived the trouble and supplied media into the reactor quickly. The concentration of the

glucose and the cell increased suddenly. The ethanol concentration decreased from 60 to 45 g/*l* because of being drawn by the membrane without media fed into the reactor. Control action was made at this moment. Fig. 6 represents that the specific growth rate increased from 0.03 to 0.065. This was due to the decrease of the ethanol inhibition and the increase of the substrate available. Though the disturbance was to the system, the values of the state variables recovered to the normal values within 4 hours.

Rule based STR is very efficient in controlling high cell concentration in cell recycled continuous bioreactor system. Even though the detailed logics behind rule bases are not clarified enough, preliminary results are very encouraging and it is believed that the rule-based adaptive control algorithm can be effectively applied in bioprocesses.

CONCLUSIONS

From the present study on the adaptive control with rule-bases of cell recycled continuous bioreactor, the followings were elucidated.

1. The control performance by self-tuning regulator without the rule-bases was not effective. Five rule-bases were built and incorporated into the adaptive control scheme.

2. The rule-based adaptive control developed in the present study was immune to the disturbances. It was stable as well as robust.

NOMENCLATURE

A : parameter matrix of state equation
A_x : row vector of matrix **A** for cell
A_G : row vector of matrix **A** for glucose
A_E : row vector of matrix **A** for ethanol
D : dilution rate [hour $^{-1}$]
B : bleeding rate [hour $^{-1}$]
E : ethanol concentration [g/L]
F : feed rate of glucose solution [L/hour]
S : glucose concentration [g/L]
S_0 : glucose concentration in feed [g/L]
Δt : time interval [hour]
V : volume [L]
X : column vector of state variables
X : cell concentration [g/L]
Y : observed variable

Greek Letters

Δ : matrix for recursive identification
Θ : parameter vector for recursive identification
θ : parameter for recursive identification
λ : forgetting factor
μ : specific growth rate of cell [hour $^{-1}$]
ξ : specific consumption rate of substrate [hour $^{-1}$]
π : specific production rate of ethanol [hour $^{-1}$]
P : information matrix for recursive identification
Φ : known function vector for recursive identification
φ : known function for recursive identification

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