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FUZZY CONTROL OF ETHANOL CONCENTRATION FOR EMULSAN PRODUCTION IN A FED-BATCH CULTIVATION OF *ACINETOBACTER CALCOACETICUS* RAG-1

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Abstract – A fuzzy control system was organized and applied to the control of ethanol concentration in a fed-batch cultivation process for emulsan production by *Acinetobacter calcoaceticus* RAG-1. The membership functions and fuzzy rules were determined by sets of data and experiences obtained from the preliminary culture experiments. The input variables, error (the difference between the set point value and the process variable) and the change of the error, were fuzzified by using the membership functions and the output variable, change of the ethanol feed rate, was inferred based on the membership functions and the given fuzzy rules. To obtain the numerical value for the output variable, the center-of-gravity method was used in the defuzzification procedure. The results showed that the ethanol concentration was well regulated around optimal level and the emulsan yield was increased compared with that of the cultivation controlled by the conventional feedback control loop.

Key words: Fuzzy Control, Fed-batch Cultivation, Emulsan, *Acinetobacter calcoaceticus* RAG-1

INTRODUCTION

Modern control theory and intelligent system instrumentation have been applied to the great number of bioprocesses as one of the key features of development in biotechnology. To achieve the optimal control of the objective process, process modeling is considered as an essential tool to be accomplished first. In process modeling, the main goal is to obtain the accurate relationship between the manipulated variables and controlled variables. However, the accurate process model describing the dynamic characteristics of the process can not be easily obtained since most of chemical or biological processes include several nonlinear elements and unexpected disturbances. Therefore, the processes are usually operated based on the information obtained from the experiences and knowledge of the experts [Stephanopoulos, 1987; Konstantinov and Yoshida, 1992].

Knowledge based control system has been developed and widely applied to complex processes, such as biological processes, with control problems due to the nonlinear and uncertain dynamic characteristics of the processes [Dohnal, 1985; Stephanopoulos, 1986; Czogala and Rawlik, 1989; Thibault et al., 1990]. Since knowledge based control system has a flexibility to handle the process information mathematically and/or linguistically, it is often chosen as an alternative control scheme to the conventional ones. The fuzzy control algorithm, one of the simplest architecture of knowledge based control systems, was developed for the effective use of uncertain and qualitative information, and has been applied to the simulation or control in many fields of the practical processes [Filev et al., 1985; Kishimoto et al., 1991; Yamakawa, 1992; Zhang and Edmunds, 1992; Alfafara et al., 1993; Katayama et al., 1993]. Considering the complexity and uncertainty of biological processes, fuzzy algorithm can be chosen as one of the most appropriate control scheme in the operation of bioprocesses.

A polyanionic heteropolysaccharide produced by *Acinetobacter calcoaceticus* RAG-1, emulsan, is an excellent bioemulsifier which can be widely applied in many fields of oil-associated and consumer product industries [Reisfeld et al., 1972; Rosenberg et al., 1979a,b, 1980; Kosaric et al., 1987]. Ethanol used mainly as the carbon source in *A. calcoaceticus* RAG-1 has an inhibitory effect on the cell growth of *A. calcoaceticus* [Abbott, 1973; Abbott et al., 1973; Schaefer, 1985; Choi et al., 1996a]. Emulsan production is affected by the ethanol concentration in culture medium since emulsan is produced with mixed growth-associated pattern and the cell growth is directly dependent on the ethanol concentration [Gutnick et al., 1980; Schaefer, 1985; Choi et al., 1996a]. Fed-batch cultivation with continuous feeding of carbon source was performed as one of the most feasible operating strategy for the enhancement of emulsan production as well as cell growth by reducing the catabolite repression effect of ethanol [Choi et al., 1996a,c].

In this study, the fuzzy control algorithm was applied to the control of ethanol concentration in fed-batch cultivation of *A. calcoaceticus* RAG-1 for emulsan production. The mem-

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bership functions and fuzzy rules were organized by trial and error method through a number of simulations and experiments. To realize the optimal control of the ethanol concentration with proposed control scheme, fed-batch cultivation experiment was done and the performance of the fuzzy controller was evaluated by comparing the sum of squared error for the process variable and the emulsan yield obtained from this work with those from early experiments with different control schemes.

MATERIALS AND METHODS

1. Microorganism

The microorganism used in this study was a gram negative bacterium, *Acinetobacter calcoaceticus* RAG-1 (ATCC 31012).

2. Medium and Culture Conditions

The organism was cultured in a medium containing 7.3 g l^{-1} of KH_2PO_4, 16.9 g l^{-1} of K_2HPO_4, 0.5 g l^{-1} of $MgSO_4$, 4.0 g l^{-1} of $(NH_4)_2SO_4$ and trace-salts solution. Ethanol (16.0 g l^{-1}) was used as the carbon source. For stoke culture, 18.0 g l^{-1} of agar was used with the above medium, and for each agar plate a filter paper wetted with 200 μl of ethanol was placed on the back cover, where the vapor of ethanol was used as the carbon source. The agar plates were incubated upside-down at 30 °C for 2 d, and then the filter paper was removed. The strain was kept on agar plates at 4 °C and transferred monthly. For shake flask culture, the liquid medium was sterilized in an autoclave at 121 °C for 15 min and pH was adjusted to 7.0 before autoclaving. The magnesium and trace-salts solution were sterilized separately and then added to the shake flask after cooling along with the addition of ethanol.

From a petri dish, three loops of cells were transferred into 100 ml maintenance medium for 250 ml shake flask and cultured at 30 °C and 180 rpm for 20 h. Three times of subculture were performed to stabilize cells before used as a seed culture for the experiments. Batch cultivations were performed in a 5 l jar reactor (B. E. Marubishi Ltd., MD-300) with an initial volume of 2 l. Before sterilize medium, pH was also adjusted 7.0. The temperature was 30 °C, the agitation speed was 500 rpm with 0.5 vvm of air flow rate. At the initial state, the medium was composed of control medium with 8.0 g l^{-1} of ethanol and 12.1 g l^{-1} of phosphate and the cultivation was performed with batch mode until the ethanol concentration fell below 6.5 g l^{-1}. Once cultivation was switched from batch mode to fed-batch mode, ethanol was fed to maintain the optimal level. Cultivation with fed-batch mode was continued until reactor working volume reached from 2 l to 3.5 l, and then operation was switched again to batch mode since emulsan was also produced after the exponential growth phase.

For the control of the substrate feed rate in fed-batch cultivation, the reactor system was interfaced with a personal computer through a D/A converter. The ethanol concentration was detected with 10 min intervals, and the data was transferred to the computer. The control output was calculated by the proposed control algorithm and the signals were transferred to a peristaltic pump (Masterflex, Model No. 7523-00, Cole-Parmer inst. Co.) to manipulate the substrate feed rate.

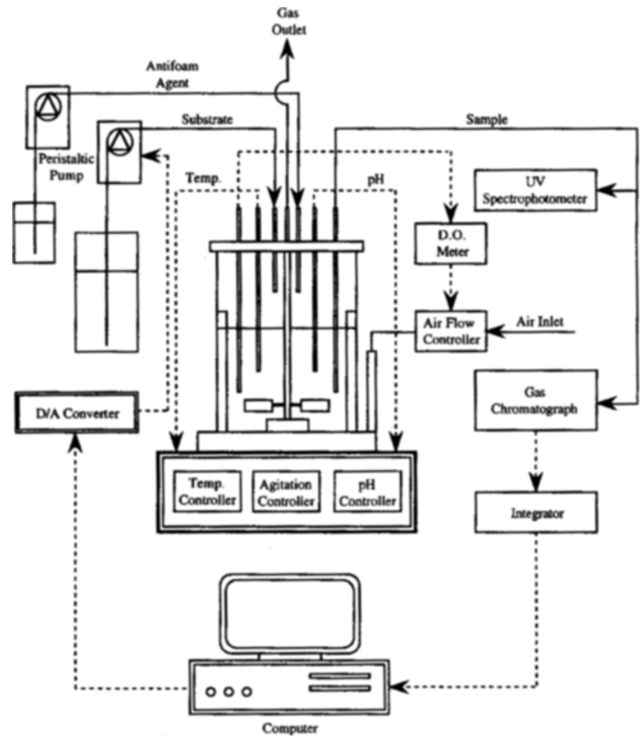

Fig. 1. A schematic diagram of the computer controlled cultivation system.

A schematic diagram of the computer controlled cultivation system is shown in Fig. 1.

3. Analytical Methods

Cell concentration was determined by a UV spectrophotometer (Shimadzu, UV-240) at 660 nm. Ethanol was measured by gas chromatography (GOW-MAC, GC model 69-550) equipped with a thermal conductivity detector using n-propanol as an internal standard. The GC column used was packed with porapak type Q (Waters, mesh 80-100) and was operated at 135 °C with helium as the carrier gas flowing at a rate of 40 ml min^{-1}. Extracellular phosphate concentration was measured by the vanadomolybdo-phosphoric acid method with detection of optical density at 400 nm. For the determination of intracellular phosphate concentration, the same method was used after sonic disruption of cells and centrifugation. Extracellular emulsan concentration was determined by the phenol-sulfuric acid method with detection of optical density at 480 nm [Dubois et al., 1959; Wang and Wang, 1989]. The concentration of cell-bound emulsan was determined by the same method after EDTA treatment to release the cell-bound emulsan.

FUZZY CONTROLLER DESIGN

1. Model of Fed-batch Cultivation

For the determination of the optimal ethanol concentration, batch cultivations with variation of initial ethanol concentration were performed and the kinetic model describing the role of ethanol and phosphate on cell growth and emulsan production was developed based on the experimental results of batch cultivations [Choi et al., 1996a,b]. The optimal ethanol concentration for the enhancement of emulsan production was determined as 6.5 g l^{-1} from the results of the early experiments and kinetic study. The generalized dynamic mathematical model of a fed-batch process is formed as follows.

- Dry Cell Weight

$$\frac{dX}{dt}=\left[\left\{1-\exp\left(-\frac{t}{t_{lag}}\right)\right\}\mu-k_d\right]X-\frac{X}{V}F \qquad (1)$$

where,

$$\mu=\frac{\mu_{max}S}{K_S+S}\left(1-\frac{S}{S_{max}}\right)^{n_s}\left[1-\exp\left\{-K_{Pi}\left(\frac{P_i}{P_{i0}}\right)^2\right\}\right]$$

- Ethanol

$$\frac{dS}{dt}=-\left[1-\exp\left(-\frac{t}{t_{lag}}\right)\right]\frac{\mu}{Y_{X/S}}X-m_SX$$
$$-\left[k_{e1}\left\{1-\exp\left(1-\frac{t}{t_{lag}}\right)\right\}\mu+\frac{k_{e2}P_i}{K_{Pi1}+P_i}\exp\left(-\frac{P_i}{K_{Pi2}}\right)\right]$$
$$\frac{1}{Y_{Et/S}}X+\frac{(S_F-S)}{V}F \qquad (2)$$

- Extracellular Phosphate

$$\frac{dP_e}{dt}=-\frac{k_{Pe}P_e}{K_{Pe}+P_e}X+(Y_{P/X}+P_i)k_dX+\frac{(P_{eF}-P_e)}{V}F \qquad (3)$$

- Intracellular Phosphate

$$\frac{dP_i}{dt}=\frac{k_{Pe}P_e}{K_{Pe}+P_e}-\alpha(Y_{P/X}+P_i)\mu-m_{P_i}$$
$$-Y_{P/Et}\left[k_{e1}\left\{1-\exp\left(-\frac{t}{t_{lag}}\right)\right\}\mu\right.$$
$$\left.+\frac{k_{e2}P_i}{K_{Pi1}+P_i}\exp\left(-\frac{P_i}{K_{Pi2}}\right)\right] \qquad (4)$$

- Cell-Bound Emulsan

$$\frac{dE_b}{dt}=\left[k_{e1}\left\{1-\exp\left(-\frac{t}{t_{lag}}\right)\right\}\mu\right.$$
$$\left.+\frac{k_{e2}P_i}{K_{Pi1}+P_i}\exp\left(-\frac{P_i}{K_{Pi2}}\right)-k_r\right]X-\xi k_dX-\frac{E_b}{V}F \qquad (5)$$

- Free (Extracellular) Emulsan

$$\frac{dE_f}{dt}=k_rX+\xi k_dX-\frac{E_f}{V}F \qquad (6)$$

where,

$$\xi=\frac{E_b}{X}$$

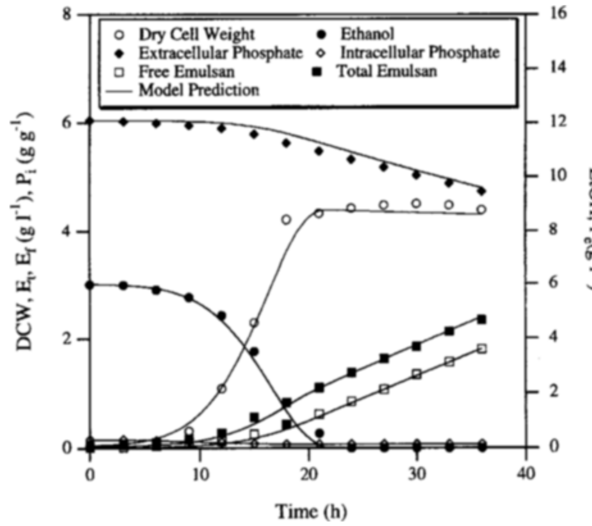

Fig. 2. Time course behavior of batch cultivation and the model prediction.

- Total Emulsan

$$\frac{dE_t}{dt}=\left[k_{e1}\left\{1-\exp\left(-\frac{t}{t_{lag}}\right)\right\}\mu\right.$$
$$\left.+\frac{k_{e2}P_i}{K_{Pi1}+P_i}\exp\left(-\frac{P_i}{K_{Pi2}}\right)\right]X-\frac{E_t}{V}F \qquad (7)$$

- Feed Rate

$$\frac{dV}{dt}=F \qquad (8)$$

The parameters used were estimated for the batch cultivations with different initial ethanol concentration (4.0, 6.0, and 8.0 g l^{-1}) by the authors [Choi et al., 1996a,b]. The model was verified by comparing with experimental results as shown in Fig. 2 and used as the bioreaction terms in simulation of the objective process operated with fuzzy control algorithm.

2. Structure of Fuzzy Controller

For the application of fuzzy control algorithm to fed-batch cultivation, the reasonable membership functions and the fuzzy rules should be organized first. Generally, the membership functions and the fuzzy rules are composed of the information based on the experiences and knowledge obtained from the experiments, and the final form is determined by trial and error method through a number of simulations and/or experiments. A schematic diagram of the fuzzy controller was shown in Fig. 3. The error, the difference between the set point value and the process variable ($e_i=S_{Opt.}-S_i$) and the change of the error ($\Delta e_i=e_i-e_{i-1}$) were considered as the input variables, and transferred into the corresponding fuzzy variable sets, [E] and [CE], through the membership functions in the fuzzification block. The output variable, the change of the feed rate, [CFR], was obtained from the fuzzy reasoning by the fuzzy rules, and then the numerical value calculated by defuzzification block was transferred into the actuator, the pump to feed ethanol.

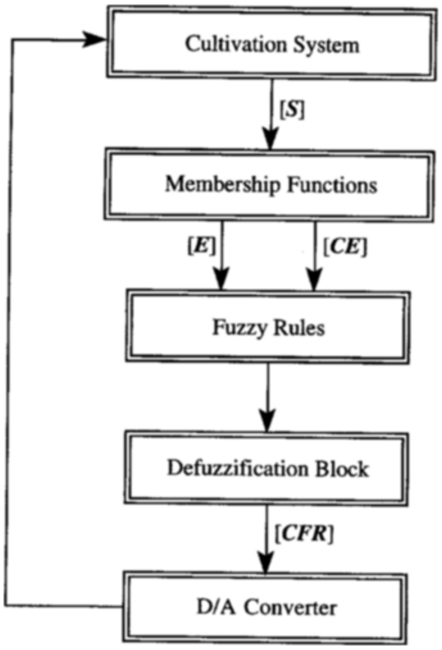

Fig. 3. A structure of fuzzy logic controller.

In the initial state, the membership functions and the fuzzy rules were organized based on the information obtained from the early experiments. Tuning of the fuzzy controller was performed by modifying the membership functions of input and output variables. Performing the simulations for the objective process with the proposed fuzzy controller, the membership functions and the fuzzy rules were further modified. The final definition of the membership functions and the fuzzy rules were obtained as shown in Fig. 4 and Table 1, respectively. The membership functions organized were composed of seven basic fuzzy subsets and used to obtain the grades of memberships for the value of [E], [CE], and [CFR]. The fuzzy subsets were NB (negative big), NM (negative medium), NS (negative small), Z (zero), PS (positive small), PM (positive medium), and (positive big). Basically, a symmetric triangular shape is used to represent a membership function, but it can be modified depending on the range of E or CE in each fuzzy subsets.

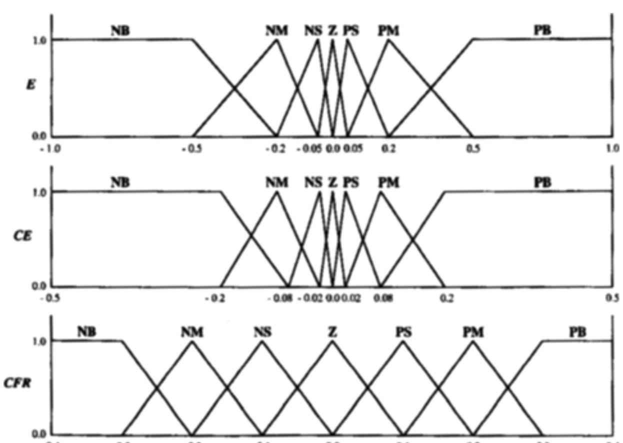

Fig. 4. Membership functions for error (E), change of error (CE), and change of feed rate (CFR).

Table 1. The look up table of the final fuzzy rules

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

Fuzzy reasoning for the input variables, [E] and [CE], with given control rules were organized as IF-THEN statements. The fuzzy reasoning for the input variables was performed by max-min composition method in which the binary operations, OR (∪: union) and AND (∩: intersection), for two fuzzy set, [E] and [CE], were defined as max- and min-operation, respectively. An example for obtaining the output variable by fuzzy reasoning was shown in Fig. 5. Since the membership functions can not be represented by a unequivocal boundary, one input variable was applied to several control rules and several outputs were obtained from the corresponding control rules as shown in Fig. 5. To obtain a final numerical value for the output variable, [CFR], defuzzification was performed by center-of-gravity method, which is most frequently used one and the validity has been verified in many practical applications [Postlethwaite, 1989; Yamakawa, 1992; Alfafara

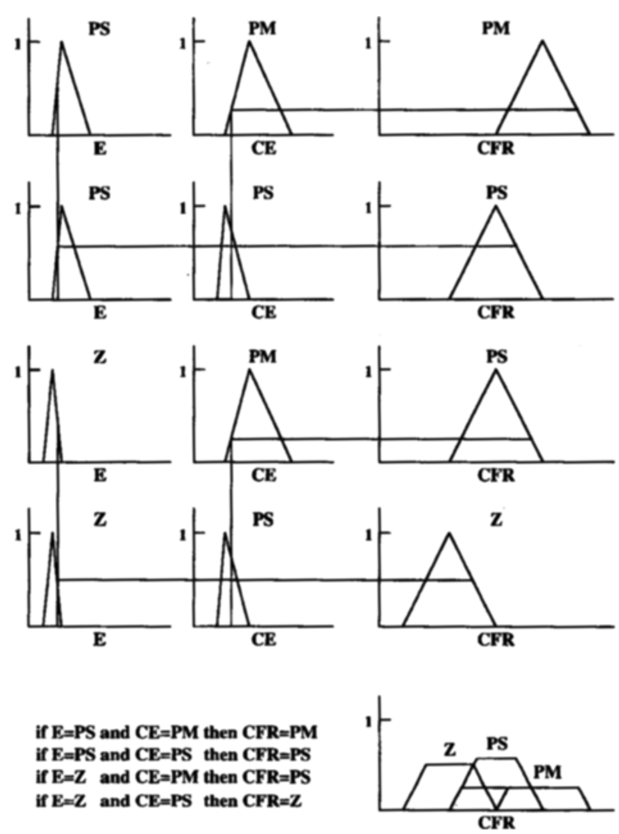

Fig. 5. An example of fuzzy inference method based on max-min composition.

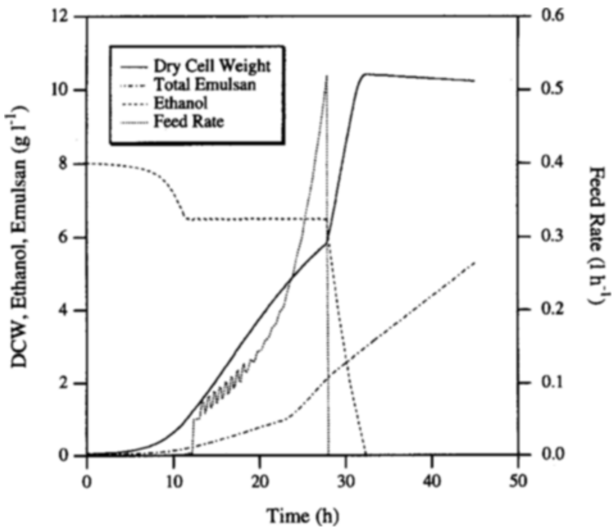

Fig. 6. Simulation results of fed-batch cultivation with the proposed fuzzy logic controller.

et al., 1993].

SIMULATION AND EXPERIMENTAL RESULTS

To examine the control performance of the proposed fuzzy controller, simulation of fed-batch cultivation was performed. The substrate feed rate was manipulated by the proposed control algorithm and the ethanol concentration was kept within the optimal range for cell growth during the fed-batch operation mode as shown in Fig. 6. The bias value was applied to the output variable in the first sampling step to achieve the more stable and faster convergence in control of the objective process. The gradual increase of output variable was found with some fluctuation in the early stage of the fed-batch operation mode and then continued to reduce the error of the process variable to the set point. The final cell density and emulsan concentration were 8.94 g l^{-1} and 5.3 g l^{-1}, respectively. The results indicate that the proposed fuzzy algorithm could be successfully applied to the objective process.

To realize the control of the practical process, the proposed fuzzy controller was applied to the experiment of fed-batch operation for emulsan production. The ethanol concentration, the process variable, was kept within the optimal range (6.5 g l^{-1}) for cell growth during the fed-batch operation mode as shown in Fig. 7 (top). As shown in Fig. 7 (bottom), the feed rate, the control variable, was manipulated to reduce the control error. The final cell density and emulsan concentration were shown in Fig. 8, which were higher than those of batch cultivation.

In the previously performed conventional feedback control, model parameters were estimated based on the approximation of the objective process to *First Order Plus Dead Time* (FOPDT) model. A proportional mode (P mode) and an integral mode (I mode) were used and the control parameters of the PI controller were tuned based on the *Integral of the Time-Weighted Absolute Value of the Error* (ITAE) tuning criterion [Smith and Corripio, 1984; Choi et al., 1996a,c]. In the feedback-assisted iterative learning algorithm, the information ac-

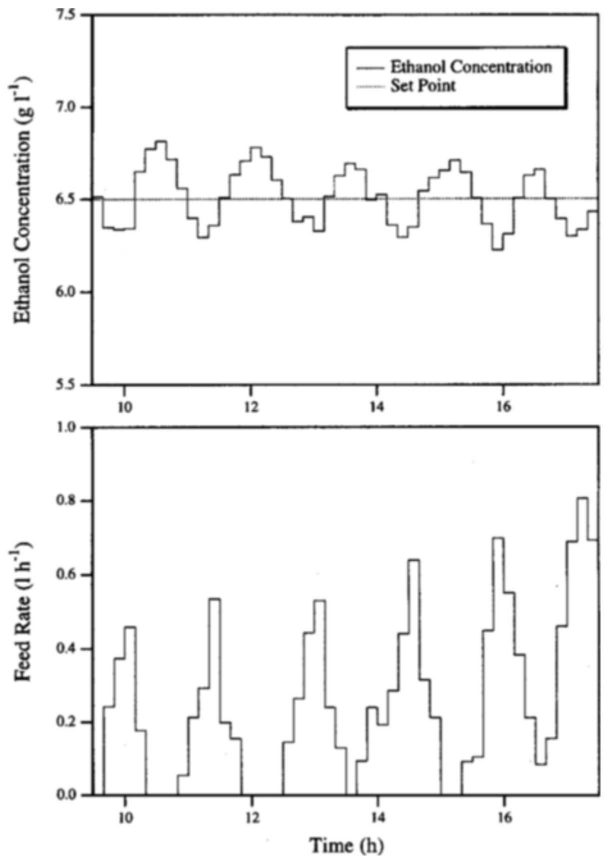

Fig. 7. Experimental results of ethanol concentration trajectory (top) and feed rate trajectory (bottom).

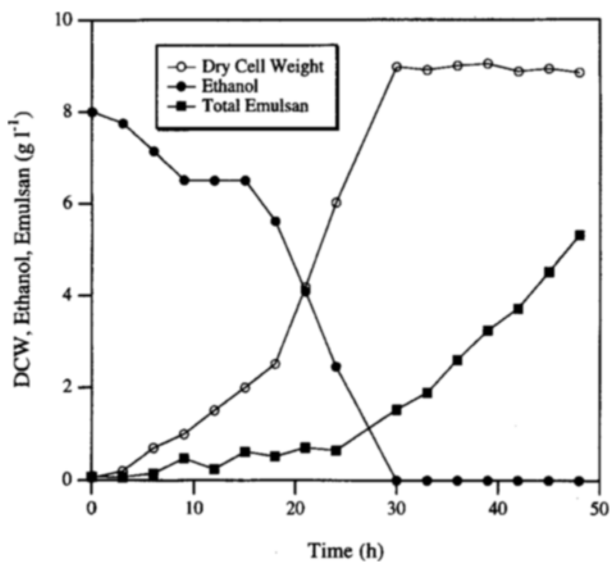

Fig. 8. Experimental results of fed-batch cultivation with the proposed fuzzy logic controller.

cumulated from the previous operations were used to enhance the control performance and the feedback loop was appropriately combined to reduce the effects of unexpected disturbances. The model for the objective process was approximated to the FOPDT model. The control parameters for learning and PI controller were determined by using the inverted model ap-

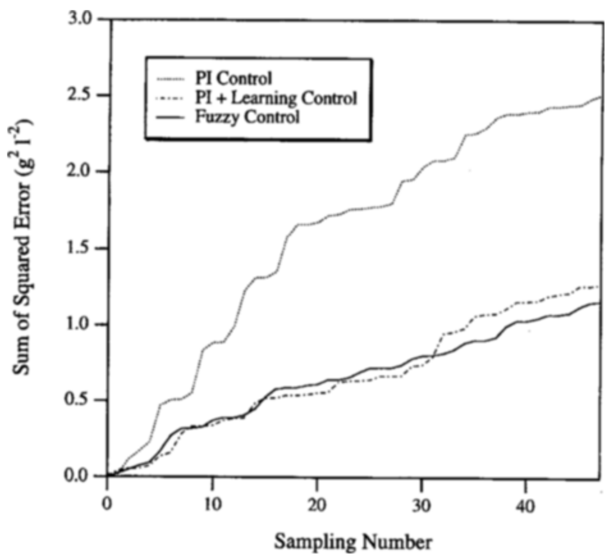

Fig. 9. Experimental results of squared error sum on the process variable (ethanol concentration) in fed-batch cultivations controlled by different schemes.

proach and ITAE tuning criterion, respectively [Choi et al., 1996c].

To clarify the control performance, the sum of the squared error for the process variable, ethanol concentration, was calculated and shown in Fig. 9. By comparing the result with those of conventional PI controller and feedback-assisted learning controller, the sum of the squared error of fuzzy controller was smaller than that of PI controller and similar to that of feedback-assisted learning controller. From these results, it can be deduced that the control performance of fuzzy controller was better than that of PI controller and similar to that of feedback-assisted learning controller. To clarify the enhancement of emulsan production, the yields of emulsan to substrate and dry cell weight were also considered and shown in Fig. 10. The production yield of fed-batch cultivation controlled by fuzzy algorithm was increased by about 18% compared with that of the cultivation controlled by a conventional feedback control algorithm and similar to that of the cultivation controlled by the feedback-assisted iterative learning algorithm, an advanced control scheme such as adaptive control method. Although the production yield of emulsan in the case of fuzzy control was not enhanced compared with that of the feedback-assisted iterative learning algorithm, it is worthwhile to adopt fuzzy algorithm in the control of bioprocess operated with fed-batch mode. Without a complex mathematical model to precisely describe the dynamic characteristics of the objective process, the process can be successfully regulated by the fuzzy control rules which can be composed linguistically based on the system information obtained from the experience.

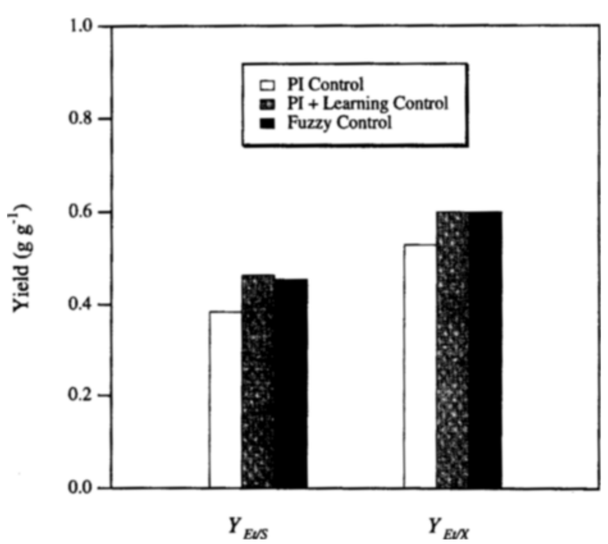

Fig. 10. Comparison of emulsan yields obtained from fed-batch cultivations controlled by different schemes.

CONCLUSIONS

The fuzzy controller was adopted to regulate the ethanol concentration in a fed-batch cultivation of *A. calcoaceticus* RAG-1. The error, $e_i = S_{Opt.} - S_i$, and the change of the error, $\Delta e_i = e_i - e_{i-1}$, used as the input variables were transferred into the corresponding fuzzy variable sets, [E] and [CE], through the membership functions in the fuzzification block. The change of the feed rate, [CFR], used as the output variable was obtained from the fuzzy reasoning by the fuzzy rules, and the numerical value was calculated by defuzzification block. The substrate concentration was successfully controlled by the proposed fuzzy controller and the emulsan production was enhanced compared with that of the previous results obtained by using conventional feedback control algorithm. It can be concluded that the proposed fuzzy control algorithm could be usefully applied to a cultivation of *A. calcoaceticus* RAG-1 operated with fed-batch mode. The control performance might be further enhanced by combining an advanced algorithm such as self-organizing fuzzy control scheme using genetic algorithm or an iterative learning algorithm.

NOMENCLATURE

E_b : cell-bound emulsan [g l^{-1}]
E_f : free emulsan [g l^{-1}]
E_t : total emulsan [g l^{-1}]
e_i : error, between the set point value and the process variable
k_d : specific death rate [h^{-1}]
k_{e1} : factor related to the growth-associated production [g l^{-1}]
k_{e2} : rate constant of non growth-associated production [g g^{-1} h^{-1}]
k_{Pe} : rate constant of active transport [h^{-1}]
K_{Pe} : saturation constant of extracellular phosphate [g l^{-1}]
K_{Pi} : growth index [k_{Pi}/k_{i0}]
K_{Pi1} : saturation constant of intracellular phosphate [g g^{-1}]
K_{Pi2} : production inhibition constant [g g^{-1}]
K_S : saturation constant of ethanol [g l^{-1}]
m_{Pi} : maintenance coefficient for intracellular phosphate [g g^{-1} h^{-1}]
m_S : maintenance coefficient for ethanol [g l^{-1} g^{-1} h^{-1}]
n_S : inhibitory index of ethanol
P_e : extracellular phosphate [g l^{-1}]
P_i : intracellular phosphate [g g^{-1}]

S : ethanol [g l^{-1}]
t : time [h^{-1}]
t_{lag} : lag time [h]
X : dry cell weight [g l^{-1}]
$Y_{Et/S}$: yield coefficient of total emulsan to ethanol [g g^{-1}]
$Y_{X/S}$: yield coefficient of cell to ethanol [g g^{-1}]
$Y_{P/X}$: yield coefficient of phosphate to cell [g g^{-1}]
$Y_{P/Et}$: yield coefficient of phosphate to emulsan [g g^{-1}]

Greek Letters

α : factor related to the growth in intracellular phosphate consumption
μ : specific growth rate [h^{-1}]
μ_{max} : maximum growth rate [h^{-1}]
$\mu_{s, max}$: maximum growth rate by ethanol contribution [h^{-1}]
ξ : cell-bound emulsan per dry cell weight [g g^{-1}]

Subscripts

Opt. : optimal value (set point value)
i : number of sampling

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