DEVELOPMENT OF INTELLIGENT CONTROL SYSTEMS FOR BIOREACTORS

Kazuyuki Shimizu* and Kaiming Ye

Department of Biochemical Engineering and Science
Kyushu Institute of Technology, Iizuka, Fukuoka 820, Japan

Abstract :Recent development of intelligent bioreactor systems has made significant evolution in terms of tools, objectives, and concepts. Stimulated by rapid progress in the field of AI backed up with powerful personal computers, AI-based computer control of bioreactors is now under remarkable progress.The present paper review the recent development of intelligent control systems for bioreactors. Paticular attention is focused on fuzzy expert systems, neuro control, neuro-fuzzy control, and genetic algorithm.

Keywords: bioprocess control, neuro-fuzzy control, genetic argorithm, bioprocess systems engineering.

1. INTRODUCTION

The ability to control fermentation processes at their optimal states accurately and automatically is of considerable interest to bioindustries since it can enable them to reduce their production costs and increase the yield while at the same time maintaining the quality of metabolic products etc. It should be noted, however, that the control system design of bioreactors is not straighty-forward due to (1)significant model uncertainty, (2)time-varying and nonlinear nature, (3)lack of reliable on-line sensors, and (4)slow response.Note that the microorganisms themselves have a complex regulatory mechanisms within the cells and that the external control system can only manipulate the extracellular environment which may indirectly affect the intracellular metabolic reactions.

Many control strategies have been proposed so far and applied in practice to overcome the above problems, and many others are still in progress(Shimizu, 1993;1994). Most of them, however, considered only one or two aspects of the above problems, and very few researchers considered properly the overall problem.

In the present paper, state of the art for the development of intelligent bioreactor systems which may overcome the above problems is described based on our recent research result.

2. MEASUREMENT, STATE ESTIMATION AND CONTROL OF BIOREACTORS

The activity of a particular enzyme is controlled by the concentration of several medium components. Well-known are the enzyme regulations by carbon compounds (e.g. glucose), nitrogen compounds(e.g.ammonia), phosphates as well as induction of enzymes by their substrates.

In order to achieve the full biological potential of the cells, the environmental conditions must be maintained optimal. The first step towards this goal is the identification of the key components. Those on-line informations are critical for the success in bioprocess control (Schugerl,1991). However, in situ techniques are restricted at present in many cases.

It is also quite important to estimate the specific rates based on the concentration data available from on-line sensors. The specific rates may be used to monitor the cell and enzyme activities. The state and parameter estimation techniques have, therefore, been developed to estimate the above secondary variables by designing several estimators such as the extended Kalman filter, adaptive Kalman filter etc..

It is by far important to control carbon sources in the culture broth, and the main problem in the fed-batch cultivation of recombinant *E.coli* is to avoid the accumulation of acetate by manipulating the feedrate of carbon source such as glucose using an appropriate control strategy. Although on-line glucose analyser has been developed(Mizutani *et al.*,1987) and is commercially available, it is rather expensive now. We, therefore, considered to indirectly control the glucose concentration making use of the pH-stat method(Yamada *et al.*,1983). The similar method such as DO-stat method(Yano *et al.*,1991) can be also applied with the same idea. We considered to manipulate the glucose feeding rate based on $\triangle T$, where $\triangle T$ is the time counted from when the glucose feeding was stopped until the time when pH value rises due to sarvation of glucose.

3. KNOWLEDGE-BASED EXPERT SYSTEMS

Traditionally, modeling and the control system design of chemical processes have been pursued by the application of complex mathematical method.The significant complexity and uncertainty of bioprocesses, however, require sophisticated logic of operation, which cannot easily fit into the mathematical framework of the traditional control approach. Namely, the conventional approaches cannot treat the variety of events and phenomena, and are often blind to the real situation. As a result, a research trend towards the exploitation of new methods, capable of manipulating and utilizing uncertain, qualitative, and informal knowledge has appeared recently (Linko, 1988; Stephanopoulos and Stephanopoulos,1990; Konstantinov and Yoshida,1992).

An important methodology was proposed by Konstantinov and Yoshida(1989) for the control of bioprocesses based on expert identification of the physiological state of the cells. The physiological state was defined qualitatively by a set of specially selected variables that form the physiological state of the culture. Upon transfer from one to another state, it often exhibits variable structure behavior, and therefore different control strategy should be applied for each state(Konstantinov and Yoshida, 1992). The important thing is, therefore, to identify adequately the physiological state on-line, and several attempts have been made by several researchers(Shimizu *et al.*,1994;Horiuchi *et al.*,1993).

Although it is fairly difficult to describe exactly the dynamic behavior of bioreactors by means of mathematical expression in many cases, it may be possible to make use of the information obtainable from operators' intuitions and experiences for the control of bioreactors. The expert system characterizes the process dynamics by symbolic and logic description to check

researchers(Shimizu *et al.*,1994;Horiuchi *et al.*,1993).

Although it is fairly difficult to describe exactly the dynamic behavior of bioreactors by means of mathematical expression in many cases, it may be possible to make use of the information obtainable from operators' intuitions and experiences for the control of bioreactors. The expert system characterizes the process dynamics by symbolic and logic description to check
data consistency, equipment failure, and contamination etc. for the efficient operation (Endo *et al.*,1989;Asama *et al.*,1991).

4. FUZZY CONTROL

Fuzzy control has also been paid recent attention, since this method does not require mathematical models and it can treat qualitative information, even vague knowledge using linguistic rules. Thus the fuzzy control is considered to be suitable in particular for bioprocesses which are difficult to treat using mathematical models.The expert knowledge can be treated as the production rules and membership functions.

The fuzzy control has so far been applied to various bioprocesses such as glutamic acid fermentation(Nakamura *et al.*,1985; Kishimoto *et al.*,1991),antibiotic fermentation (Fu *et al.*,1988; Chen *et al.*,1988),SCP production (14), coenzyme Q_{10} production (Yamada *et al.*,1991), sake brewing (Koizumi *et al.*,1990; Oishi *et al.*,1991; Matsuura *et al.*,1991; Hara *et al.*,1993), baker's yeast cultivation (Park *et al.*,1993), and glutathione production using yeast(Alfafara *et al.*,1993). Siimes *et al.* (1992) considered object oriented fuzzy expert system for the on-line diagnosis and control of bioprocesses. Jitsufuchi *et al.*(1992) considered to adjust the membership functions based on the experimental data.

We also applied fuzzy control for the efficient production of gene product using recombinant *E.coli* (Jin *et al.*,1994). *Escherichia coli* has been widely employed as the host strain to obtain various kinds of gene products such as β-galactosidase, trypsin, human leukocyte interferon, and β-lactamase. Many factors influence the efficiency of the expression of gene products. Those may be (i)plasmid stability, (ii)growth inhibition due to the accumulation of inhibitory metabolites, (iii)the timing of induction, (iv)gene dosage . The increase of the gene dosage in the recombinant strain can be attained using strong promoters or high copy number plasmids. Among many factors that contribute to the increase in the productivity of gene products, it is quite important to optimize the culture condition to attain high cell density of recombinant *E. coli*, since the volumetric productivity of gene product can often be increased in accordance with the increase of the cell density in the culture broth (Iijima,1991).

Let F(t) be the feed rate predetermined by the mass balance based on the nominal operating condition. Since F(t) so determined is a rough approximation, without taking into account the cell activity, environmental *coli*, since the volumetric productivity of gene product can often be increased in accordance with the increase of the cell density in the culture broth (Iijima,1991).

Let F(t) be the feed rate predetermined by the mass balance based on the nominal operating condition. Since F(t) so determined is a rough approximation, without taking into account the cell activity, environmental changes, etc., some correction needs to be made and let it be △F, and consider adjusting △F by the fuzzy control algorithm. Fuzzy inference is carried out for example as

IF {specific growth rate is PL(Positive large)
and △T is PS(positive small)},
THEN {△F(t) be NS(negative small)}.

where the specific growth rate estimated from the on-line data of cell concentration and △T as mentioned above for the pH-stat method may be used as the input variables for fuzzy inference. Output of fuzzy inference is the correction of the feeding rate of carbon source.

5. NEURO CONTROL

Although the fuzzy control has been applied to the variety of bioprocesses and made some success, its performance heavily relies on the soundness of the knowledge obtained by the domain experts and the reliability of the fuzzy inference. Furthermore, it is a cumbersome procedure and it takes too much time to decide IF-THEN type of linguistic rules and membership functions.

The use of ANNs (artificial neural networks) has been paid recent attention because of its ability of knowledge acquisition focusing on the application to the identification and control of chemical and biochemical processes. ANNs are highly interconnected networks of nonlinear processing units of which connection weights (strength) are adjustable by "learning". ANNs having appropriate dimension can approximate large classes of nonlinear functions and are capable of adjusting dynamically to environmental changes by performing generalization from the specific data available. Several researchers, therefore, considered of applying ANNs to the estimation and prediction of bioprocess variables (Thibault *et al.*, 1990; Linko and Zhu,1991; Karim and Rivera,1992).

Figure 1 shows the structure of the neural network employed in our study, where y(k) denotes the ethanol concentration at k sampling instant and u denotes the glucose feeding rate. n is the order of the system and d is the dead time. The experimental data of on-off control were used to train the neural network off-line.The u(k) was determined so that one-step-ahead prediction of the ethanol concentration, y(k+1) obtaind by the neural network be equal to the set value $y_{sp}(k+1)$ (Ye *et al.*,1994). In our study, the number of neurons of input, hidden and output layers were 9,30 and 1, respectively. The identified order of the process was 4 and the deadtime was 1 sampling instant, where 1 sampling time was 1 min.

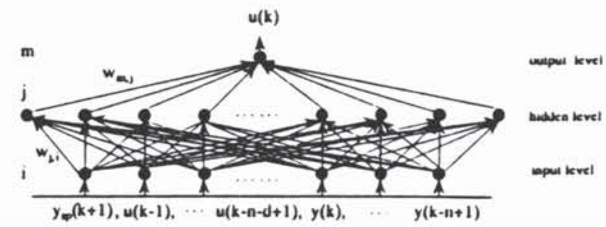

Fig.1 The structure of the neural network employed for fed-batch baker's yeast cultivation.

6. NEURO-FUZZY CONTROL

Although the neural network has the ability of learning, the structure of the above-stated neuro control is a kind of black box. It is quite difficult, though not impossible, to understand how each neuron affects the control quality. On the other hand, the structure of fuzzy control is transparent, but the fuzzy control lacks the ability of learning.

We, therefore, considered a neuro-fuzzy control strategy which retains both advantages of neural net and fuzzy control. Namely, we considere to adjust the membership functions of fuzzy control in accordance with the changing patterns of the state variables where the patterns were recognized on-line by neural networks (see Fig. 2) (Shi and Shimizu, 1992).

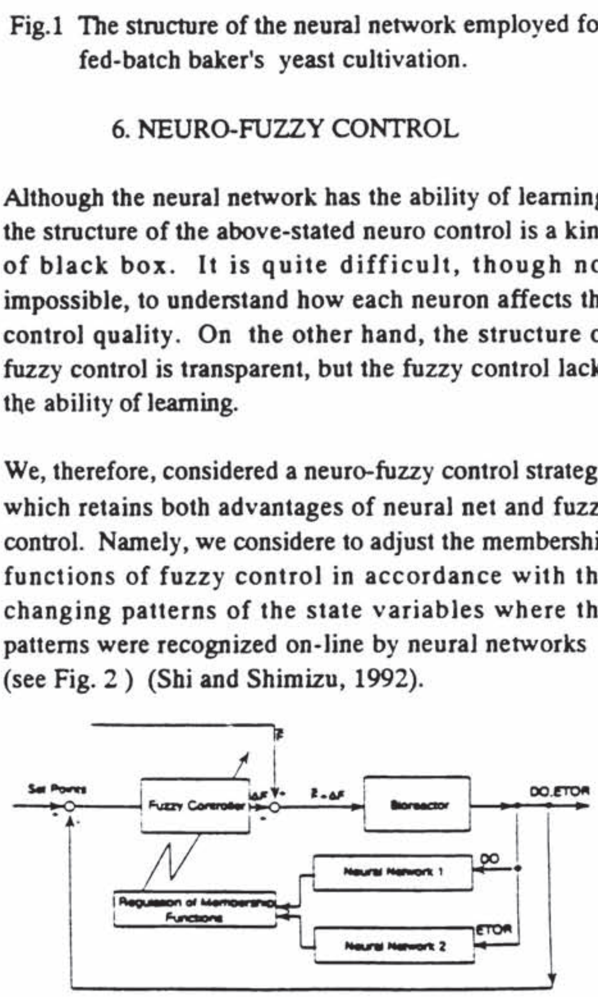

Fig.2 Neuro-fuzzy control configration.

In baker's yeast cultivation, certain relationships exist between the glucose concentration in the fermentor and the changing patterns in the DO and ethanol concentrations; an excess amount of glucose causes the Crabtree effect and the ethanol concentration tends to increase, while if glucose is not present in the fermentor the accumulated ethanol is consumed as a substrate and the ethanol concentration tends to decrease. Moreover, if the substrate is consumed and no substrate is present in the fermentor, the cell growth stops and the DO concentration abruptly increases because of the mass balance for oxygen. In this situation, if glucose is supplied the DO concentration returns to its original level. When the cell concentration becomes high, the supplied glucose is consumed quickly, and the repetition of this sequence makes the DO concentration tend to oscillate. Now the idea is to make use of these relationships for indirect but efficient monitoring of the glucose concentration in the fermentor. We considered to recognize these patterns on-line with the aid of neural networks(Shi and Shimizu, 1993).

7. FUZZY NEURAL NETWORK

Noting that the above neuro-fuzzy control can adjust the membership functions on-line by scaling but cannot adjust each membership function, we considered another different architecture which merges fuzzy inference and neural network(Ye *et al.*, 1994).

Consider the multilayer network structure as shown in Fig.3, which we call FNN (fuzzy neural network). At the layer 0 of FNN,the input variables e and c are scaled. At the layer 1, the outputs of the nodes are given by the following equations:

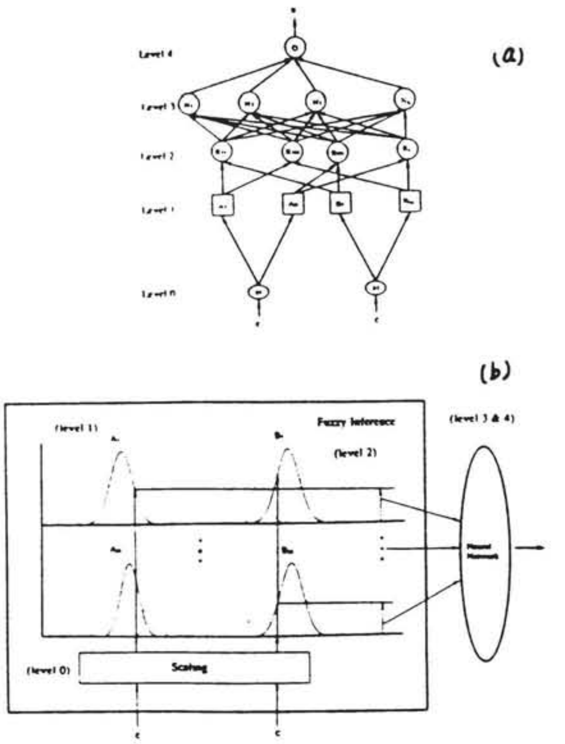

Fig.3 Fuzzy neural network: (a) Architecture of FNN; (b) Schematic illustration of FNN.

$$O_{Ai1}(e) = \exp[-\{(e-e_i)/\sigma_{ai}\}^2], \qquad (1)$$

$$O_{Bk1}(c) = \exp[-\{(c-c_k)/\sigma_{bk}\}^2] \qquad (2)$$

where A_i and B_k are the fuzzy predicates referred to several linguistic labels such as PL (positive large), PS (positive small) etc. The right hand side of the above equations are the membership functions expressed by Gaussian distribution functions. e_i, c_k, σ_{ai}, σ_{bk} are the parameters which determine the location and the shape of the membership function, and are determined by learning with neural network. At layer 1, each node gives the degrees to which the given e and c satisfy the fuzzy predicates A_i and B_k.At layer 2, the following dot("AND") operation is made:

$$O_{m2} = O_{Ai1} \cdot O_{Bk1} \qquad (3)$$

The layers 3 and 4 represent the neural network. It should be noted that the layers 3 and 4 are referred to as the consequent part in the fuzzy inference system, where defuzzified decision is expressed by the set of weights. Therefore, logic inference can be adjusted through learning the appropriate data by mapping the output signal to the input signal using the error back propagation algorithm. Note that the parameters of the membership functions are determined by learning the experimental data with this FNN. Figure 4 shows the membership functions so obtained, and Fig.5 shows the experimental result for the recombinant *E.coli* cultivation under FNN control, which indicates the highest productivity among the experiments we conducted so far.

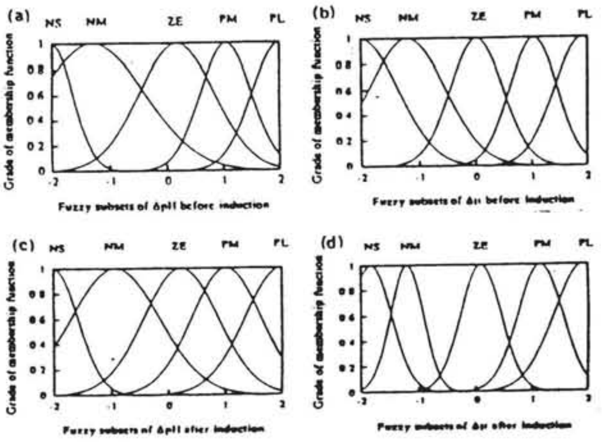

Fig.4 The membership functions before and after induction for FNN.

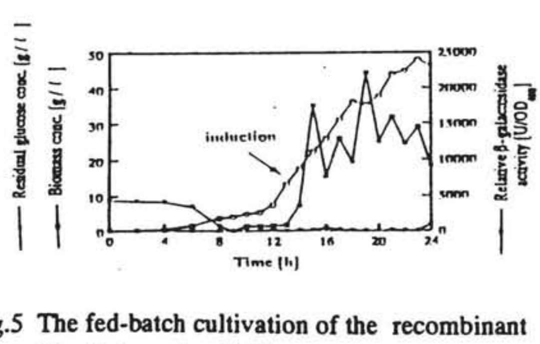

Fig.5 The fed-batch cultivation of the recombinant *E.coli* by using FNN.

8.GENETIC ALGORITHM

The genetic algorithm(GA) is a probabilistic search proceduce based on the mechanics of natural selection and natural genetics. Recently, the GA has received considerable attention in various fields since it has the ability of global optimization.

The GA was applied for on-line determination of the culture temperature for ethanol fermentation. The optimization was made to maximize the final ethanol concentration with respect to culture temperature , and cell concentrations were measured on-line. Two computers were employed, one for the monitoring, control and data processing and the other for the calculation of GA (See Fig.6).

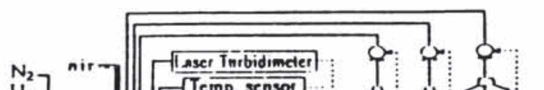

Fig.6 Configuration of experimental system.

It was shown that about 14 % of increase in the productivity could be attained by this method as compared with the coventional constant temperature policy (See Fig.7).

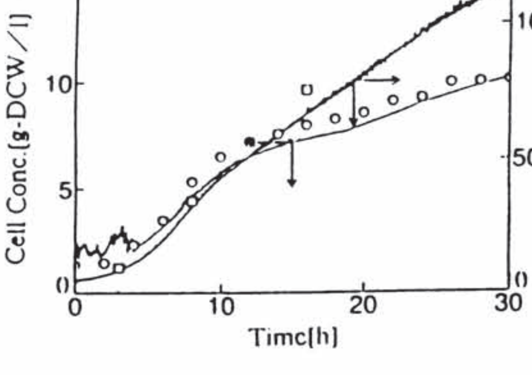

Fig.7 Experimental result using modified GA.

9. DISCUSSION AND CONCLUSION

In the present paper, several approaches were described for the development of intelligent bioreactor systems. Although significant improvements have been made for expert systems and fuzzy control in AI field, the neural networks have been paid recent attention because of their ability of knowledge aquisition by learning.We considered several types of neuro-fuzzy architectures which retain both advantages of neural network and fuzzy control to overcome the difficulties associated with bioprocess control. Other types of neuro-fuzzy control strategies have also been developed recently by other researchers(Hayashi and Umano,1993). Genetic algorithm is also quite promising for optimization of bioprocesses, and AL(artificial life) may have some potential in the near furture.

Recent development in on-line measurement techniques covers various items such as on-line aseptic sampling, on-line continuous flow analysis, on-line flow injection analysis, on-line HPLC (Ohara *et al*, 1993), and on-line MS (Chauvtcharin and Yoshida, 1994). Inexpemsive MS is becoming popular for monitoring exhaust gas composition of cultivations. Recently, dissolved gas and volatile components have also been analyzed on-line (Heinzle and Dunn, 1991). Fully automated analyzer systems allow the real-time elemental balancingand evaluating nonmeasurable process variables (Bellgardt *et al*, 1986). The on-line measurement of the concentrations of all precursers and key medium components in bioreactors allowed the investigation of the in vivo biosynthesis of secondary metabolites during their production as a function of the medium composition(Holzhauer *et al.*,1990) as well as the evaluation of dynamic relationship between cell regulation and reactor control. These are prerequisites for the development of a realistic dynamic model for advanced process control. NADH and NADPH in the viable cells can be excited by UV light, and the induced fluorescence can be measured by suitable detectors. This NAD(P)H fluorescence has a large potential as a tool to monitor, study and control cultivations.

It should be noted that the important thing in considering the control problem is not simply to apply sophisticated control theory but to consider it with deep understanding of the dynamics of the physiological state changes. It is, therefore, quite important to investigate how the physiological state changes in relation to genetic change and environmental change, and the so-called "metabolic engineering" seems to be quite promising(Stephanopoulos, 1991; Bailay, 1991).

REFERENCES

Asama.H., T.Nagamune, I.Endo, M.Hirata, A.Hirata (1991) *Kagaku Kougaku Ronbunshu* **17**,579.

Alfafara.C.G., K.Miura, H.Shimizu,S.Shioya, K.Suga, K.Suzuki (1993) *Biotechnol. Bioeng.* **41**,493.

Bailey.J.E.(1991) *Science*, **252**. 1668.

Bellgardt.K.H.,W.Kuhlmann,H.D.Meyer,K.Schugerl, M.Thoma (1986) *IEE Proc.* **133**,226.

Chauvtcharin.S., T.Yoshida (1994) *Chemical Engineering* (Japanese), **39**,27.

Chen.Q, S.Q.Wang, J.C.Wang(1988) *Proc. of the 4th Int. Conf. on Comp. Appl. in Ferm. Tech., Cambridge*, 253.

Endo.I., H.Asama, T.Nagamune, (1989)A Data-base System and an Expert system for Realizing Factor Automation in the Bioindustries. in *Bioproducts and Bioprocesses* (Fiechter *et al.* eds.) (Springer-Verlag,)

Fu.C.S, S.Q.Wang, J.C.Wang,(1988) *Proc. of the 4th Int. Conf. on Comp. Appl. in Ferm. Tech.*, Cambridge, 411.

Hara.J., M.Yuta, K.Takatsuna, Y.Tsuchiya,K.Suenari, J.Koizumi, S.Nagai,(1993)*Hakkoukogaku* **71**.15.

Hayashi.I, M.Umano (1993) *J.Fuzzy Theory and Systems* (Japanese), **5**,4.

Heinzle.E., I.J.Dunn (1991) Methods and Instruments in Fermentation Gas Analysis In : Rehm H J and Reed G(eds.) *Biotechnology* (VCH. Weinheim) 27-74.

HolzhauerRieger.K.,Zhouw,K.Schugerl (1990) *J.of Chromatography*,**499**,609.

Horiuchi.J., M.Kamasawa, H.Miyakawa,M.Kishimoto, H.Momose(1993) *J.Ferment. Bioeng.*, **76**,207.

Iijima.S.(1991) *Hakkokogaku* **69**,507.

Jin.S. ,K.Ye, K.Shimizu(1994) *J.Chem.Tech.Biotech.* **61**,273-281.

Jitufuthi.T, H.Ishikawa, H.Tanaka, K.Matsushima (1992) *J. Ferment.Bioeng.* **74**,312.

Karim.M.N. , S.L.Rivera (1992) *Adv.Biochem.Eng./ Biotech.* **46**, 1.

Kishmoto.M.,Y.Kitta,S.Takeuchi,M.Nakajima, T.Yosida (1991) *J.Ferm. Tech.* **72**,110.

Koizumi.J., Y.Tsuchiya,K.Suenari,Y.Teshiba, S.Nagai (1990) *Proc.of APBio ChEC'90*, Apr.22-25, Kyongju 284.

Konstantinov. K.B. and T.Yoshida (1989) *Biotech. Bioeng.* **33**,1145.

Konstantinov. K.B. and T.Yoshida (1990) *J.Ferment. Bioeng.* **70**,48.

Konstantinov.K.B. and T.Yoshida (1992) *Biotech. Bioeng.* **39**,479.

Linko.P.(1988) *Ann. N.Y. Acad. Sci.* **542**, 83.

Linko.P., Y.Zhu (1991) *J.Biotechnol.* **21**,253.

Matsuura. K., M. Hirotsune, F. Nakada, M.Hamachi, (1991) *Hakkokougaku*, **69**,455.

Mizutan.S., S.Iijima, M.Morikawa, K.Shimizu, M. Matsubara, Y. Ogawa, R. Izumi, K. Matsumoto, T. Kobayashi (1987) *J.Ferment.Technol.* **65** ,325.

Nakamura.T., T.Kuratani, Y.Morita (1985) *Proc. of IFAC Modeling and Control of Biotechnological Procecess* (Noordwijkerhout,) 231.

Ohara.H., T.Hiraga, I.Katasho, T.Inuta , T.Yoshida (1993)*J.Ferment.Bioeng.*, **75**,470.

Oishi.K., M.Tominaga, A.Kawato, Y. Abe, S.Imayasu, A.Nanba (1991) *J. Ferm.Tech.* **72**,115.

Park.Y.S., Z.Shi, S.Shiba, C.Chantal, S.Iijima, T. Kobayashi (1993) *Appl. Microbial. Biotechnol.* **38**,649.

Rumelhart.D.E.,J.L.McClelland,PDP Research Group (1986) *Parallel Distributed Processing* (MIT Press, London).

Schugerl. K. (1991) *Biotechnology*, eds. H.J.Rehm and Reed(VCH, Weinheim,) 5.

Shi.Z, and K.Shimizu (1992) *J. Ferm. Bioeng.* **74**,39.

Shi.Z, and K.Shimizu (1993) *Kagakukogaku Ronbunshu* **19**,692.

Shimizu.H.,K.Miura,S.Shioya,K.Suga(1994) *Proc.of PSE'94*, Kyongju, Korea .

Shimizu.K.(1993)*Adv.Biochem. Eng./Biotech.* **50**,65.

Shimizu.K.(ed.) (1994) *Bioprocess Systems Engineering* (IPC, Tokyo,) .

Siimes.T., M.Nakajima, H.Yada, H.Asama, T.Nagamune, P.Linko, I.Endo, (1992) *Appl. Microbial. Biotechnol.* **37**,756.

Stephanopoulos.G., G.Stephanopoulos(1990) *Trends Biotechnol.* **4**,241.

Stephanopoulos.G.(1991) *Science*, 252,1675.

Thibault.J. (1990), V.V. Breusegem, A. Cheruy *Biotech.Bioeng.* **36**,1041.

Yamada.Y., K.Haneda, S.Murayama,S.Shiomi (1991) *J. Chem. Eng. Japn.*, **24**,94.

Yamada.H.,T.Suzuki,K.Shimizu (1993)*Process Cont. and Qual.* **5**, 29.

Yano.T., T.Endo, T.Tsuji, Y.Nishizawa(1991) *J.Ferment. Bioeng.* **71**,35.

Ye.K., K.Fujioka, K.Shimizu,(1994) *Process Cont. and Qual.* **5**, 245.

Ye.K, S.Jin, K.Shimizu (1994) *J. Ferment.Bioeng.* **77**,663-673.