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Transient Behavior of Ethanol Fermentation in Immobilized Cell Bioreactors*1

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Abstract

The dynamic behavior of ethanol fermentation catalysed by an immobilized cell has been studied in batch and continuous stirred tank bioreactors, changing the operating conditions in a stepwise fashion. The rate of ethanol fermentation in the flow reactor reaches a new steady state within 60 min for the stepwise change in temperature or flow rate at 15~30°C and the residence time t_R=40 hr. The rate of fermentation obeys the Lineweaven-Burk plot and the Michaelis constant is calculated.

1. Introduction

In the immobilized enzyme or microbial reactor system, a large number of reactor types has been used such as a backmix reactor, fixed bed, fluidized bed, membrane and suspension bubble tower reactor[1~4]. For these reactors, the optimum operating conditions should be chosen to maximize the amount of products while minimizing the undesired products and without bacteria infection. The heterogeneous catalytic reaction using solid catalysts and immobilized biocatalysts have operated under steady state conditions with a constant flow rate, temperature, pressure, and reactant composition, because of ease of operating the system and analying the reaction data. In the heterogeneously catalysed chemical reaction system, the unsteady state operating system has increasingly been employed, such as the forced cyclic operation of feed reactants, reactor temperature or feed flow rate in a flow reactor[5~13]. Because those cyclic operations sometimes enhance the product yields through averaged operating time more than the fixed operating conditions. Huanz and Chen[14] have reported the enhancement of ethanol production under the temperature cycling system compared with the fixed temperature system. Very recently, Wu and Weng[16] have applied the transient response method to elucidate the mechanism of reaction

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over immobilized enzymes. The transient response method was first developed to analyse the reaction mechanism in gas-solid heterogeneous catalysis by Kobayashi and Kobayashi[17~19] and Bennett[20].

In the present study, our interests are focused on the forced cyclic operation of temperature, reactant concentration and flow rates which will influence the average yield of products, using alcohol fermentation with a typical bioreactor.

2. Experimental Method

(1) Immobilization Procedure of Yeast

Fig. 1 illustrates the schematic immobilization procedure. Saccharomyces cerevisiae 2HY-1 (DC-2) which was kindly supplied by Dr. Shimobayashi of the Industrial Institute of Hokkaido was used as a biocatalyst. The yeast cells were precultured in a culture medium containing 10.0% glucose, 3.0% yeast extract, 5.0% pepton and 3.0% malt extract for 24 h at 30°C. The precultured suspension was mixed into a sodium alginate solution (1.5 wt%) so as to become 10^6 cells/cm^3-gel. The mixture was then added dropwise to a gently stirred calcium chloride solution of 0.09 M by a syringe. The detailed composition of the culture medium is presented in Table. 1.

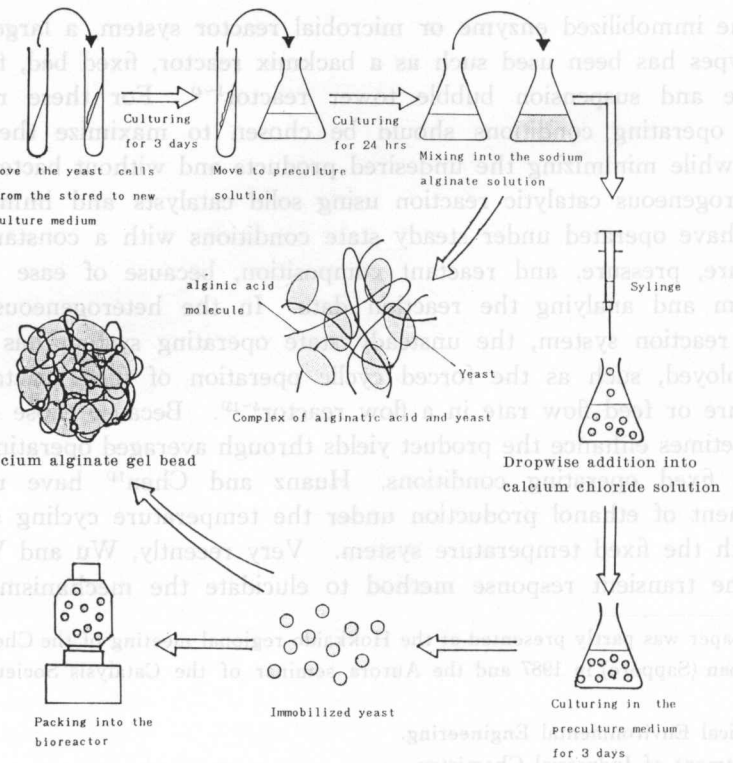

Fig. 1. Schematic drawing of the immobilization procedure of yeasts using calcium alginate gel.

Table 1. Composition of the used media

Medium components	Precultured solution (g/l)	reactant solution (g/l)
Glucose	10.0	200
Yeast extract	3.0	1.5
Malt extract	3.0	—
Pepton	5.0	—
NH_4Cl	—	2.5
KH_2PO_4	—	5.5
$MgSO_4 \cdot 7H_2O$	—	0.25
NaCl	—	1.0
$CaCl_2$	—	0.01
Citric acid	—	3.0
Top water	1000	1000

(2) Analytical Methods

The viable cell number was calculated by colony counts using the dilute, spread and plate technique in a malt extract medium. 20 of the gel beads were dissolved in a flask containing 100 cm^3 of citrate buffer of PH7 or a NaCl solution, by shaking the flask at 40°C for 60 min. The cell suspension was appropriately diluted 10^{-6} times, and 1 cm^3 or 0.1 cm^3 of the diluted cell suspension was plated in the agar medium. The plates were then incubated at 30°C for 48 hr after which the number of colonies was counted.

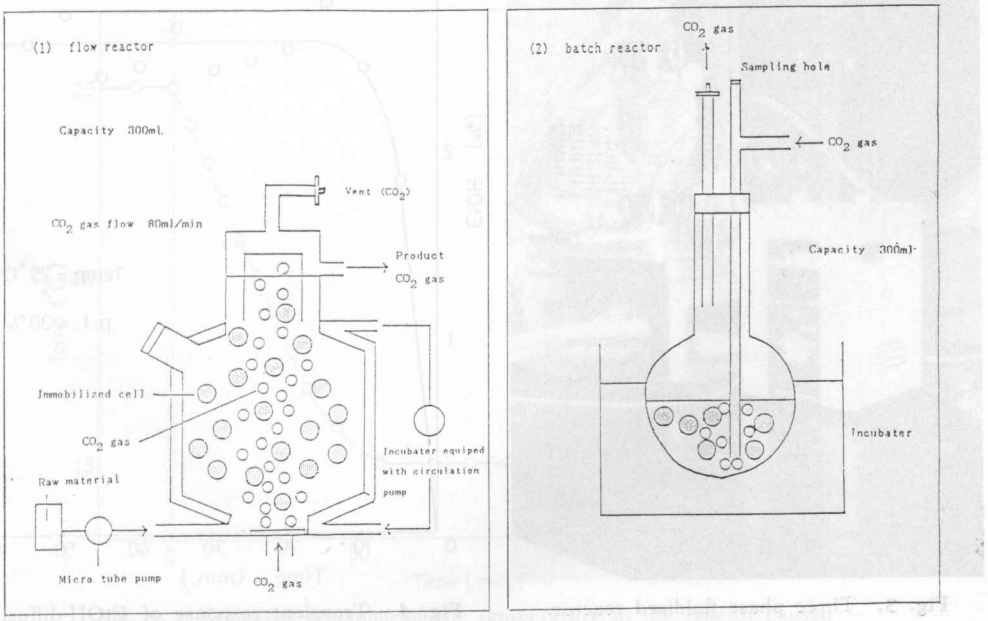

Fig. 2. Batch and continuous ethanol fermentation reactor.

The ethanol concentration was analysed by using a gas chromatograph equipped with a FID. The sugar concentration was determined by both the conventional Modified Somogyi Method and a liquid chromatograph equipped with a TSK-GEL SCX column with a flowing Perchloric acid buffer solution at 40°C.

(3) Experimental Set-Up

Figs. 2 and 3 illustrate a schematic diagram of the reaction systems, batch and continuous reactors and a photograph of the continuous reactor, respectively. A 500 cm^3 flask was used for the batch reactor, and a 300 cm^3 fluidized type fermentor (15 cm ID × 20 cm in height) equipped with a draft line was used as a flow reactor. The flow reactor used was a three phase (gel beads, substrate solution and CO_2-gas) fluidized type as has been reviewed by Schuzerl[15]. 70 of the gel beads were packed into the reactors in which the packing factor (p. f) was 30% throughout the experiments except for specified cases. Low agitation by the CO_2-gas flow (about 30 cm^3/min) was provided for the gel beads in the reactor.

3. Experimental Results and Discussion

3-1. Evaluation of the System Parameters

A large number of papers concerning the application of fluidization principles

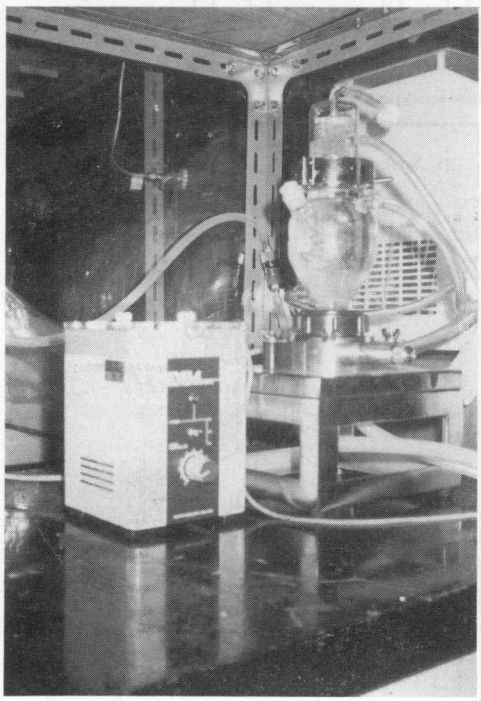

Fig. 3. Three phase fluidized reactor.

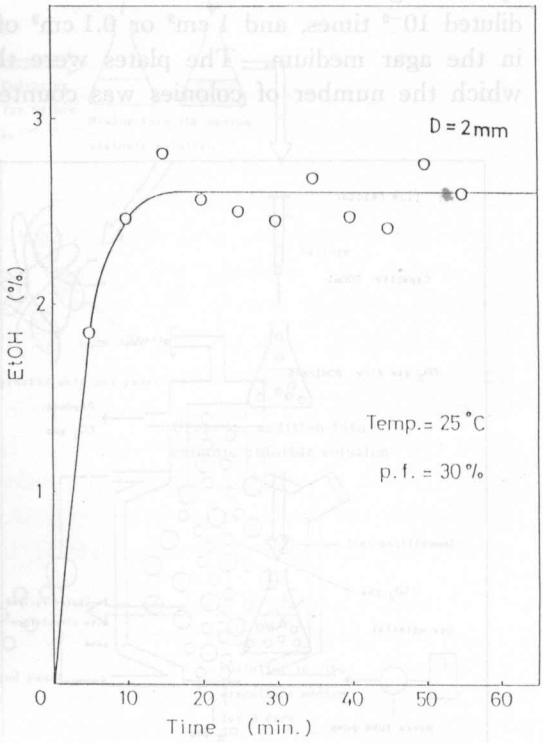

Fig. 4. Transient response of EtOH-diffusion from the calcium alginate gel.

in biotechnology has been published in the last ten years. The main application of these fluidization studies is in the field of environmental biotechnology. Several researchers have studied fluidized-bed reactors consisting of solid particles with immobilized enzymes, even though no commercial application is known. A batch-scale fluidized bed reactor was described by Scott[21] with which he obtained ethanol production utilizing flocculating zymomonas mobilis with biomass recycling. A typical reactor used is a three phase reactor which is also used in the present experiment. To operate this reactor, one should pay special attention to the effectiveness factors of bed porosity, liquid phase mass transfer and solid mixing.

Fig. 4 shows the transient response of ethanol in a three phase batch reactor at 25°C and p. f.=30%. The calcium alginate gel beads (2 mm in diameter) were immersed in 10% ethanol solution for 5 hr, and then the beads were taken out and washed for a few seconds and put into 100 cm^3 of water which was stirred by a magnetic stirrer. The change in the concentration of ethanol was followed and the response curve obtained reached an equilibrium level of 2.5% EtOH within 10 min as can be seen from the curve. Since the transient response of actual fermentation reaction needs more than 180 min, no serious influence of the diffusion of alcohol formed in the gel beads may be considered to be present through any the transient response data.

Fig. 5 illustrates the transient response of the reactor temperature caused by the step change in the temperature of heating water. The temperature of the water was changed within five minutes by using an excellent temperature controller. The reactor temperature reached a new temperature level within 60 min, whereas the actual response of reaction caused by the stepwise change

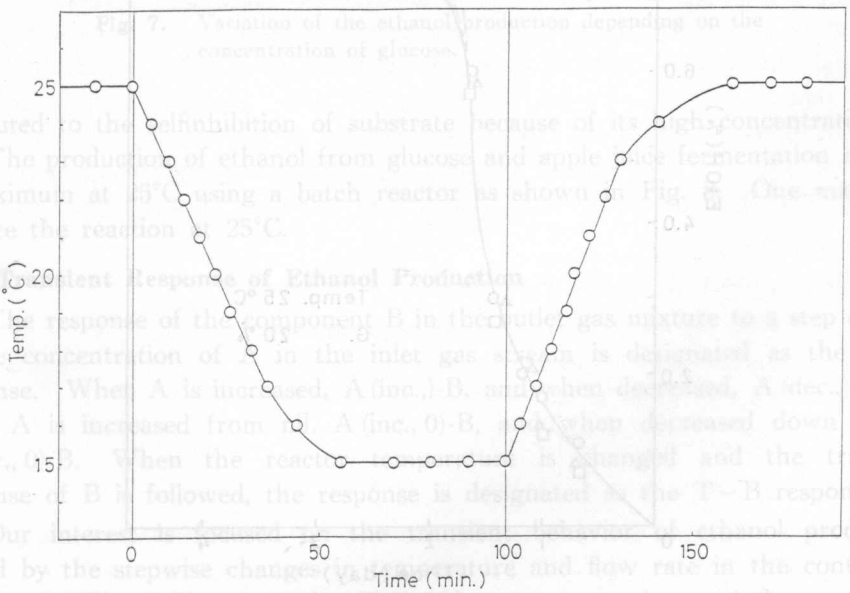

Fig. 5. Transient response of the temperature in the reactor caused by the temperature jump.

in temperature needs more than 1800 min. One may thus presume that the delay of the response in this system does not influence the reaction response seriously.

3-2. Sensitivity of Operating Conditions

The reactor efficiency is sensitively influenced by the operating conditions used such as the packing factor (p. f.), concentration of substrate, reaction temperature, flow rate etc.

Fig. 6. illustrates the effect of p. f. on the reactor efficiency (batch reactor) at 25°C. The higher the p. f., the higher the efficiency, therefore 30% of p. f. is used throughout the experiment. In addition, the time to reach the maximum reaction rate for p. f.=30% is within two days, which is shorter than the other two. Fig. 7 shows the ethanol production depending on the concentration of glucose in the reactor. The production of ethanol increases with increased concentrations of glucose, and the maximum is reached at around 20% of glucose since 30% of glucose shows a decrease in ethanol production. This may be

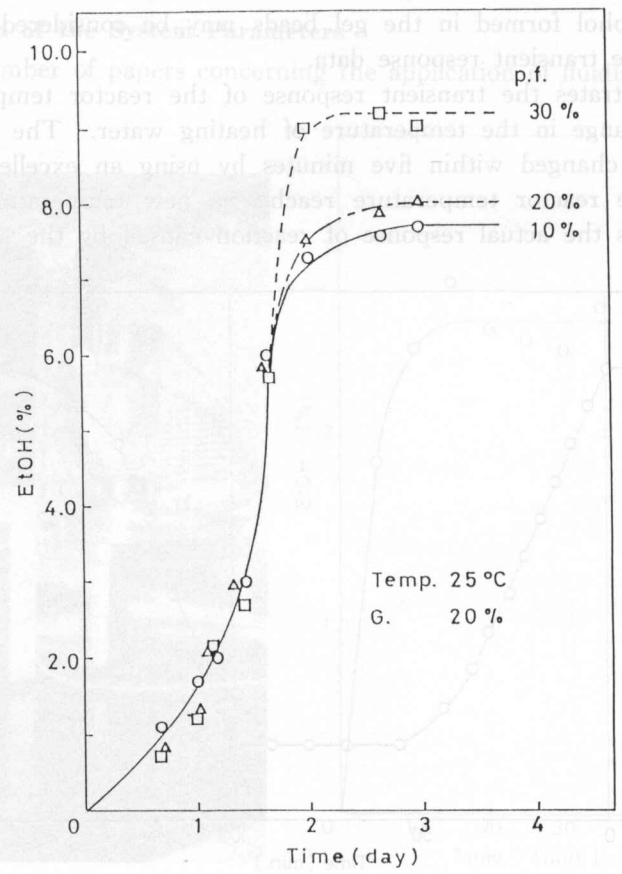

Fig. 6. Variation of the reactor efficiency to form alcohol by three packing factors.

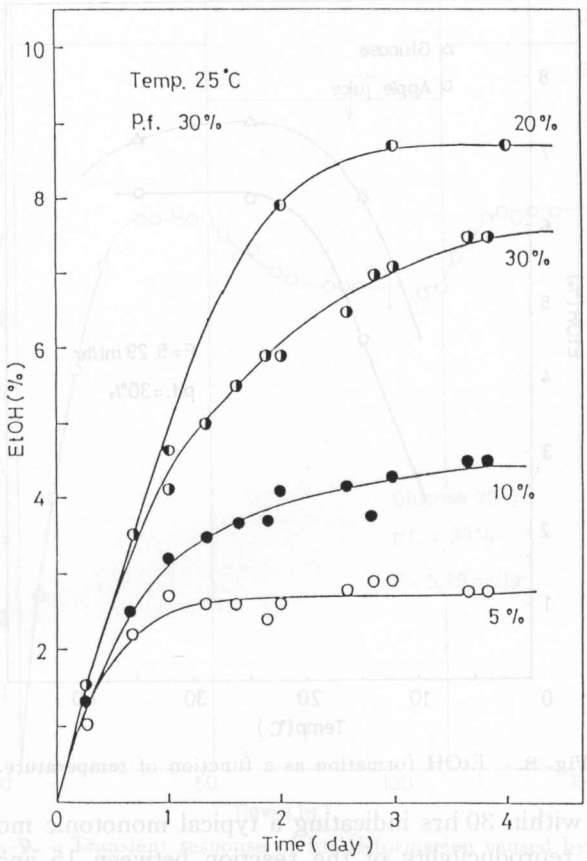

Fig. 7. Variation of the ethanol production depending on the concentration of glucose.

attributed to the selfinhibition of substrate because of its high concentration.

The production of ethanol from glucose and apple juice fermentation reaches a maximum at 25°C using a batch reactor as shown in Fig. 8. One may thus operate the reaction at 25°C.

3-3. Transient Response of Ethanol Production

The response of the component B in the outlet gas mixture to a step change in the concentration of A in the inlet gas stream is designated as the A−B response. When A is increased, A (inc.,)-B, and when decreased, A (dec.,)-B and when A is increased from nil. A (inc., 0)-B, and when decreased down to nil, A (dec., 0)-B. When the reactor temperature is changed and the transient response of B is followed, the response is designated as the T−B response.

Our interest is focused on the transient behavior of ethanol production caused by the stepwise changes in temperature and flow rate in the continuous bioreactor. Fig. 9 illustrates the T-EtOH response at the total flow rate $F=5.29\times10^{-3}$ 1/hr (glucose 20% and p. f.=30%). The ethanol (EtOH) reached a

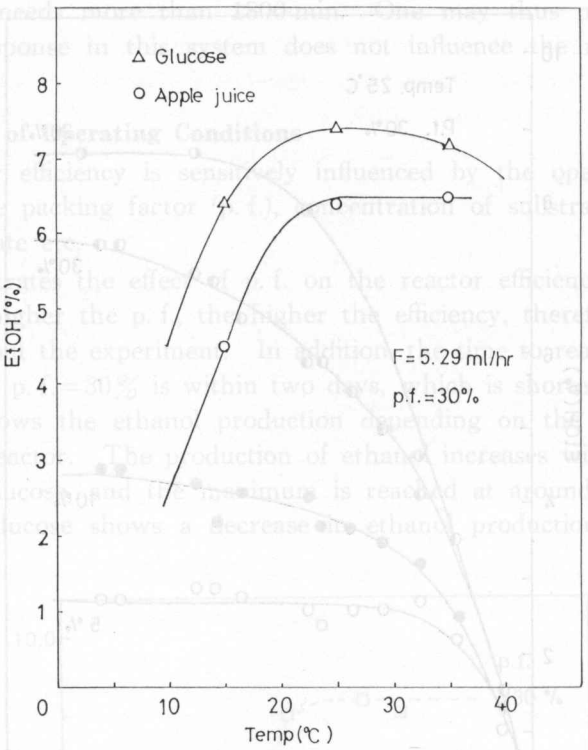

Fig. 8. EtOH formation as a function of temperature.

new steady state within 30 hrs indicating a typical monotonic mode, and one may recognize a good reproducibility of the reaction between 15 and 25°C.

Fig. 10 illustrates the v_0-EtOH response at 25°C, $C_G=20\%$ and p. f.$=30\%$. The total flow rate is changed stepwisely such as $F=12$ (Run 1)$\rightarrow F=0$ (Run 2) (Batch reactor system)$\rightarrow F$ (Run 3)$=5.54\times10^{-3}$ 1/hr. In all runs, the transient state was observed for more than 30 hrs similar to the case of the temperature jump experiments. The reactor efficiency of the batch reactor completely agrees with the flow reactor at the total flow rate of less than 60×10^{-2} l/day as shown in Fig. 11.

3-4. Kinetic Analysis in the Flow Reactor

The activity of biocatalysts was determined by measuring the alcohol production rate. Two moles of carbon dioxide and two moles of ethanol were produced from one mole of glucose.

$$\underset{\text{glucose (G)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{alcohol (EtOH)}}{2C_2H_5OH} + 2CO_2 \qquad (1)$$

Using the immobilized enzyme, the reaction may be rewritten as follows.

$$G + E \underset{k_1'}{\overset{k_1}{\rightleftarrows}} E\cdot G \xrightarrow{k_2} E + 2EtOH + 2CO_2 \qquad (2)$$

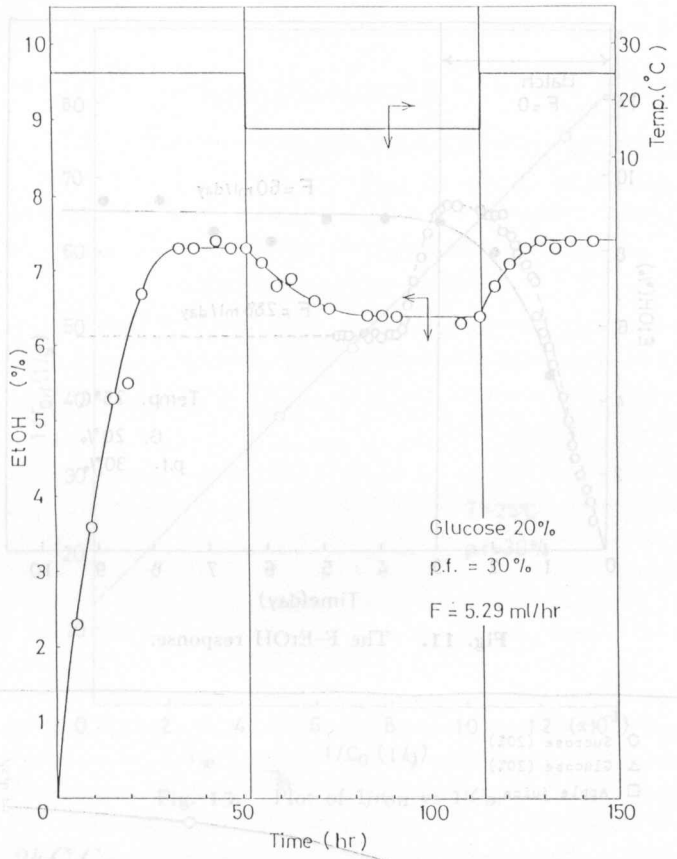

Fig. 9. Transient response of EtOH-formarion caused by the temperature jump.

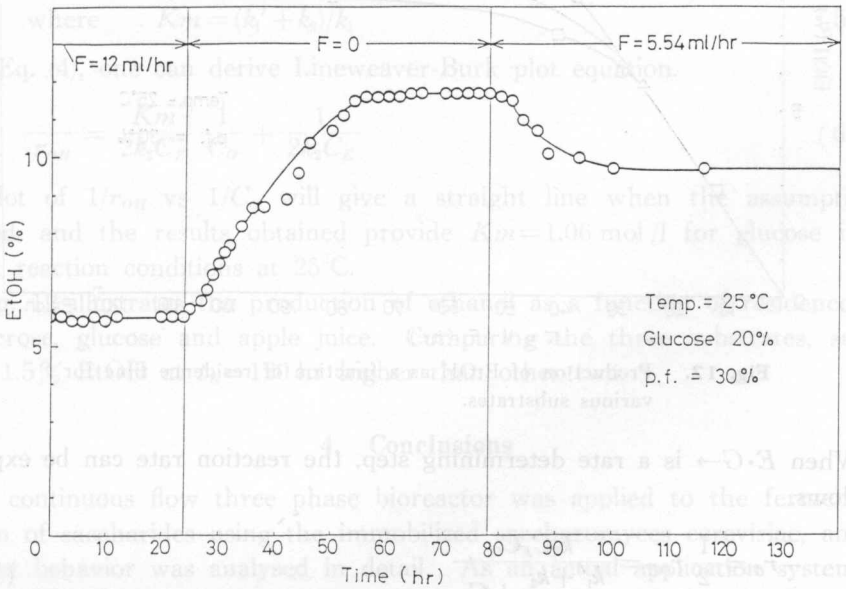

Fig. 10. Transient response of EtOH-formation caused by the flow rate jump.

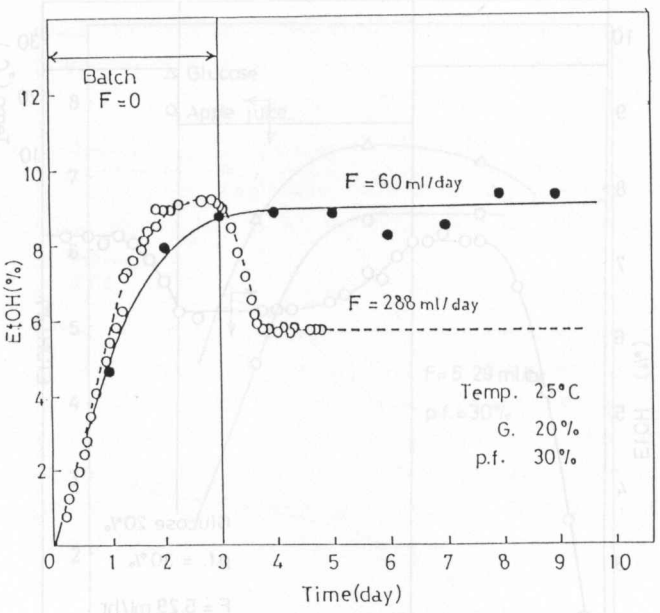

Fig. 11. The F-EtOH response.

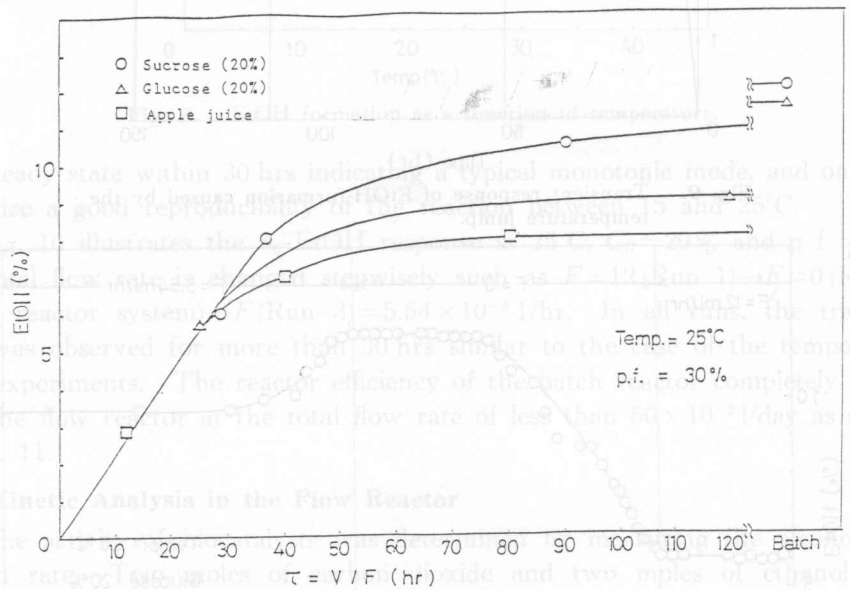

Fig. 12. Production of EtOH as a function of residence time for various substrates.

When $E \cdot G \rightarrow$ is a rate determining step, the reaction rate can be expressed as follows.

$$-r_G = \frac{1}{2} r_{OH} = \frac{k_2 C_E C_G}{\dfrac{k_1' + k_2}{k_1} + C_G} \qquad (3)$$

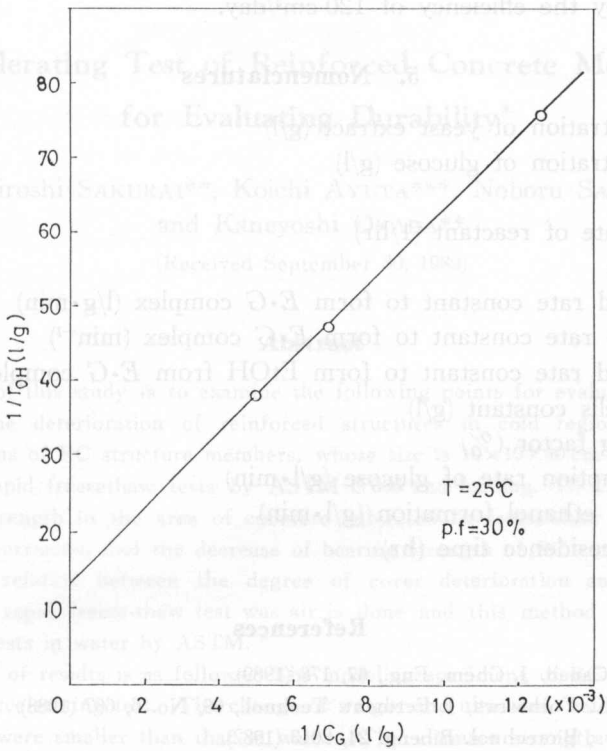

Fig. 13. Plot of $1/r_{OH}$ vs $1/C_G$.

$$r_{OH}=\frac{2k_2C_EC_G}{Km+c_G} \tag{4}$$

where $$Km=(k_1'+k_2)/k_1 \tag{5}$$

From Eq. (4), one can derive Lineweaver-Burk plot equation.

$$\frac{1}{r_{OH}}=\frac{Km}{2k_2C_E}\frac{1}{C_G}+\frac{1}{2k_2C_E} \tag{6}$$

The plot of $1/r_{OH}$ vs $1/C_G$ will give a straight line when the assumption is accepted, and the results obtained provide $Km=1.06$ mol/l for glucose in the present reaction conditions at 25°C.

Fig. 12 illustrates the production of ethanol as a function of residence time for sucrose, glucose and apple juice. Comparing the three substrates, sucrose gives 11.5% EtOH at $t_R=120$ hr higher than other two.

4. Conclusions

A continuous flow three phase bioreactor was applied to the fermentation reaction of saccharides using the immobilized saccharomyces cerevisiae, and the transient behavior was analysed in detail. As an actual application system, the continuous production of apple wine was carried out at 25°C and a good wine

was produced by the efficiency of 120 cm^3/day.

5. Nomenclatures

C_E: concentration of yeast extract (g/l)
C_G: concentration of glucose (g/l)
E: yeast
F: flow rate of reactant (1/hr)
G: glucose
k_1: forward rate constant to form $E \cdot G$ complex (l/g·min)
k_1': reverse rate constant to form $E \cdot G$ complex (min^{-1})
k_2: forward rate constant to form EtOH from $E \cdot G$ complex (min^{-1})
Km: Michaelis constant (g/l)
p. f.: packing factor (%)
$-r_G$: consumption rate of glucose (g/l·min)
r_{OH}: rate of ethanol formation (g/l·min)
t_R: mean residence time (hr)

References

1) K. Schugerl, Canad. J. Chem. Eng., **67**, 178 (1989).
2) K. Sato and K. Yoshizawa, J. Ferment. Technol., **66**, No. 6, 667 (1988).
3) G. F. Andrews, Biotechnol. Bioeng. **24**, 2013 (1982).
4) G. F. Andrews and Przezdziecki, Biotechnol. Bioeng., **28**, 802.
5) Y. E. Li, D. Willcox and R. D. Gonzalez, AIChE Journal, **35**, No. 3, 423 (1989).
6) K. Rigopoulos, X. Shu and A. Cinar, AIChE Journal, **34**, No. 12, 2041 (1988).
7) G. Varrciyen, A. Annapragada and E. Gulari, Chem. Sci., **43**, No. 11, 2957 (1988).
8) S. Sivakumar, M. Chidambaram and H. S. Shankar, Canad. J. Chem. Eng., **65**, 767 (1987).
9) A. A. Adesina, R. R. Hudgins, P. I., Silveston, Canad. J. Chem. Eng., **64**, 447 (1986).
10) X. Zhou and E. Gulari, Chem. Eng. Sci., **41**, 883 (1986).
11) B. K. Cho and L. A. West, IEC. Fund., **25**, 158 (1986).
12) S. C. Capsaskis and C. N. Kenney, J. Phys. Chem., **90**, 4631 (1986).
13) L. Chiao and R. G. Binken, Chem. Eng. Sci., **44**, 9 (1989).
14) S. Y. Huang and J. C. Chan, J. Ferment. Technol., **66**, No. 5, 509 (1988).
15) K. Schugerl, Canad. J. Chem. Eng., **67**, 178 (1989).
16) J. Y. Wu and H. S. Weng, Biotechnol. Bioeng., **33**, 415 (1989).
17) H. Kobayashi and M. Kobayashi, Catal. Rev. Sci. Eng., **10**, 139 (1974).
18) M. Kobayashi, Chem. Eng. Sci. **37**, 393 (1982).
19) M. Kobayashi, Chem. Eng. Sci, **37**, 403 (1982).
20) C. O. Bennett, Catal. Rev. Sci. Eng., **13**, 121 (1976).
21) Ch. D. Scott, Fifth Symposium on Biotechnology for Fuels and Chemicals" Biotecnol. Bioeng. Symp. No. 13, Ch. D. Scott, Ed., John Wiley & Sons, New York (1983), pp. 287.