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콤퓨터 제어에 의한 빵 酵母의 流加培養

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Computer-aided Fed-bath Culture of Baker's Yeast

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요 약

PDP 11—03 마이크로 콤퓨터를 발효 시스템에 성공적으로 연결하고 개스 분석기를 주된 측정 기기로 사용하여 효모 *Sacchararomyces cerevisiae* 의 배양을 수행하였다. 필요한 방정식과 콤퓨터 프로그램을 개발하여 시스템 운전에 사용하였다.
최적 휘드 백 정책을 콤퓨터 프로그램하여 콤퓨터에 기억시킨 후 측정치를 사용하여 적정한 값의 제어시그날을 발생시키도록 하였다.
간접적으로 얻은 균체농도는 직접 측정치를 상당히 정확하게 예측하였다. 온 타인 휘드 백 제어를 수행한 결과 얻어진 호흡지수는 아주 흡족한 상태를 보였으며 전반적인 세포 생산 수율은 회분 배양과 비교했을 때 상당히 증가되었다.

ABSTRACT

A PDP 11—03 microcomputer was successfully interfaced to the fermentation system and yeast *Saccharomyces cerevisiae* cultivation with this computerized system was carried out using the gas analyzers as the primary measuring elements. The necessary equations and computer programs were developed and used in the system operation. The optimal feed-back control policy computerized and stored in the system utilizes these monitored variables in order to generate the output control signals at proper level. The indirectly determined cell-concentration has predicted the direct measurement rather accurately. The profile of the respiratory quotient obtained upon exercising the on-line fee-back control

has shown a very smooth and desirable behaviour and the ob erall cell yield has been improved quite significantly as compared with the batch system without control.

Ⅰ. Introduction

The fed-bathch fermentation is a method by which a specific limiting substrate is fed into the fermentor during fermentation and the cell and/or the metabolite as product is harvested only after the fermentation is finished. Lim et al.[1] reported on the results of a fed-batch culture using a programmed feed-rate of constant value or exponentially increasing one. Aiba et al.[2] demonstrated the controllability of bakers' yeast culture using respiratory quotient as directly controlled variable. He could exercise automatic feed-rate manipulation to exert an indirect control on glucose concentration via the indication from R.Q. and thus to achieve the maximum cellular yield and productivity.

Cooney et al.[3~5] described the use of an on-line computer-aided material-balancing routine for the continuous assessment of cell growth during bakers' yeast production. The information obtained from this indirect monitoring of cell mass was used to develop an on-line feed-back control strategy for the addition of molasses as carbon source to the fermentation vessel. They carried out the optimal feed-back fed-batch fermentation utilizing R.Q. values, oxygen uptake rate and carbon dioxide evolution rate as the primary measurements. For the cultivation of bakers' yeast it is very imporant to formulate a glucose feeding-schedule for maintaining the glucose concentration in the culture medium at a low but optimal value at all time. According to Cooney et al.[5] for the optimal culture of bakers' yeast the residual glucose concentration was around 0.13g glucose/liter. When glucose concentration in an aerobic culture medium reaches 70mg/liter glucose tends to be partly metabolized to ethanol and CO_2 during fermentation.[6,7]

In this work a PDP 11—03 microcomputer was sucessfully interfaced to the fermentation system producing yeast cell mass, and both the programmed and the feed-back on-line controls were practiced following the general methodology used by previous people.[3~5] Two of the differences from the previous works are: (1) a microcomputer is used for the first time; (2) the indirect assessment of cell mass concentration is based upon the glucose consumption, not the ammonia consumption.

Ⅱ. Materials and Methods

Ⅱ-1 Analytical Methods

The cell concentration was measured by taking the optical density at 600nm (Shimazu, Spectronic 20). The optical density was related to the cell mass concentration with a priori determined calibration curve. The dry cell weight was measured by centrifuging a 20-ml sample at 3000 rpm, suspending the pellet in 20-ml of distilled water, recentrifuging, and drying the pellet in a drying dish overnight at 90°C.

The glucose assay was done using the Somogyi-Nelson method.[8] An enzymatic method via ethanol dehydrogenase was used for

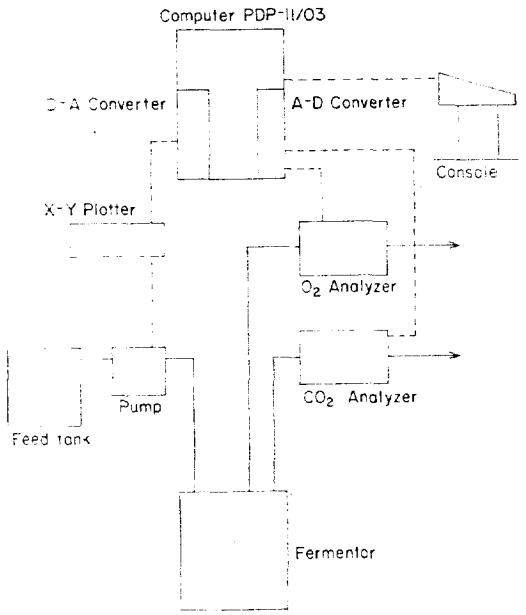

Fig. 1. Schematic diagram of the fermentation system

the determination of ethanol concentration (Sigma).

Ⅱ-2 Equipment

A 10-liter jar-fermentor (Marubish Co. LTD, Tokyo, model MD-500) equipped with a pH controller, a D.O. indicator, a thermostat, an anti foam controller, and an rpm controller was used for fed-batch culuture under aerobic condition. The on-line control and data processing were carried out on the PDP 11-03 microcomputer system (Digital Equipment Corp., Maynard, MA, U.S.A.).

The PDP 11-03 system contains an ADV 11-A analog-to-digital convertor, an AAV 11-A digital-to-analog converter, and a Kwv 11-a programmable real time clock. The core memory size of the computer was 32 k words, a console with typing speed of 30 characters/sec was used as a hard copy terminal, and the programs and the output data were stored on the dual floppy disk (128 K words x 2).

The CO_2 gas analyzer was of IR type and the O_2 gas analyzer was of paramagnetic type. For the coninuous feeding of glucose solution to the fermentor a microtubing pump (Tokyo Rikakikai) was used.

Fig. 1 is a schematic diagram of the interfaced control system composed of the computer, the fermentor, and all the process sensors.

Ⅱ-3. Culture Media and Methods

A commerial strain of bakers' yeast (*Saccharomyces cerevisiae*) was used in the fed-batch culture. The strain was maintained by monthly transfer on Y.M. agar slant and stored at 4°C.

Culture medium used in the glucose fed-batch culture was composed of $(NH_4)_2$ SO_4 10g/l, KH_2PO_4 6g/l, $MgSO_4$ $7H_2O$ 3g/l, $CaCl_2$ $2H_2O$ 0.1 g/l, NaCl 0.1 g/l, and yeast extract (Difco) 0.5 g/l.

The initial medium was sterilized by heating up at 120°C for 25 mins and the glucose solution at 120 g/l was sterilized separatey in a 2-liter bottle. The initial working volume was 5 liter, the temperature of cultivation was kept at 30°C and the impeller was rotated at 600 rpm. During the culture pH was controlled at 5.0 with addition of concentrated ammonia solution using an analog pH controller.

Ⅲ. Theoretical Development

Ⅲ-1. Material Balance on Yeast Growth

The glucose, oxygen, and other nutrients in culture medium are used for producing cell mass, carbon dioxide, intermediate met-

abolite, and water as schematically shown in the following equation.

Glucose+Oxygen+Ammonium⟶Cell mass+Water+Carbon dioxide +Ethanol (1)

If products other than biomass are not formed, we can write a stoichiometric relationship on yeast growth. where a, b, c, d,

$$aC_6H_{12}O_6 + 6O_2 + cNH_3 + \frac{X_0}{M_c}C_5H_{10}NO_3 \longrightarrow \left(d + \frac{X_0}{M_c}\right)C_6H_{10}NO_3 + eH_2O + fCO_2 \quad (2)$$

e, and f are moles of the reactants and the products. The molecular formula of cell mass is $C_6H_{10}NO_3$.[5] The cell growth is autocatalytic and the initial cell mass influences the cell production rate.

If ethanol is the only product without cell growth, the stoichiometric equation can be written as:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad (3)$$

For the general case where both cell growth and ethanol formation occur, Eqs. (2) and (3) can be linearly combined to give

$$aC_6H_{12}O_6 + (1-F')6O_2 + (1-F')cNH_3 + \frac{X_0}{M_c}C_6H_{10}NO_3 \longrightarrow \left[\frac{X_0}{M_c} + (1-F')d\right]C_6H_{10}NO_3 + (1-F')eH_2O + [(1-F')f + 2aF']CO_2 + 2aF'C_2H_5OH \quad (4)$$

where F' is the fraction of total glucose consumption diverted to the ethanol production.

From this stoichiometric relationship, one can set up the material balance equations on carbon, hydrogen, oxygen, and nitrogen. Equation for carbon:

$$6a = 6(1-F')d + [(1-F')f + 2aF'] + 4aF' \quad (5)$$

Equation for hydrogen:

$$12a + 3(1-F')c = 10(1-F')d + 2(1-F')e + 12aF' \quad (6)$$

Equation for oxygen:

$$6a + 2(1-F')b = 3(1-F')d + (1-F')e + 2[(1-F')f + 2aF'] + 2aF' \quad (7)$$

Equation for nitrogen'

$$(1-F')c = (1-F')d \quad (8)$$

If the inlet air is assumed not to contain carbon dioxide and is also assumed to be saturated with water vapour, one can have the following expressions for the oxygen uptake rate and the carbon dioxide evolution rate.

$$OUR = \frac{F_N}{V}\left(\frac{0.21}{1.0-0.21} - \frac{P_{0(out)}}{1.0-P_{0(out)}-P_{c(out)}}\right) \quad (9)$$

$$CER = \frac{F_N}{V}\left(\frac{P_{0(out)}}{1.0-P_{0(out)}-P_{c(out)}}\right) \quad (10)$$

The oxygen uptake rate (OUR) has the following relationship with cell growth.

$$OUR = \frac{\mu X}{60\,M_c\,Yo_2} \quad (11)$$

From the Eq. (4), the cell yield on oxygen, Yo_2 is

$$Yo_2 = \frac{(1-F')d}{(1-F')b} = \frac{d}{b} \quad (12)$$

From Eqs. (11) and (12),

$$OUR = \frac{\mu}{60}\left[\frac{X_0}{M_c} + (1-F')d\right]\frac{b}{d} \quad (13)$$

The carbon dioxide evolution rate (CER) can be written as:

$$CER = \frac{1}{60\,M_c}\mu X \cdot Y_{CO_2} \quad (14)$$

Eq. (4) can be rewritten to give the expression for the carbon dioxide production per unit cell mass formed, Y_{CO_2}, as:

$$Y_{CO_2} = \frac{(1-F')f + 2aF'}{(1-F')d} \quad (15)$$

Eqs. (14) and (15) are combined to give

$$CER = \frac{\mu}{60}\left\{\frac{X_0}{M_c} + (1-F')d\right\} \frac{(1-F')f + 2aF'}{(1-F)d} \quad (16)$$

The balance on nitrogen when the nitrogen feed is taken as the only fed-batch variable is

$$\frac{dS_N}{dt} = \frac{F_{SN}[S_N(\text{in}) - S_N]}{V} - \frac{\mu X}{Y_N} \cdot 0.82 \quad (17)$$

where S_N is the concentration of nitrogen source in equivalent amount ofrnitrogen and Y_N is the yield coefficient based on nitrogen source as given by gram cells produced per gram of ammonia consumed. Eq. (17) can be utilized in estimating the growth rate based upon the nitrogen source consumption as indicated by the nitrogen feed rate to keep the pH constant.

When we take the stoichimetric coefficient for cell mass in Eq. (4) itself as the cell mass produced per unit reactor volume in time Δt, an estimate for the specific growth rate can be given by:

$$\mu = \frac{(1-F')d \cdot M_c}{[X(t) + d \cdot M_c \cdot (1-F')] \cdot \Delta t} \quad (18)$$

Ⅲ-2. Respiratory Quotient and Ethanol Production

Oxygen starvation (Pasteur effect) and/or high sugar concentration (Crabtree effect) in yeast leads to the production of ethanol and the CO_2 production accompanying this ethanol formation does not need the oxygen consumption via the *TCA* cycle and the electron transport chain, which causes the higher respiratory quotient.

The respiratory quotient (*RQ*), which is the ratio of the carbon dioxide evolution rate (*CER*) to the oxygen uptake rate (*OUR*), can be correlated with the fraction of glucose converted to ethanol using Eq. (4).

$$RQ = \frac{[(1-F')f + 2aF']}{(1-F')b} = \frac{f}{b} + \frac{2aF'}{(1-F')b} \quad (19)$$

The term f/b is the respiratory quotient (RQ_0) for the biomass synthesis when ethanol is not produced during the culture. The maximum cellular yield that can be achie ved in the absence of ethanol production is 0.5g cell/g glucose and the respiratory quotient in this case is found to be 1.04 because most of the carbon dioxide produced comes from the consumption of oxygen in the oxidation of carbon source to yield just enough energy for cellular biosynthesis.

Eq. (19) in another form is

$$EPR = (RQ - RQ_0)(OUR) \quad (20)$$

where *EPR* is the ethanol production rate (m moles/liter-hr). We can use the *RQ* value as a quantitative indicator of ethanol formation.

Ⅲ-3. Indirect Measurement of Cell Mass

The carbon in the glucose consumed appears in cell mass, ethanol and carbon dioxide being produced. By taking the carbon balance the increase in cell mass (ΔX_1) and a preliminary value of cellular yield can be calculated and tested against the oxygen balance. The calculated cell mass (ΔX_2) from oxygen balance can be compared with that from carbon balance (ΔX_1) and, if these valuse are not in agreement, the average of these two valuse are computed and another cycle of iteration is initiated until the convergence within prespecified limits is achieved. Actual cell concentration, growth rate and the cellular yield coefficient

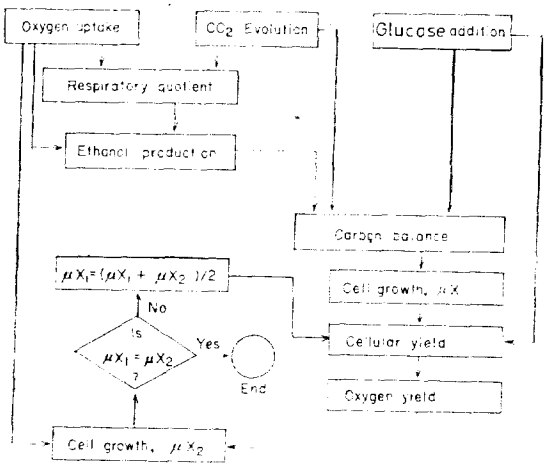

Fig. 2. Flow sheet for computation of cell growth. The three monitored variables are the oxygen uptake rate, the carbon dioxide evolution rate, and the glucose addition rate

can be calculated by using the finally converged value of ΔX. *Figure 2* shows an indirect measurement routine for such a purpose.

Ⅲ-4. Theory of Fed-batch Culture

In a bakers' yeast fed-batch culture, it is very important to formulate a glucose feeding-schedule to maintain the glucose concentration in the fermentor at low but optimal value at all times. Variations in the behavior of the culture are commonly caused by changes in the size and/or condition of the seed inoculum, or by over or underfeeding of glucose. If the glucose feed rate is held at too low level, the cell productivity will be lower than the maximum.[7] Conversely, if the feed rate is too high, glucose will accumulate in the culture medium and lead to the production of ethanol even in the presence of sufficient oxygen (Crabtree effect). By feeding the glucose just enough to match the optimal requirement by the culture as indicated by the culture state monitored the productivity and cellular yield can be improved to a significant degree.

In fed-batch culture, the system equation for the cell mass can be written as follows:

$$\frac{d(VX)}{dt}=\mu(V.X) \tag{21}$$

where VX and μ are, the total yeast cell mass in the fermentor and the specific growth rate (hr^{-1}) of yeast at any given time.

Integrating Eq. (21) from initial time O to a given time t,

$$VX=V_0X_0\ exp\left[\int_0^t \mu dt\right] \tag{22}$$

where X_0V_0 is the amount of the initial yeast cell mass in the culture vessel. In order to maximize the cell mass, specific growth rate will always have to be kept at its maximum possible value at each point of the time interval.

However, maximum value of the specific growth rate does not necessarily mean the best yield because the high glucose concentration required by the maximum specific growth rate will certainly result in the reduced yield due to the Crabtree effect. The maximum glucose concentration that one can maintain without causing the ethanol production through the Crabrtee effect was determined by Cooney[5] to be 0.13 g/l.

The balance equation for the substrate in the fed-bateh culture is

$$\frac{dS}{dt}=\frac{F}{V}(S_F-S)-\frac{\mu X}{Y} \tag{23}$$

where F is the feed rate, S_F the concentration of feeding substrate, S substrate concentration in culture medium, and Y the cellular yield.

If the cencentration of the limiting substrate is very low and slightly fluctuates in the medium during the culture, the derivative term in Eq. (23) can be put equal to

zero and the feed rate required to keep the constant substrate concentration is given as follows.

$$F = \frac{\mu V X}{Y(S_F - S)} \tag{24}$$

$$F = \frac{\mu\, V_0\, X_0}{Y(S_F - S)}\, exp\left[\int_0^t \mu\, dt\right] \tag{25}$$

With the optimal substrate concentration, *So*, kept at constant value for a constant μ_0 and with the constant yield coefficient, Y_0, Eq. (25) becomes

$$F = \frac{\mu_0\, V_0\, X_0}{Y_0(S_F - S_0)}\, exp(\mu_0 t) \tag{26}$$

If the water loss through evaporation, the variation of specific gravity, and the addition of alkali solution are ignored, we can account for the change in volume by

$$\frac{dV}{dt} = F \tag{27}$$

From Eqs. (26) and (27), the volume of culture medium is:

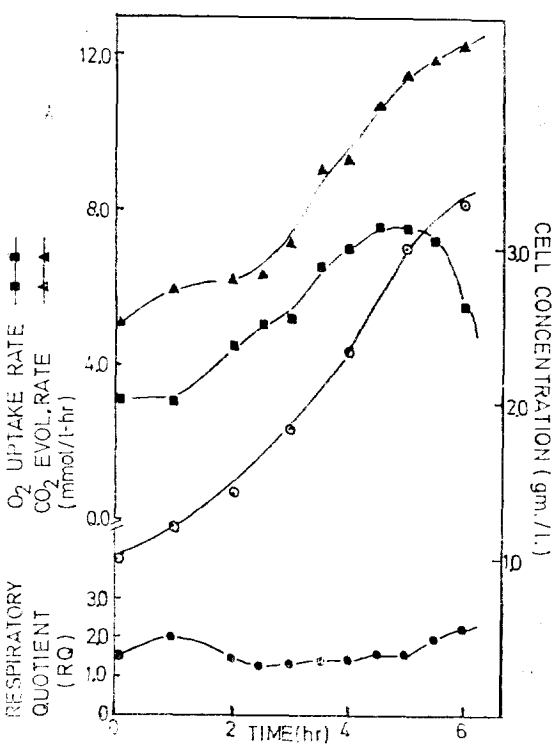

Fig. 3. Result of the programmed control.

$$V = V_0 + \frac{V_0\, X_0}{Y_0(S_F - S_0)}[exp(\mu_0 t) - 1] \tag{28}$$

From Eq. (22), the cell concentration is

$$X = \frac{V_0\, X_0\, exp(\mu_0 t)}{V} \tag{29}$$

From Eqs. (26) and (28)

$$X = \frac{X_0}{[(1-c)e^{-\mu_0 t} + c]} \tag{30}$$

$$C = \frac{X_0}{Y_0(S_F - S_0)} = \frac{F/V_0}{\mu_0}\, e^{-\mu_0 t} \tag{31}$$

Ⅳ. Results and Discussions

Ⅳ-1. Programmed Fed-batch Culture

We can carry out the programmed fed-batch culture of bakers' yeast according to the a priori-set feed schedule:

$$F = \frac{\mu_0\, X_0\, V_0}{Y(S_F - S_0)}\, exp(\mu_0 t) \tag{32}$$

where we assume that the specific growth rate, the cellular yield coefficient and the residual glucose concentration in the medium are constant. The residual glucose concentration which is usually kept at constant value, *So*, can be quite properly neglected in comparison with the feed glucose concentration, S_F, because of their relative difference in magnitude.

One can also make allowance for the cell death and the maintenance requirement in the calculations for feed rate and volume change. The feed rate is given by

$$F = \frac{1}{S_F - S_0}\left(\frac{\mu_0}{Y_0} + m\right) V_0\, X_0\, exp\,[(\mu_0 - k_e)t] \tag{33}$$

The volume change can be expressed as

$$V = V_0\left(1 + \frac{X_0}{(\mu_0 - k_e)(S_F - S_0)}\right)\left(\frac{\mu_0}{Y_0} + m\right)[exp[(\mu_0 - k_e)t] - 1] \tag{34}$$

where k_e and m are, cell death ceofficient (hr^{-1}) in fermentor and maintenance coefficient respectively.

The result of a programmed fed-batch culture of bakers' yeast is shown in *Fig. 3.* The parameter values utilized in this experimental run are: the specific growth rate (μ) = 0.21hr^{-1}; cellular yield (Y) = 0.5 gram cell/gram glucose. After 5 hour from the start the oxygen uptake rate (*OUR*) decreased and the RQ value rose to 2.0 because of the glucose overfeeding causing the accumulation in the medium and hence the ethanol production even in the presence of sufficient oxygen. The *RQ* value during the programmed fed-batch culture fluctuates quite a bit and is consistently kept at values higher than the desired point near 1.03. This significant deviation from the desired behaviour is caused by the difference in the physiological state of the microorganism between the actual and the expected system operations.

Ⅳ-2. Feedback Fed-batch Culture

The indirect cell measurement routine mentioned in previous section is utilized in carrying out the feedback fed-batch culture. The standard glucose feed-rate trajectory shown in the following equation is initially taken for selecting the time-dependent glucose feed rate.

$$F = \frac{\mu}{Y_{x/s}} \frac{X \cdot V}{S_F} \cdot AB \qquad (35)$$

Every 10 minutes a new cell mass concentration and other system parameter values are computed using the previously mentioned routine based on material balance. The primary goal is to achieve the maximum cell yield and productivity in the absence of the ethanol production. Whether the ethanol is being produced or not can be deter mined from the relationship among *CER*, *OUR*, *RQ* and *RQo.*

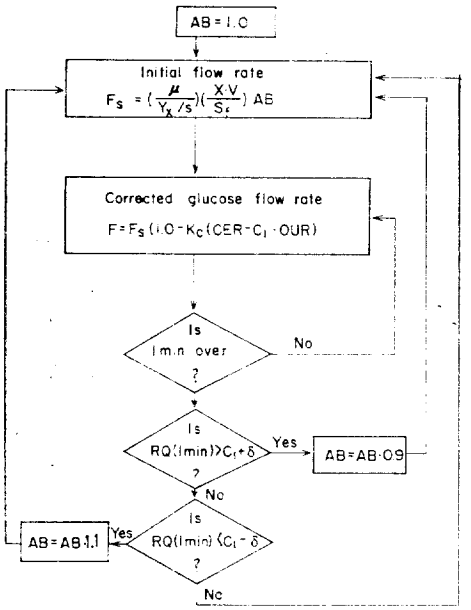

Fig. 4. Flow sheet for the optimal feed-back cotrol

$$EPR = CER - RQo \cdot OUR \qquad (36$$

The *EPR*, *i,e,* the difference between *CER* and *RQo OUR*, can be selected as the system variable to be controlled for the intended objective. This idea is incorporated into the proportional feedback control scheme to correct for the standard feed trajectory based upon no ethanol production.

$$F_s = F[1 - K_c(CER - C_1 \cdot RQ_0 \cdot OUR)] \qquad (37)$$

where F_s is the corrected glucose feed rate, K_c the controller gain, and C_1 the properly chosen parameter to adjust the set point. In the experiments the values of these parameters used are: $K_c = 0.01$, $RQo = 1.0$ and $C_1 = 1$. The particular value chosen for K_c was decided upon through a compromise between the response speed and the system stability. To achieve a greater vessatility in control scheme, a correction factor, *AB*, with an initial value of 1.0 is employed in the glucose feed rate calculation. (*Fig. 4*). After every 10 min, an overall *RQ* value is

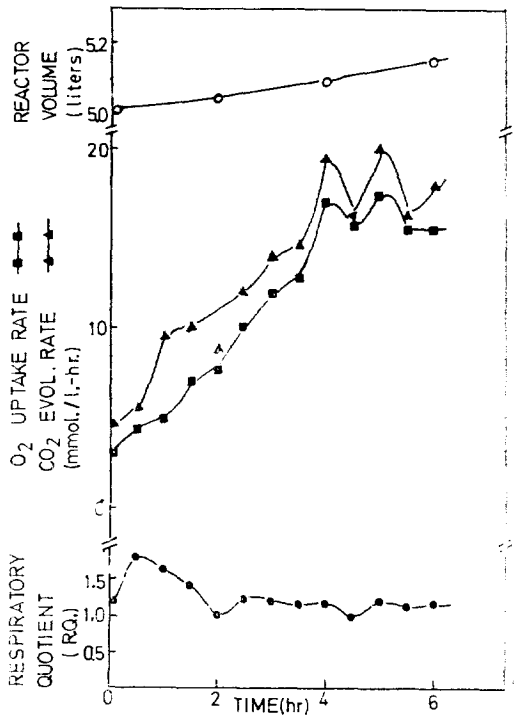

Fig. 5. Result of the feed-back control. Control with RQ as the only controlled variable.

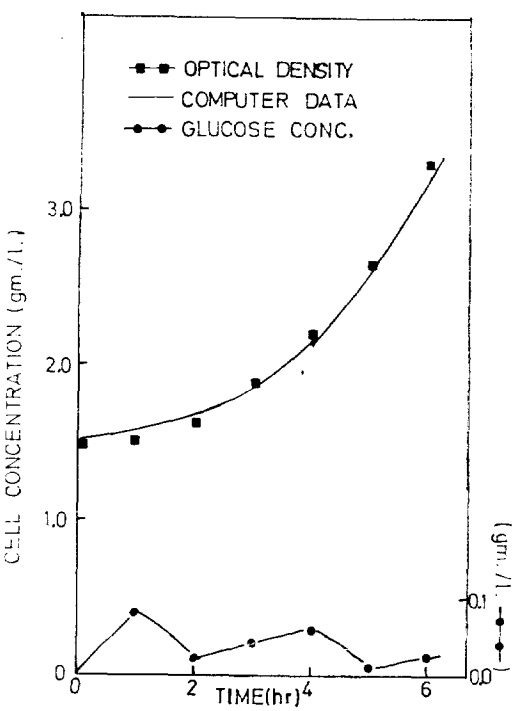

Fig. 6. Result of the feed-back control. Control with RQ as the only controlled variable.

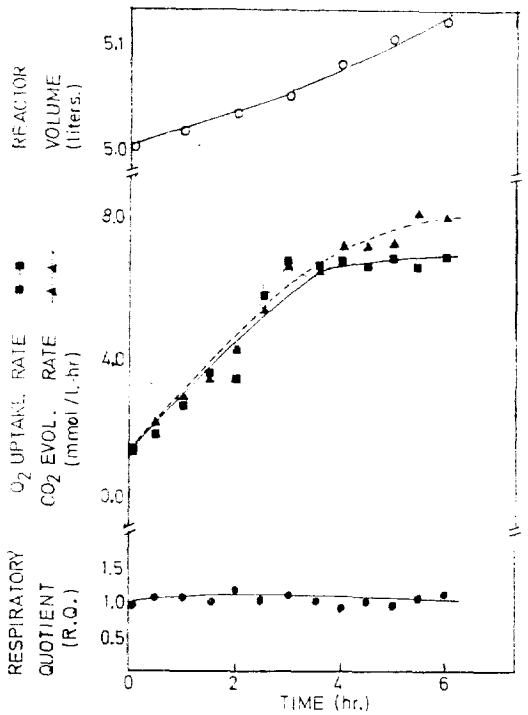

Fig. 7. Result of the feed-back control

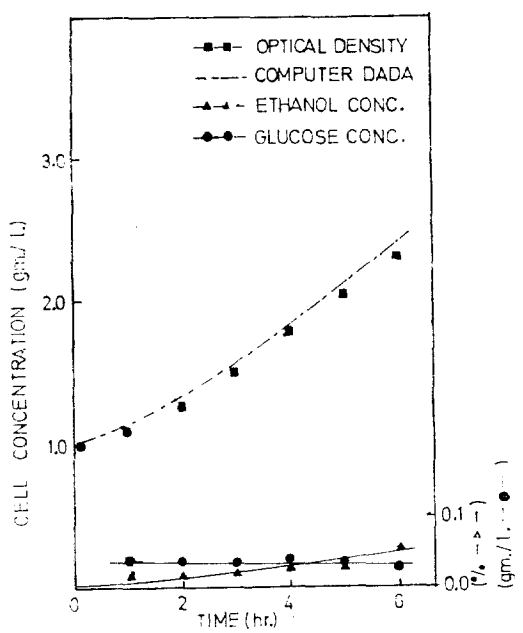

Fig. 8. Result of the feed-back control with two controlled variables. Computer predictions and the experimental results. For glucose the experimental alone are plotted.

calculated and compared with the set point. If it is persistently higher than the set point, then this correction factor is reduced accordingly to adjust the standard feedrate trajectory itself. Similarly, if the *RQ* value is held at low values, the correction factor is readjusted to higher values, effecting the return of the specific growth rate of yeast to the maximum possible value without ethanol formation. Two kinds of feed-back control experiments were performed. In the first case we chose *RQ* as the only controlled variable. Here the proportional part in *Fig. 4* is dropped out and used the multiplicative factor, *AB*, as the only manipulated parameter. In the second case two controlled variables, i.e. *RQ* and the difference between *OUR* and *CER*, were used, which corresponds to the routine depicted in *Fig. 4*.

With *RQ* as the only controlled varable we get rather unsatisfactory experimental results shown in *Fig. 5* where we see rather severely fluctuating values of *CER*, *OUR*, and glucose concentration after 4hours on from the beginning. However, the cell concentration indirectly determined by the on-line calculation computer system could quite correctly predict the real cell concentration throughout the culture period. (*Fig. 6*) The *RQ* value during the early phase of the culture period was considerably higher than the desired set point ($RQ = 1.03$). Afterwards the *RQ* dropped quite a bit but still stays at the value of 1.2 consistently higher than the set point. This means that we cannot accomplish an acceptable level of control with the *RQ* alone as the controlled variable and the very simple-minded control scheme.

The experimental results from the case with two cotrolled variables are shown in

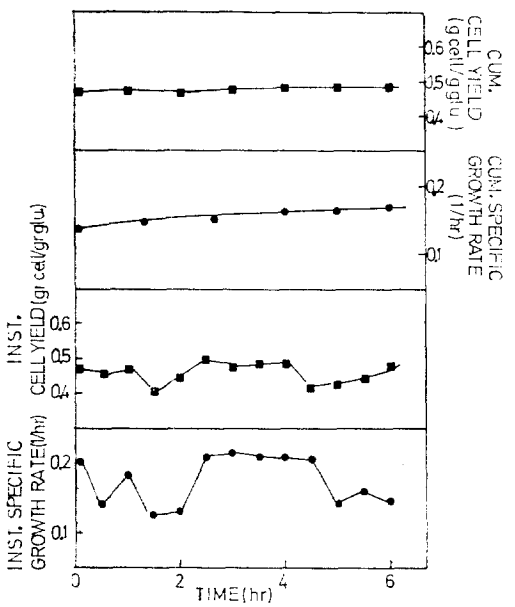

Fig. 9. Calculated instantaneous and cumulative valuses for cell yield and growth rate during bakers' yeast growth on glucose.

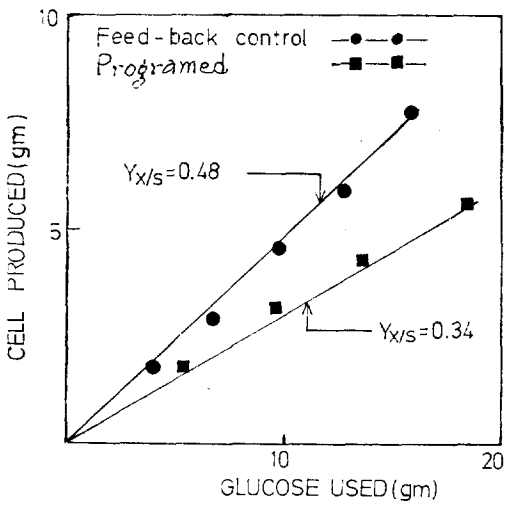

Fig. 10. Correlationbeteen the glucose addition and the cell growth.

Figs. 7-9. In *Fig. 7* we see that both *OUR* and *CER* increase steadily with time and *CER* maintains its values slightly higher than *OUR* from 4th hour on, which means that the *RQ* stays at a value quite close to

the desired set point slightly higher than 1.0. *Fig. 8* shows that our indirect feedback control scheme can automatically contol the glucose concentration quite satisfatorily around the constant value of 40 mg/l. Also the predictions for the cell mass concentration and the ethanol Concentration almost exactly coincide with the experimentally determined values. cumulative average value of the specific growth rate stays quite near $0.16hr^{-1}$. (*Fig. 9*) The overall cellular yield from this feedback control is 0.48 which is significantly higher than the one from the programmed fed-batch culture. (*Fig. 10*)

Acknowledgment

The financial support from the Korean Science and Engineering Foundation is gratefully acknowledged.

Nomenclature

a: moles of glucose consumed
b: moles of oxygen consumed
c: moles of ammonia consumed
d: moles of unit cell mass produced
e: moles of water produced
f: moles of carbon dioxide produced
k_e: cell death coefficient (hr^{-1})
m: maintenance coefficient (hr^{-1})
AB: correction factor used in control strategy
C: constant
C_1: the properly chosen parameter to adjust the set point
CER: carbon evolution rate (mols/min.liter)
F: feed rate of substrate (liter/hr)
F': the fraction of total glucose consumption diverted to the ethanol production
F_N: molar flow rate of inlet (N_2) gas/min
F_s corrected glucose flowrate (liter/hr)
F_{S_N}: flow rate of ammonia addition (liter/hr)
K_C: controller gain
M_c: molecular weight of cell mass (158)
OUR: oxygen uptake rate (mols/min. liter)
$P_C(in)$: partial pressure of carbon dioxide in inlet gas
$P_C(out)$: partial pressure of carbon dioxide in outlet gas
$P_O(in)$: partial pressure of oxygen in inlet gas
$P_O(out)$: partial pressure of oxygen in outlet gas
RQ: respiratory quotient (mol CO_2/mol O_2)
RQ_0: respiratory quotient when ethanol was not produced (mol CO_3/mol O_2)
S: substrate concentration in culture medium (g/liter)
S_F: concentration of feeding substrate (g/liter)
S_N: concentration of nitrogen source in equivalent amount of nitrogen (N g/liter)
$S_N(in)$: inlet concentration of nitrogen source in equivalant amount of nitrogen (N g/liter)
S_0: optimal substrate concentration in culture medium (g/liter)
Y: cellular yield (g cell/g glucose)
Y_N: the yield coefficient based on nitrogen source (g cell/g ammonia)
Y_{CO2}: the ratio of carbon dioxide produced per unit of cell mass formed (mol CO_2 /mol cell)
Y_0: cellular yield at optimal substrate concentration (g cell/g glucose)
Y_{O_2}: the cell yield on oxygen (mol cell/mol oxygen)
V: liquid volume (liters)
X: concentration of cell mass in culture

medium (g/liter)

X_0: initial cell mass concentration (g/liter)

$X(t)$: concentration of cell mass in culture medium at time t (g/liter)

Greek letters

μ: specific growth rate based on cell mass concentration (hr^{-1})

μ_0: specific growth rate based on cell mass concentration at optimal substrate concentration (hr^{-1})

Subscript

O: initial time O

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