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Microcomputer-coupled Baker's Yeast Production

AKIRA NANBA, FUMITO HIROTA*, and SHIRO NAGAI

Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Senda-machi 3-8, Hiroshima 730

A microcomputer system providing feedforward and feedback control of sugar feed-rate to avoid the glucose effect in baker's yeast production was examined. A sensor with high sensitivity and a rapid response to combustible substances was used to monitor the dynamic behavior of ethanol during aerobic cultivation. Ethanol formation began when glucose concentration exceeded ca. 130 mg/*l*, whereas ethanol was simultaneously consumed with glucose when glucose concentration was less than ca. 70 mg/*l*. The oscillatory phenomena due to ethanol formation and consumption were used as the feedback signal in controlling the sugar feed-rate, which was programmed in anticipation. This simple and reliable control strategy afforded an almost maximal growth yield from glucose of 0.5 g cell/g glucose and a specific growth rate of 0.17 hr^{-1} by preventing overproduction of ethanol by the glucose effect.

One major limitation in the application of computer control to microbial cultivation processes is the lack of suitable sensors allowing automatic analysis of the state variables. Recently, a sensor for combustible substances has been used for the continuous determination of ethanol in aerobic fermentation of *Saccharomyces cerevisiae*.[1] The operating principle is based on the detection of ethanol vapor in the exit gas, which is in equilibrium with the ethanol concentration of the broth at constant temperature. The sensor had a high sensitivity, measuring up to 1 mg ethanol/*l*, and a short response time of 10 sec. The shortcomings of the sensor include its response to other combustible substances and its susceptibility to influence by the partial oxygen pressure (pO_2) in the exit gas. But since the output signal of the sensor is not affected by pO_2 at relatively high aeration rates,[1] this problem can be overcome by selection of an appropriate aeration rate.

A porous membrane tube immersed in the broth is also of use for continuous determination of diffusible substances from broth. The diffusible substance can be quantitatively removed from the broth by a carrier gas flowing through the tube.[2-4] This method, while avoiding the pO_2 problem, is however inferior in response and sensitivity to direct measurement in the exit gas.

In baker's yeast production from cane sugar molasses, it is necessary to maintain sugar concentration at a low but optimal value so as to prevent ethanol formation while maintaining a relatively high growth rate. To control sugar concentration at an appropriate level in fed-batch culture, Aiba *et al.*[5] attempted to control the sugar feed-rate with a feedback signal of respiratory quotient (RQ), which was to be maintained between 1.0 and 1.1. Recently, Wang *et al.* have controlled yeast production using an on-line computer control system based on material balance equations in which RQ was used as a parameter for indirect monitoring of ethanol formation.[6,7] A feedforward control capable of supplying sugar required for exponential growth, and a feedback control capable of stopping the sugar supply when its concentration exceeded an predetermined level, were combined to enhance the productivity of baker's yeast production.[7]

As the systems for computer-aided baker's

* Present address, Kanegafuchi Chemical Industry Co. Ltd., Takasago-shi, Hyogo 676

yeast production examined so far[5,7] involve intricate problems in application to large scale production, we have attempted to construct a simplified microcomputer-aided process capable of providing feedforward and feedback control of sugar feeding based on the output signal of a reliable ethanol sensor.

Materials and Methods

Microorganism, culture medium and condition A commercial strain of *Saccharomyces cerevisiae* (baker's yeast, Kyowa-Hakko Co., Ltd., Hofu) was used. The medium composition for preculture was as follows (g/*l*): glucose, 0.9; $(NH_4)_2SO_4$, 1.7; KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1, and yeast extract, 0.1. In addition, 10 ml of 1 % Adecanol (LG109, Asahi-denka Co., Ltd., Tokyo) was added as antifoam. The medium composition for the fed-batch culture was (g/*l*): glucose, 180; KH_2PO_4, 10; $MgSO_4 \cdot 7H_2O$, 2; yeast extract, 2; and 1 % Adecanol, 10 ml. Aqueous ammonia (2N) was used for pH adjustment and as a nitrogen source during fed-batch culture. Culture temperature and pH were maintained at 30°C and 4.5 throughout. Dissolved oxygen concentration (DO) was maintained above ca. 1 mg/*l* by automatic adjustment of agitation speed (described later). Aeration rate was fixed at a relatively high value of 2 vvm to maintain pO_2 in exit gas at ca. 19 vol % throughout the cultivation.

A 5-*l* fermentor (diameter 15 cm) with a 6-vaned disc impeller (diameter 5 cm) capable of agitating up to 1,500 rpm was used with an initial medium of ca. 2 *l*. After glucose had been almost completely consumed in batch culture, fed-batch culture was began with computer controlled sugar feeding (described later).

Microcomputer system A schematic diagram of the microcomputer-coupled system and a flow sheet of the computer program are shown in Figs. 1 and 2. A commercial microcomputer system TLCS-12A (Tokyo-Shibaura Electric Co., Ltd., Tokyo) was interfaced to a 5-*l* fermentor. The memory system of the computer consists of RAM (0.5K words) and ROM (0.5K words). A hexadecimal-data input key and 6-digital LED display were used as input-output system. Programming was done by means of a hand assembling.

The analog signals from the three sensors, i.e., pH, DO in the broth and ethanol concentration in the exit gas (see Fig. 1), were digitized through a 3.5-digit digital voltmeter (TR 6911 B, Takeda-Riken Co., Ltd., Tokyo) with an 8-channel analog multiplexer (see Fig. 1). The computer scanned the sensor signal at intervals of 1 sec and repeated periodically the same procedure at intervals of 5 sec (see Fig. 2). In Fig. 2, the five subroutines connected to the analog multiplexer

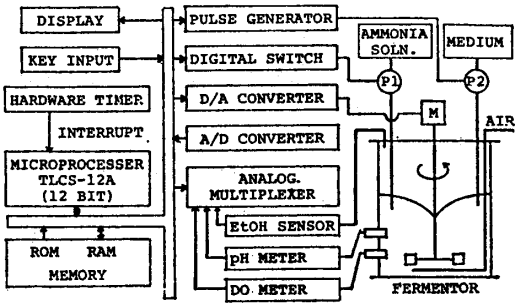

Fig. 1. Block diagram of microcomputer-coupled culture system for baker's yeast production.

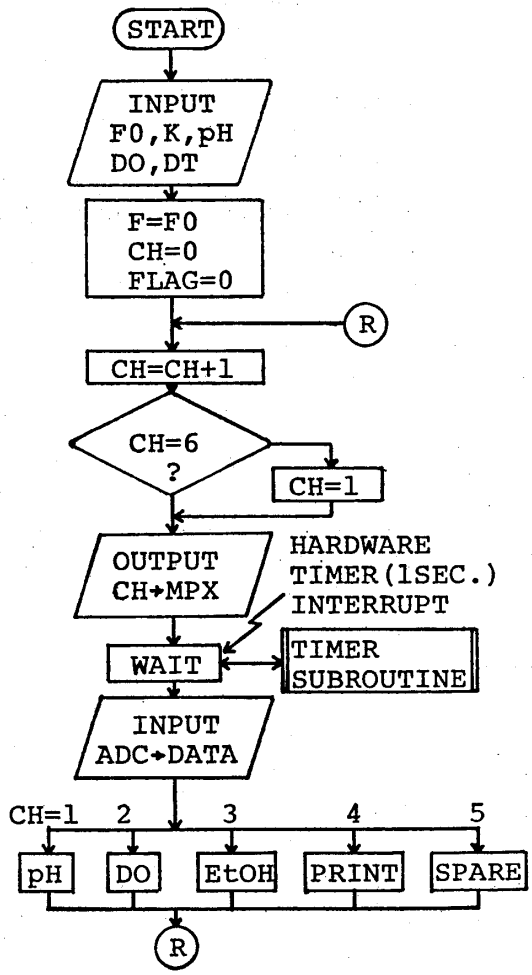

Fig. 2. Flow sheet of computer program for logging data from sensors. The three variables monitored are DO, pH in the broth and ethanol concentration in the exit gas. Algorithms of the acquired data are described in the text. *F0*, initial flow rate of medium; *K*, exponent of feed-rate function (see Eq. 9); DO, minimum level of DO, 1 ppm; CH, multiplexer channel; MPX, multiplexer; FLAG, signal of ethanol change; ADC, A/D converter; *F*, flow rate of medium (see Eq. 9); DT, time interval, Δt (see Eq. 9).

consist of pH measurement and control (CH: 1), DO measurement and control (CH: 2), ethanol measurement to control the feed-rate of medium (CH: 3), printing (CH: 4) and one spare part (CH: 5).

In addition, a pulse generator (Oriental Motor Co., Ltd., Tokyo), a digital-analog converter and a digital switch were interfaced with the computer to control respectively the feed-rate of medium, the DO by agitation speed, and pH level by alkali addition (see Fig. 1, details given later).

Sensors A sensor (TGS 812, Figaro Engineering Inc., Osaka) consisting of a sintered SnO_2 ceramic semi-conductor[1] was used to measure the ethanol concentration in the exit gas from the fermentor. A preliminary test was carried out to examine the response and sensitivity of the sensor by stepwise addition of ethanol to 2 *l* of water in a 5-*l* fermentor to give concentrations between 10 and 200 mg/*l*, and by variation of aeration rate between 0.5 and 2.0 vvm, and agitation speed between 440 and 1,400 rpm. The sensor showed a rapid response to the ethanol in the exit gas, with a dead time of 5 to 10 sec and a relaxation time to the saturation level of ethanol of 40 to 174 sec. The sensor was sensitive and reliable in the range of 10 to 200 mg/*l* water, although the response was nonlinear at more than 500 mg/*l*.

The sensor output under aeration and agitation could be maintained at maximum level for ca. 10 min without being influenced by evaporation due to aeration. To avoid the effect of pO_2 on the sensitivity,[1] a relatively high aeration rate (2 vvm) was used in order to maintain pO_2 above 19 vol %. The results confirmed the suitability of the sensor for monitoring ethanol concentration under this condition.

Ethanol concentration was also measured by gas chromatography (GCG-555 FT, Yanagimoto Co., Ltd., Kyoto, and Chromosorb 101) to double-check the validity of the ethanol sensor output. To prevent condensation of water vapor on the sensor, it was fixed in a glass tube fitted with a heating device to maintain its temperature at 40°C.

A polarographic electrode for DO (Toshiba-Beckman Co., Ltd., Fieldlab 1008, Tokyo) and a pH electrode (Hitachi-Horiba Co., Ltd., M-5, Tokyo) were used as sensors.

Feedforward control of medium feed-rate For yeast cells to grow exponentially in a fed-batch culture, the growth-limiting substrate must be fed exponentially while the other nutrients must be maintained in sufficient supply. To achieve this in computer-aided cultivation, the mass balance equation between glucose supply and consumption must be considered.

$$\frac{dS}{dt}=F(t)\,S_r-\frac{1}{Y}\frac{dX}{dt} \tag{1}$$

where $F(t)$=flow rate of fresh medium *l*/hr; S, residual glucose amount in fermentor, g; S_r, glucose concentration of fresh medium, g/*l*; t, culture time, hr; Y, growth yield from glucose, g/g

Exponential growth can be expressed as:

$$\frac{dX}{dt}=\mu X \tag{2}$$

$$X=X_0 \exp(\mu t) \tag{3}$$

$$X_{i+1}=X_i \exp(\mu \Delta t) \tag{4}$$

where X_0, biomass in fermentor at $t=0$, g; X_i and X_{i+1}, X at $t=t_i$ and t_{i+1}, g; Δt, a small time interval, $t_{i+1}-t_i$; μ, specific growth rate, hr^{-1}

At a quasi-steady state in Eq. 1, the substitution of Eq. 2 into Eq. 1 yields

$$F(t)=\frac{\mu X}{YS_r} \tag{5}$$

Then,

$$F(t_0)=\frac{\mu X_0}{YS_r} \tag{6}$$

$$F(t_{i+1})=\frac{\mu X_{i+1}}{YS_r} \tag{7}$$

The substitution of Eq. 4 into Eq. 7 yields

$$F(t_{i+1})=\frac{\mu X_i}{YS_r}\exp(\mu\Delta t)$$

$$=F(t_i)\exp(\mu\Delta t) \tag{8}$$

If μ in Eq. 8 is replaced by the exponent of the feed-rate function, K, hr^{-1}, then Eq. 8 becomes

$$F(t_{i+1})=F(t_i)\exp(K\Delta t) \tag{9}$$

Flow rate of the medium at arbitrary time, $F(t_{i+1})$, can be sequentially assessed by Eq. 9, if K, Δt and $F(t_0)$ are given. If Eq. 9 is programmed in the computer, the output of $F(t_{i+1})$ can be converted to 'a pulse signal' by a pulse generator. Then, the pulse signal can act upon 'a driving unit' of a stepping motor (Type 2CPH-005, Oriental Motor Co., Ltd., Tokyo) connected with the head part of a peristaltic pump (Cole-Parmer, Chicago, Ill.), (see Fig. 1). Thereby the medium can be supplied into the fermentor based on Eq. 9.

Feedback control of medium feed-rate Should the sugar concentration of the broth exceed the critical level at which the yeast cells start to produce ethanol by the Crabtree effect,[8] the sugar supply on Eq. 9 must be temporarily stopped. For this, the signal of ethanol sensor was used to control the scheduled sugar supply. This will be described in Results (see Fig. 5).

DO and pH controls DO during the cultivation was maintained above 1 mg/*l*. This was effected through the computer by using DO signal: when the DO signal fell below 1 mg/*l*, agitation speed was increased by 60 rpm by means of a digital-analog converter connected to the induction motor of stirrer (Type 51K-40 RA, Oriental Motor Co., Ltd., Tokyo) (see Fig. 1). By this means, agitation speed could be increased up to 1,500 rpm. In compliance with the

instructions of the pH subroutine, 2N aqueous ammonia was added to the fermentor *via* a digital switch to adjust pH at the setting point of 4.5 (see Fig. 1).

Analyses Ethanol was measured with the ethanol sensor and, if necessary, by gas chromatography. Glucose was measured by the Nelson-Somogyi method or by the glucostat method (Fujisawa Medical Supply Co., Ltd., Osaka). Cell weight was measured as follows: cells were harvested by centrifugation, washed with distilled water, then dried at 105°C for 6 hr. The optical density of broth was measured at 570 nm with a spectrophotometer (Model 101, Hitachi Co., Ltd., Tokyo), and optical density was converted to dry cell weight based on an experimentally determined calibration curve.

Results and Discussion

In the aerobic cultivation of *S. cerevisiae* on a glucose medium, ethanol formation was initiated when the glucose concentration in the broth exceeded ca. 130 mg/*l* at a dilution rate of 0.25 hr^{-1} in chemostat.[7,9] In fed-batch culture, to prevent the ethanol formation in aerobic growth of *S. cerevisiae*, it will be necessary to select an exponent of feed-rate function, K (see Eq. 9), of less than 0.25 hr^{-1}, since K corresponds to dilution rate in chemostat.

However, in fed-batch culture, when the glucose concentration unexpectedly exceeds the critical concentration of ca. 130 mg/*l*, the feedforward control alone can no longer reduce the excess sugar concentration without a feedback control device.

To ascertain the necessity of feedback control, ethanol formation as a function of glucose concentration was examined in a fed-batch culture with the feedforward control of sugar feeding at K of 0.09 hr^{-1} (Fig. 3). In the quasi-steady state in terms of glucose concentration, the glucose concentration was at a low level of ca. 70 mg/*l*, and consequently the concentration of ethanol formed was low: ca. 2 mg/*l* in Fig. 3-a, and 15 mg/*l* in Fig. 3-b. During the fed-batch culture, a small amount of glucose was suddenly injected into the fermentor, and this was repeated when the glucose concentration had returned almost to the original level (ca. 70 mg/*l*). The excess of glucose over that being continuously fed at K of 0.09 was rapidly consumed by the

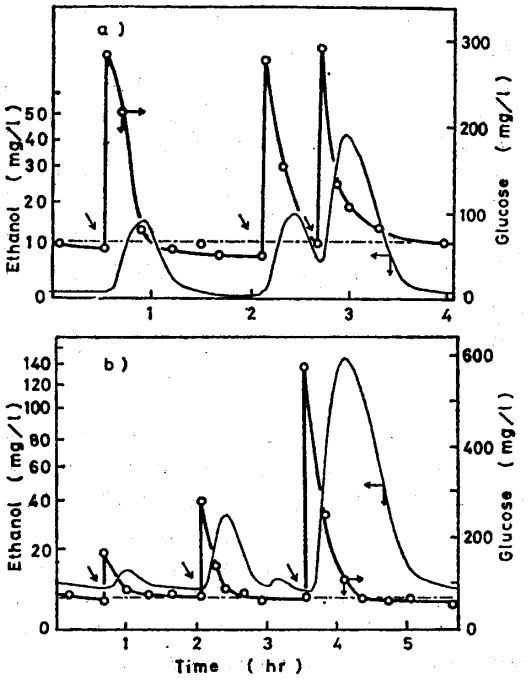

Fig. 3. Effects of glucose concentration on ethanol formation and consumption in a fed-batch culture of *Saccharomyces cerevisiae* at sugar feed-rate of K= 0.09 hr^{-1}. Glucose was suddenly added to the fed-batch culture at K=0.09 and this was repeated (see downward arrows) when the glucose concentration had returned almost to the steady-state level (ca. 70 mg/*l*, dotted line). a) X=3.8 g/*l* at t=0, b) X =11.8 g/*l* at t=0.

yeast cells, and the glucose concentration returned to the level before the addition.

The sudden addition of glucose stimulated ethanol formation by the glucose effect, and the maximum ethanol concentration reached appeared to be a function of the maximum glucose concentration reached (see Fig. 3-b). The relationship between ethanol formation and glucose concentration is depicted in Fig. 4, from which is evident that ethanol formation was neglegible at glucose concentrations up to ca. 100 mg/*l*, but was remarkably stimulated at concentrations above ca. 130 mg/*l*, as observed by Wang *et al.*[7]

Another striking feature of Fig. 3 is that when glucose concentration was lower than 70 mg/*l* (indicated by the dotted lines), the yeast cells started to consume ethanol simultaneously with glucose, when glucose was being supplied at K=0.09 hr^{-1}. This con-

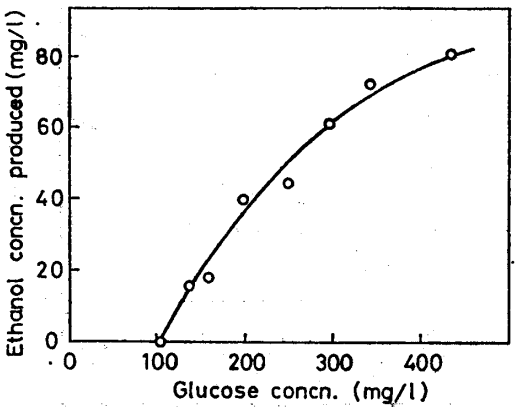

Fig. 4. Ethanol produced by *Saccharomyces cerevisiae* after sudden addition of glucose at a quasi-steady state of fed-batch culture at $K=0.09$ hr^{-1}. Ethanol produced, plotted on the abscissa, is the difference between the steady state and the maximum levels (see Fig. 3).

trasts with the well-known diauxie consumption of ethanol which occurs after glucose is almost completely consumed.[10] The initiation of ethanol formation and consumption were closely linked to the glucose concentrations of 130 and 70 mg/*l*, respectively, and these phenomena allowed the change in ethanol concentration to be used as the feedback signal to control, if necessary, the anticipatory sugar feed-rate.

The computer program for the feedback control of sugar feeding was based on the analysed ethanol tendency during cultivation (Fig. 5). The difference (DC) between two ethanol outputs at intervals of 5 sec (see Fig. 2) was continuously pushed onto a stack capable of holding 6 consecutive DCs, and the averaged value of 6 consecutive differences ($\overline{DC}$) was used as the control signal of sugar feeding.

The control strategy in Fig. 5 is that when $\overline{DC}$ is positive, i.e., ethanol is being produced because the glucose concentration is above 130 mg/*l*, the anticipatory feed-rate at that moment based on Eq. 9 is stored in the memory, and the medium feeding pump is temporarily stopped (see route 1). Then, when the 'flag' becomes 1, the signal flows to route 3, and if $\overline{DC}$ has a negative value, i.e., ethanol is being consumed because the glucose concentration is below 70 mg/*l*, then the anticipatory feed-rate must be recalled so

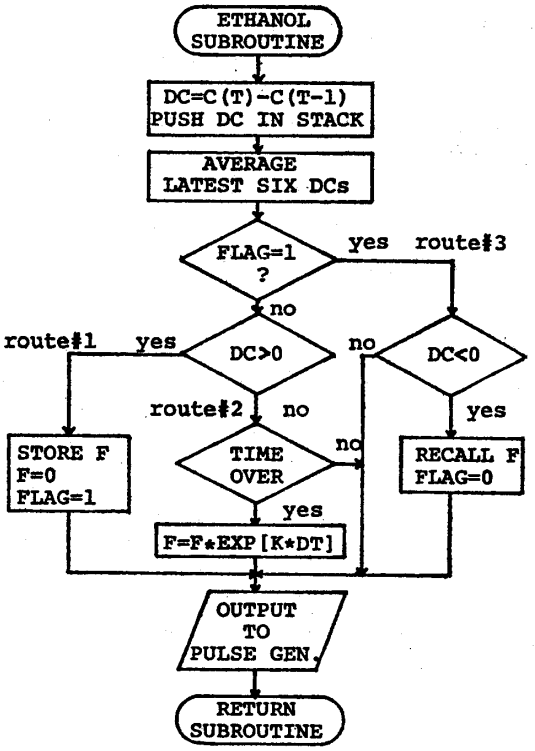

Fig. 5. Flow sheet of computer program for the feed-forward and feedback control of sugar feed-rate in fed-batch culture for baker's yeast production. Ethanol outputs automatically acquired from the exit gas (see Fig. 2) are used as the feed-back signal to control the projected sugar feed-rate based on Eq. 9.
C(T), ethanol output at arbitrary time; DC, difference in ethanol outputs at intervals of 5 sec; $\overline{DC}$, average of six consecutive DC values; FLAG =1, ethanol production phase; FLAG=0, ethanol consumption phase; F, sugar feed-rate based on Eq. 9; K, exponent of sugar feed-rate; DT, time interval, Δt (see Eq. 9).

as to restart the medium feeding and the 'flag' becomes 0. If a negative value of $\overline{DC}$ can not be obtained in route 3, the signal is returned and the cycle is repeated. When $\overline{DC}$ is zero, i.e., ethanol is being neither formed nor consumed, the medium is fed at the anticipatory feed-rate based on Eq. 9 (see route 2).

This computer-aided cultivation system is expected to maintain the aerobic growth of *S. cerevisiae* at a relatively high growth rate by maintaining the sugar concentration at a low but optimal level capable of minimizing the ethanol formation, provided that nutrients other than sugar are in sufficient supply.

To ascertain the applicability of computer-aided culture system, first, a feedforward (anticipatory) control program of sugar feed-rate without feedback control was used in fed-batch culture of the yeast cells in which K=0.09 or 0.18 hr^{-1}, $F(t_0)$=11 or 22 ml/hr and Δt =5 min (see Eq. 9). The results are shown in Fig. 6. As might be expected, in the run at K=0.09 (Fig. 6-a), typical aerobic growth was observed throughout cultivation, in which glucose concentration remained below 70 mg/l. Corresponding to the K value (0.09), the overall specific growth rate was 0.08 hr^{-1}, which was a considerably lower than that (0.18) attained in a baker's yeast factory, although the growth yield from sugar was a reasonable value of 0.48 g cell/g glucose as a result of the typical aerobic growth.

In contrast, in the run at K=0.18 (see Fig. 6-b), peaks of comparatively high ethanol concentration were frequently observed, suggesting that ethanol was more frequently formed and consumed. In the first 7 hr of cultivation, glucose concentration apparently remained in the optimal range in which the glucose effect was narrowly avoided, but after 7 hr, glucose concentration exceeded the critical level of ca. 130 mg/l, which caused the production of ethanol by the aerobic fermentation.[9]

Secondly, a fed-batch culture with the feedforward and feedback control of sugar feeding obtained in Fig. 5 was conducted at K of 0.20 hr^{-1} with the aim of achieving aerobic cell growth without the glucose effect (Fig. 7). In contrast with the case of only feedforward control of sugar feeding at K of 0.18 (Fig. 6-b), a fairly stable oscillation of ethanol was observed, together with a low sugar concentration of ca. 50 mg/l. During the cultivation, the anticipatory sugar feeding was temporarily stopped by the feedback control signal when ethanol concentration rose, and restarted when ethanol concentration fell. Consequently, the glucose concentration during the cultivation could be maintained at less than ca. 130 mg/l, preventing the overproduction of ethanol. The specific growth rate and the growth yield

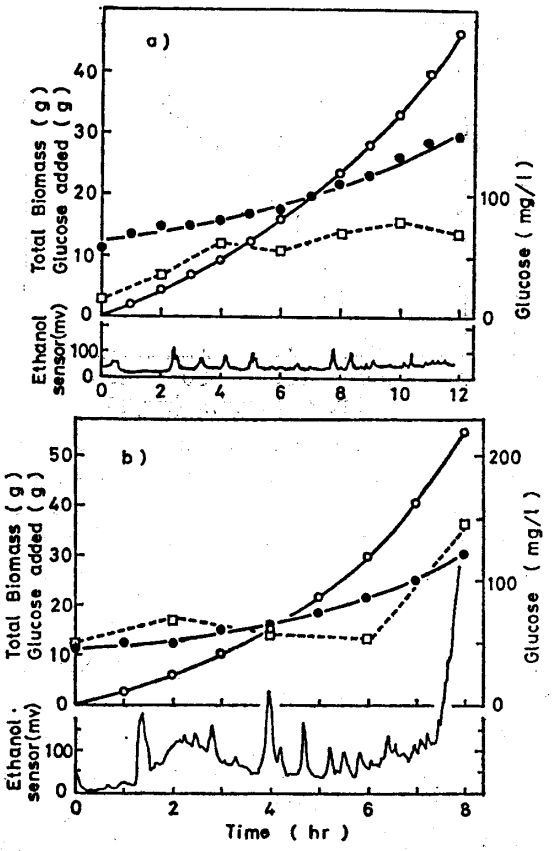

Fig. 6. Baker's yeast production by the feedforward control of sugar feed-rate in fed-batch culture.
●, Total biomass; ○, Glucose added; □, Glucose concentration. Operating conditions (see Eq. 9):
a) X_0=11 g, K=0.09 hr^{-1}, Δt=5 min, Y=0.5 g/g, S_r=180 g/l, $F(t_0)$=11 ml/hr ($=KX_0/YS_r$).
b) X_0=11 g, K=0.18 hr^{-1}, Δt=5 min, Y=0.5 g/g, S_r=180 g/l, $F(t_0)$=22 ml/hr.

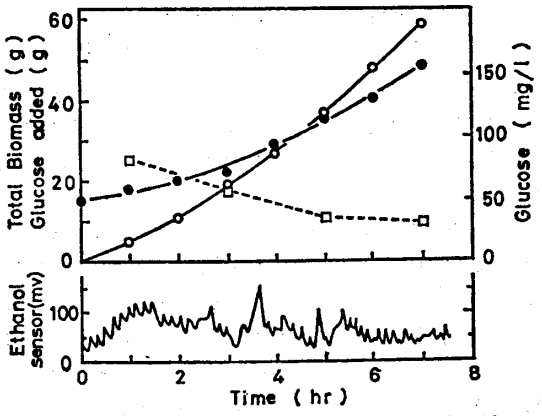

Fig. 7. Baker's yeast production by the feedforward and feedback control of sugar feed-rate (see Fig. 5) in fed-batch culture.
●, Total biomass; ○, Glucose added; □, Glucose concentration. Operating conditions (see Eq. 9): X_0=15 g, K=0.2 hr^{-1}, Δt=5 min, Y=0.5 g/g, S_r =180 g/l, $F(t_0)$=33 ml/hr.

Table 1. Baker's yeast production by the feedforward and feedback control of sugar feed-rate in fed-batch culture.

V_0 l	V l	X_0 g	X g	t hr	μ hr^{-1}	Y g/g
2.1	2.4	13.8	50.0	7.6	0.17	0.55
2.0	2.3	14.6	48.4	7.0	0.17	0.48
1.9	2.4	11.0	55.2	10.0	0.18	0.52

Operating conditions: $K=0.2$ hr^{-1}, $\Delta t=5$ min, $Y=0.5$ g/g, $S_r=180$ g/l, $F(t_0)=KX_0/YS_r$.
V_0, initial culture volume; V, final culture volume; X_0, initial cell mass; X, final cell mass; t, culture time; μ, specific growth rate; Y, growth yield from glucose.

from sugar were 0.17 hr^{-1} and 0.48 g/g, respectively.

Other data obtained with the feedforward and feedback control of sugar feeding in fed-batch cultures are summarized in Table 1. The growth yield from glucose was a practically acceptable value, suggesting that the glucose was mainly converted to cell mass in the aerobic pathways, while specific growth rate was slightly low compared with K (0.20), probably because of limitation by the relatively low glucose concentration.

It is concluded that microcomputer-aided baker's yeast production with feedforward and feedback control by the ethanol sensor could be applicable for economical production of yeast cells from glucose, provided that the sensor has a high sensitivity and a rapid response to the ethanol behavior during the cultivation.

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