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Computer Control of Bakers' Yeast Production

HENRY Y. WANG, CHARLES L. COONEY,* and DANIEL I. C. WANG, *Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

Summary

The study presented here was undertaken to demonstrate the usefulness of computer control for the production of yeast from molasses. A flexible control system was developed by using an on-line computer for the monitoring of cell mass and employing anticipatory control to maintain the maximum productivity. Process disturbances were minimized by employing a multivariable feedback control system to prevent ethanol formation. The control strategy acted to keep overall conversion yield at its maximum level, about 0.5 g cell/ g sugar, while maintaining high volumetric productivity between 3 and 5 g/liter-hr. Results are presented to show the effectiveness of simultaneous anticipatory and feedback control in overcoming problems of oxygen starvation, molasses quality, and variable inoculum size.

INTRODUCTION

In previous papers we have described the use of an on-line computer-aided material balancing routine for the continuous assessment of growth during bakers' yeast production.[1,2] The information obtained from this indirect monitoring of cell mass has been used here to develop an on-line anticipatory control strategy for the addition of molasses to a fed-batch fermentation. The objective function of this control system is the maintenance of high volumetric productivity while maximizing the cell yield. This control system alone, however, was found not to work in certain situations: specifically, in the application of anticipatory control, the assumption is made that growth is limited by the availability of sugar. It is possible, however, that certain changes (e.g., oxygen limitation, unhealthy inoculum, or low-grade molasses supply) could necessitate a change in the control strategy.

The limitations of anticipatory control can easily be overcome by

* Please address correspondence to: Dr. C. L. Cooney, 16-229, M.I.T., Cambridge, MA 02139.

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augmenting and fine-tuning with a feedback scheme. In this way anticipatory control can be used to adjust the sugar feed rate to account for increases in cell growth and fermentor volume, while feedback control can provide a safeguard against ethanol formation.

Many sensors have been suggested as possible parameters for the monitoring of yeast growth, e.g., cell concentration,[3] oxygen uptake rate,[4] dissolved oxygen,[5] respiratory quotient,[6,7] ethanol in the gas stream,[8] ethanol in the fermentation broth,[9] and sugar in the fermentation broth.[10] However, the problem is to choose the specific state variables that can be monitored easily and reliably, as well as with sufficient sensitivity for control purposes.

Experimental studies in batch culture have shown that the ethanol production rate is a function of the specific growth rate[2] and both these parameters are related to the residual sugar concentration. It is therefore important that the actual sugar concentration in the fermentor be maintained at a low but optimal value at all times. **Ethanol formation aerobically in the presence of high sugar concentration, known as the Crabtree Effect,[11] is distinctly different from the Pasteur Effect where the ethanol is formed under conditions of oxygen starvation.**

The **respiratory quotient (RQ)** was shown by Wang et al.[2] to be related to the **ethanol production rate (EPR)** by the following equation:

$$\mathbf{EPR} = (\mathbf{RQ} - C_1)\,(\mathbf{OUR}) \qquad (1)$$

where C_1 is the RQ value in the absence of ethanol formation and OUR is the oxygen uptake rate in mmol/liter-hr. Thus, used in combination with the other measurements in eq. (1), RQ may provide a parameter for indirectly monitoring ethanol production.

As early as 1959, Dietrich[6] suggested the use of RQ as a control parameter for yeast production. More recently, Aiba et al.[7] controlled yeast production by controlling the sugar feed rate and thus maintaining the RQ within a range of 1.0 to 1.1. However, their experiments were performed at a "pseudo"-steady state, and their final cell concentrations never exceeded 10 g/liter, whereas industrial systems always produce 40 g/liter or more of cells by the end of fermentation.[11] The experiments reported in this paper approximate more closely the conditions of actual industrial fermentations.

MATERIALS AND METHODS

The computer-coupled fermentation system used in this study has been described in detail by Wang et al.[2] This system has been

modified by the incorporation of a feedback control signal from the computer to control the molasses flow rate during the fermentation. A precision peristaltic pump (Leeds and Northrop, North Wales, PA) is used for molasses addition. Details of this system have been described by H. Y. Wang (Ph.D. thesis, M.I.T., 1977).

The medium used for yeast propagation contained: 10 g/liter $(NH_4)_2SO_4$; 10 g/liter KH_2PO_4; and 3 g/liter $MgSO_4 \cdot 7H_2O$. This modification of the medium used by Wang et al.[2] was made to permit high cell densities. A mixture of equal volumes of cane molasses and beet molasses (which contain 30% and 39.5% reducing sugar by weight, respectively) was used; no vitamin addition was necessary. The analytical procedures for determining cell mass and the concentrations of ethanol, reducing sugar, and glucose have been described previously.[2] The organism used in this study was a commercial strain of *Saccharomyces cerevisiae*.

RESULTS AND DISCUSSION

Monitoring of a Fed-Batch Fermentation for Yeast Production

In order to implement automatic process control it is necessary to monitor pertinent parameters accurately and reliably. In the production of bakers' yeast, for example, the important objective parameters are substrate conversion yield (g cell/g sugar) and volumetric productivity (g cell/liter-hr). Once these parameters can be monitored continuously, it is possible to evaluate the effect of operating variables on process performance and to select the desired set points for an optimal control strategy. In the production of bakers' yeast from molasses in a fed-batch fermentation, the effects of variables such as pH, temperature, and dissolved oxygen are known,[10] and their set points can be chosen. On the other hand, the most critical variable, the flow rate for molasses addition, is not defined and the demand for molasses will change during the fermentation process. **Therefore, to achieve optimal control, it is necessary to continuously assess the state-of-the-fermentation and to anticipate the demand for sugar, which, in turn, determines the optimum rate for molasses addition.**

The demand for molasses will depend on the cell concentration, specific growth rate, and cell yield. In the absence of a sensor for direct measurement of cell mass, a computer-aided material-balancing technique was developed **to calculate continuously the cell concentration, specific growth rate, sugar consumption rate, and other**

growth-related parameters from measurements of the rates of air flow, CO_2 production, and oxygen consumption.[1] Our objective here is to utilize this indirect method for monitoring growth as a basis for computer control of yeast production by regulating the addition of molasses to achieve high volumetric productivity with high cell yield.

The application of this indirect method for monitoring a yeast fermentation is illustrated in Figure 1. The principal measured variables are: the molasses reservoir weight, the ammonia reservoir weight, and the carbon dioxide and oxygen concentration in the exit gas (Fig. 1(a)). The abrupt changes in the concentrations of oxygen and carbon dioxide in the exit gas at 12 and 15 hr were caused by manual changes in the air flow rate (Fig. 1(b)); the resulting transient behavior of the gas-exchange data, however, is not important in subsequent calculations. The calculated values for oxygen uptake rate, carbon dioxide evolution rate, and respiratory quotient are shown in Figure 1(c). These intermediate results are necessary for the calculation of other biological parameters. The final oxygen uptake rate reached 300 mmol/liter-hr, and the respiratory quotient remained at approximately 1.0 throughout the fermentation. The molasses addition rate (Fig. 1(c)), which was under computer control (to be discussed later), increased exponentially as growth proceeded.

During the fermentation, samples were taken from the fermentor and analyzed for dry cell weight and glucose and reducing sugar concentration (Fig. 1(d)). The glucose concentration was always low (less than 0.5 g/liter) while the concentration of reducing sugar rose during the fermentation. The final, unmetabolizable level of reducing sugar was 5–6 g/liter, or about 2–3% of the total amount of reducing sugar in the molasses. This is consistent with the values reported by Reed and Peppler.[11] Although the data are not shown, the ethanol concentration was low during the fermentation reaching a final value of 0.8 g/liter.

Using the material balances described previously by Cooney et al.[1] the cell concentration, specific growth rate, and cellular yield coefficients are then calculated every 10 min and represent instantaneous values. Representative results are presented in Figure 2 as discrete points. The same parameters are calculated on a cumulative basis by integration of the instantaneous data (Fig. 2(b)). The calculated cell concentration agrees well with the experimentally determined value. The final cell concentration was 57 g/liter. The

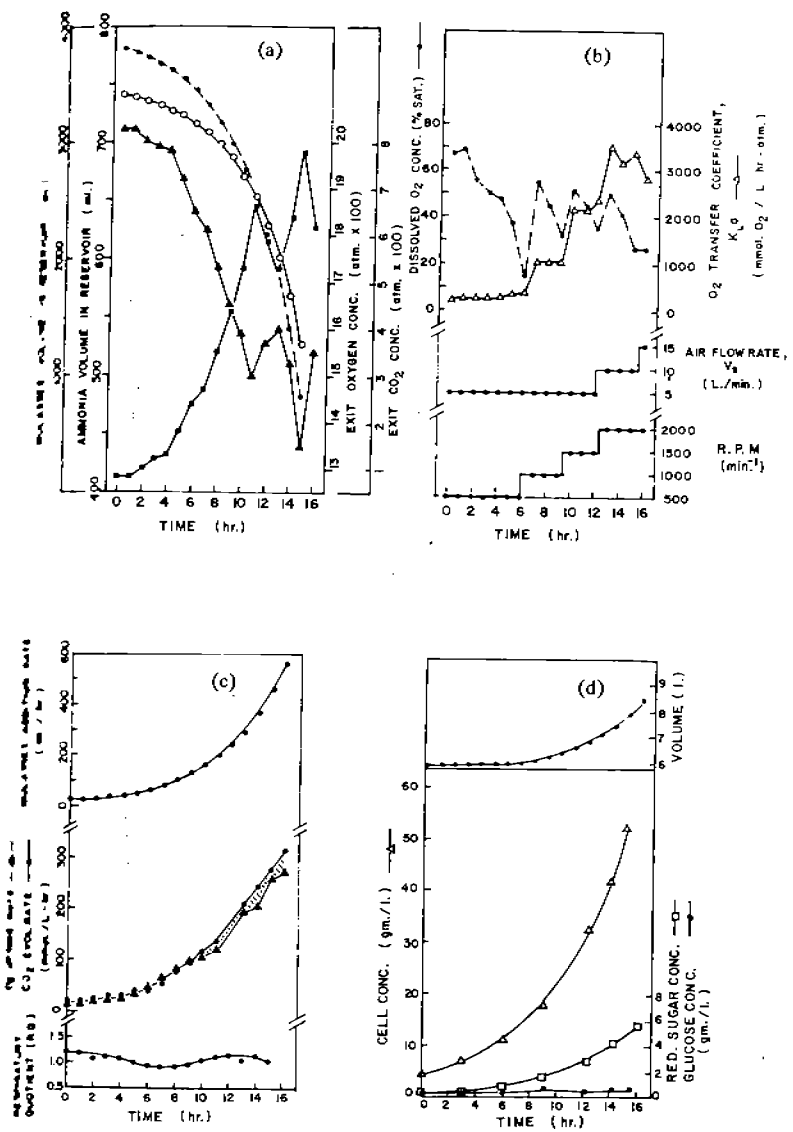

Fig. 1. Fermentation kinetics for the production of bakers' yeast on molasses at 30°C. (a)-(b) Values for directly measured parameters and the calculated oxygen-transfer coefficient (●) ammonia; (○) molasses; (▲) oxygen; (■) CO_2 (△) k_La. (c) Calculated values for (▲) oxygen uptake rate, (●) carbon dioxide evolution rate, RQ, and molasses addition rate. (d) Fermentor volume and measured values for (△) cell and sugar concentration; (□) reducing sugar, (●) glucose.

specific growth rate remained constant at about 0.2 hr^{-1}, and the overall cell yield was 0.51 g cells/g sugar.

Development of a Sugar-Feeding Schedule

The Zulauf, or fed-batch process for bakers' yeast production has been described by White[12] and, more recently, by Reed and Peppler.[11] The first step in developing a fed-batch process is to formulate a feeding schedule. This is usually done empirically, and the resulting fixed schedule does not allow for variations in the behavior of the system. Variations in the behavior of the fermentation are commonly caused by changes in inoculum size or condition, or by over- or underfeeding of sugar. Consequently, high conversion yields cannot be assured for every batch. If the sugar is fixed and its rate is too low, the productivity will then be lower than max-

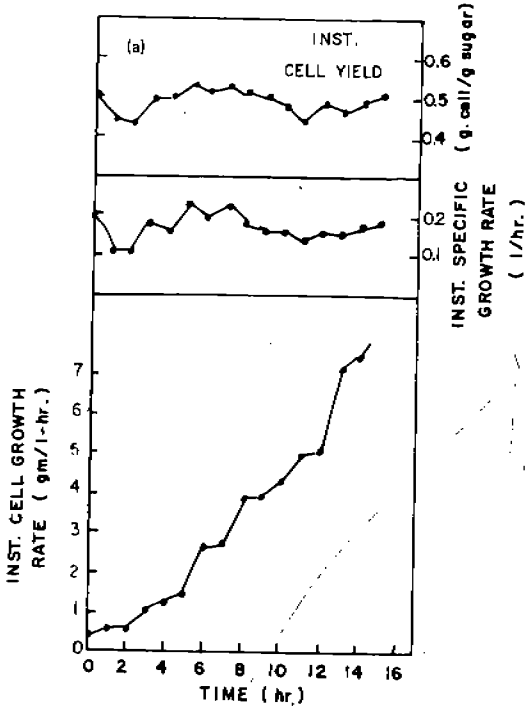

Fig. 2. Calculated instantaneous (a) and overall or cumulative (b) values for cell yield and growth rate during bakers' yeast growth on molasses. (△) Values for cell concentrations measured taken from Fig. 1(d) and shown for comparison.

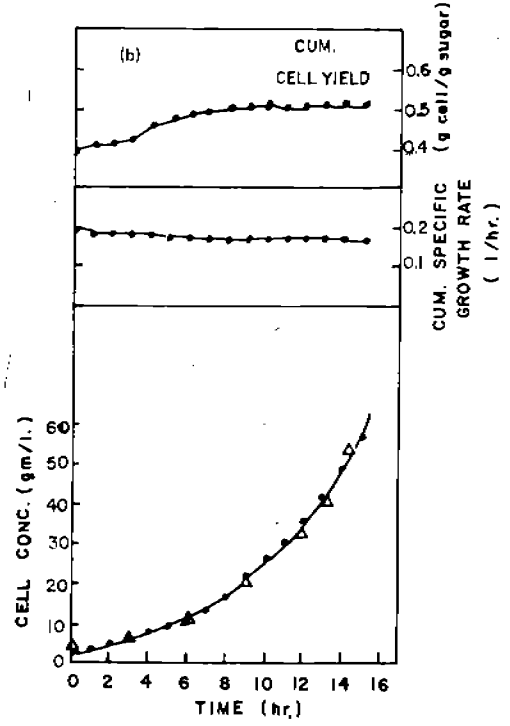

Fig. 2. *(Continued from previous page.)*

mum. Conversely, if the rate is too high, sugar will accumulate in the medium and lead to expression of the Crabtree effect,[13] in which ethanol is produced even in the presence of sufficient oxygen. Our objective is to develop a method for continuously assessing the state-of-the-fermentation and then calculating the demand for sugar.

In a fed-batch process, both the yeast concentration and the fermentation volume increase with time:

$$\frac{d}{dt}[X(t)\ V(t)] = \mu(t)\,[X(t)\ V(t)] \tag{2}$$

where $[X(t)\ V(t)]$ and $\mu(t)$ are, respectively, the total yeast biomass in the fermentor and the specific growth rate (hr^{-1}) of the yeast at any given time. Thus, the rate of molasses addition should increase accordingly. In an actual yeast fermentation, the final fermentation volume, V, can increase by 30 to 40%. If water loss through evaporation is ignored, one can account for the changes in

volume by

$$\frac{dV(t)}{dt} = F_s(t) + F_a(t) + F_n(t) \quad (3)$$

$F_s(t)$, $F_n(t)$, and $F_a(t)$ are the flow rates for molasses, other nutrients, and ammonia, respectively.

From a material balance for sugar, the following equation can be developed to express the rate of change of sugar concentration during fermentation:

$$\frac{d}{dt}[S(t)\ V(t)] = F_s(t)\ S_f - \frac{\mu(t)\ [X(t)\ V(t)]}{Y(t)} \quad (4)$$

$S(t)$ is the residual sugar concentration in the fermentor at any given time; S_f is the feed sugar concentration in the molasses and $Y(t)$ is the cellular yield coefficient.

To prevent the expression of the Crabtree effect, the concentration of sugar in the broth should be low, and to maintain optimal performance, it should be kept constant at its optimal value. Therefore the time derivative should be

$$\frac{dS(t)}{dt} = 0 \quad (5)$$

To fulfill this requirement, the molasses feed rate must equal the cellular demand at all times during the fermentation. It can be shown that the molasses feed rate $F_s(t)$ is a function of the total yeast biomass $[X(t)\ V(t)]$; specific growth rate, $\mu(t)$; and cellular yield coefficient, $Y(t)$, and is expressed as

$$F_s(t) = \frac{\mu(t)\ [X(t)\ V(t)]}{Y(t)S_f} \quad (6)$$

In order to achieve maximum cellular yield at high volumetric productivity, the relationship between the specific growth rate and the cell yield must also be known. Continuous-culture[14] and our batch-culture[2] studies have shown that the cellular yield coefficient is dependent on the specific growth rate (Fig. 3). At high specific growth rates, the cell yield is low because of ethanol formation and, at low growth rates the cell yield is low because of the maintenance energy requirement. **According to von Meyenburg,[14] the maximum specific growth rate without significant ethanol formation was around 0.25 hr^{-1} for *S. cerevisiae*; the maximum cellular yield was 0.5 g cell/g sugar. In the yeast industry, the feed schedule is**

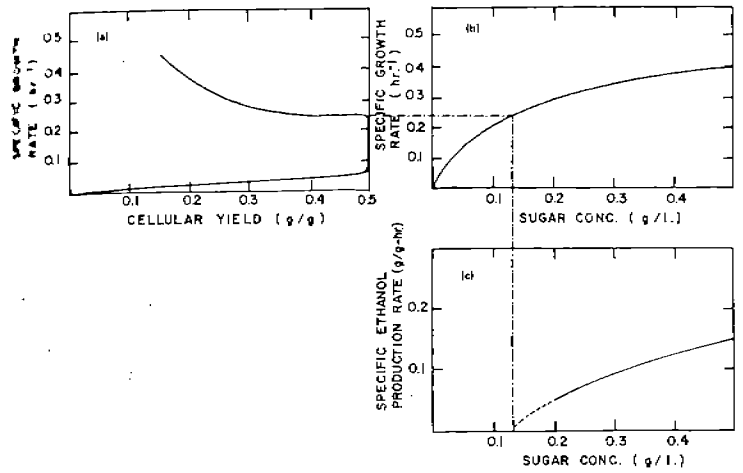

Fig. 3. Relationships between (a) specific growth rate and cell yield, (b) sugar concentration and specific growth rate, and (c) sugar concentration and ethanol production rate in a bakers' yeast fermentation.

usually designed to maintain a specific growth rate lower than 0.25 hr^{-1} in order to minimize ethanol formation.[11]

As the sugar concentration rises, the specific growth rate increases. The ethanol production rate, however, remains negligible until a value of 0.13 g glucose/liter is reached (Fig. 3(c)). This critical point correlates with a specific growth rate of 0.25 hr^{-1}. The relationships shown in Figure 3 define a range of acceptable growth rates and corresponding sugar concentrations that must be maintained in order to maximize the cell yield.

In order to control the molasses feed rate, it is necessary to know the total amount of yeast in the fermentor $[X(t)\ V(t)]$, at all times. This can be calculated by the indirect material-balancing method and knowledge of the fermentor volume (eq. (3)). Equation (5) is then used to calculate the rate, using the relationship in Figure 3 to define acceptable values. This approach is based on the specific demands of the yeast and is much more flexible than a fixed schedule.

A control strategy based on anticipation of the required sugar demand is a form of feed-forward control. However, in true feed-forward control, the controlled variable is not used as one of the inputs. Here, the controlled variable, molasses flow rate (F_s) is used to calculate the fermentor volume which in turn is used to

calculate F_s. Therefore, we have chosen to call this aspect of the control system anticipatory control.

To test this method of anticipatory control, experiments were conducted at preset specific growth rates (Fig. 4). Dissolved oxygen was maintained above 15% of air saturation. When the growth rate was maintained at 0.25 hr^{-1}, the resulting cell yield was only 0.36 g cell/ g sugar. The respiratory coefficient was continually greater than 1.0, indicating ethanol formation,[2] which is the most likely reason for the low yield. When the growth rate was controlled at 0.2 hr^{-1}, the productivity was predictably lower, but the cell yield rose to 0.49 g/g; the respiratory quotient was lower, indicating less alcohol formation.

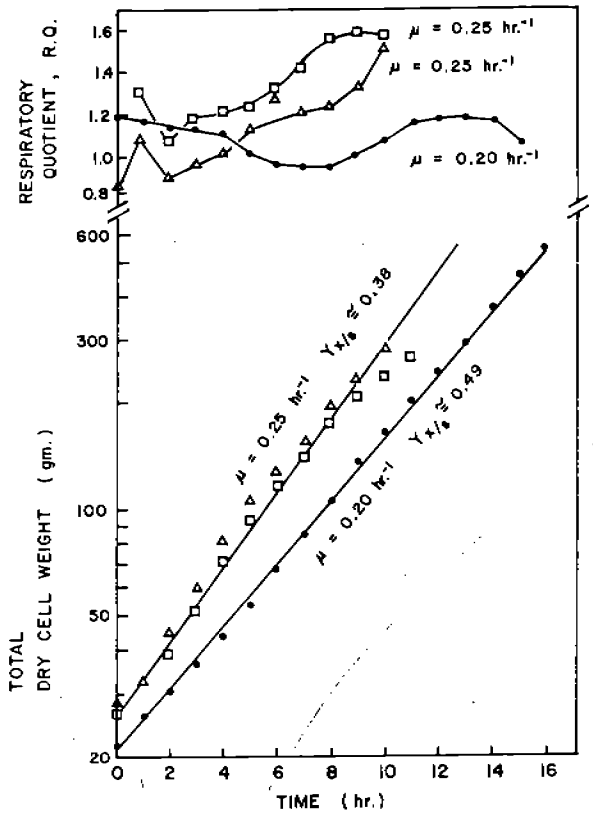

Fig. 4. Cell mass and RQ for bakers' yeast fermentations run under anticipatory control at growth rates of 0.25 and 0.20 hr^{-1}.

Oxygen-Limited Growth

Implicit in the use of eq. (5) is the assumption that growth is limited by the rate of sugar addition. However, as the concentration of yeast increases, so does the demand for oxygen and if this demand exceeds the oxygen-transfer capacity of the fermentor, the oxygen-transfer rate becomes growth limiting. If sugar is being added to maintain a high growth rate, problems will arise because glucose is metabolized to ethanol under oxygen limitation. One approach to this problem is to monitor the dissolved oxygen and when it falls below a critical point (i.e., less than 10% of air saturation) a constraint is placed on eq. (5). The major problem with this approach is that the routine measurement of low levels of dissolved oxygen is difficult.

An alternative approach is to utilize a direct or indirect method to monitor ethanol production and then use this signal to decrease the flow rate of molasses addition.

Detection of Ethanol Production

The respiratory quotient (RQ) correlates well with the ethanol production rate.[2] This has also been substantiated by Aiba et al.[7] Since the oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER) are already computed for the indirect assessment of cell growth, no additional instrumentation is needed for calculating the RQ value.

In experiments where only the RQ value was used to control the molasses addition by allowing the molasses flow rate to increase until RQ rose above 1.0, the molasses flow rate was found to oscillate. Moreover, ethanol production at a RQ value greater than 1.0 at a low cell concentration is quite different than at a high cell concentration; this is seen by the comparison in Table I. The ab-

TABLE I
EtOH Production Rate at Different O_2 Uptake Rates with the RQ Maintained at 1.2

O_2 uptake rate (mmol/liter-hr)	CO_2 evolution rate (mmol/liter-hr)	EtOH production rate (mmol/liter-hr)
30	36	6
50	60	10
100	120	20
150	180	30
300	360	60

$EPR = CER - C_1(OUR)$ $C_1 = 1.2$ in absence of EtOH ie. EtOH = 0

solute value of the EPR is actually proportional to the differe... between the CER and the OUR:

$$\text{EPR} = \text{CER} - C_1(\text{OUR})$$

where C_1 is the RQ value in the absence of ethanol formation.

For these reasons, we elected to use the difference between CE... and OUR as our feedback control parameter, as shown in t... following equation:

$$F_{ss} = F_s\,[1 - K_c\,(\text{CER} - (C_1)\,\text{OUR})] \qquad (...$$

where F_{ss} is the corrected molasses flow rate and K_c is the co... troller gain. Theoretically, the difference between CER and OU... should be more sensitive than RQ values for control of yeast fe... mentations. It should be noted, however, that the difference fun... tion for gas exchange data requires accurate measurement of th... air flow rate, while calculation of RQ does not.

Combined Anticipatory/Feedback Control

A complete strategy for controlling molasses flow in fed-batch fermentations, based on the indirect cell measurement technique and incorporating eq. (8) for feedback control, has been developed as outlined in Figure 5. The calculations are initiated by introducing values for inoculum size and fermentor liquid volume. Values for the desired specific growth rate and cell yield coefficient are also specified. The starting value for F_s is arbitrary, but here it is the lowest value of the controlled pump which is 10 ml/hr.

Every 10 min, a new cell concentration and liquid volume are computed through material balances.[1,2] The molasses flow rate is constantly calculated and adjusted every 5 sec, according to eq. (8). In this manner, the respiratory quotient can be maintained at a value around 1.0 throughout the fermentation. To achieve greater versatility in the control scheme, a correction factor (A8 in Fig. 5) with an initial value of 1.0 is incorporated into the molasses flow rate calculation. After every 10 min, an overall RQ value is calculated and compared with the set point. If it is persistently higher than the set point, then the correction factor would be decreased accordingly to adjust the flow rate to the physiological condition of the cells. Similarly, if the RQ value decreases during the fermentation, the correction factor would increase, causing the specific growth rate of the yeast to return to the maximum permissible without ethanol formation.

Exact values for the control parameters needed to be identified

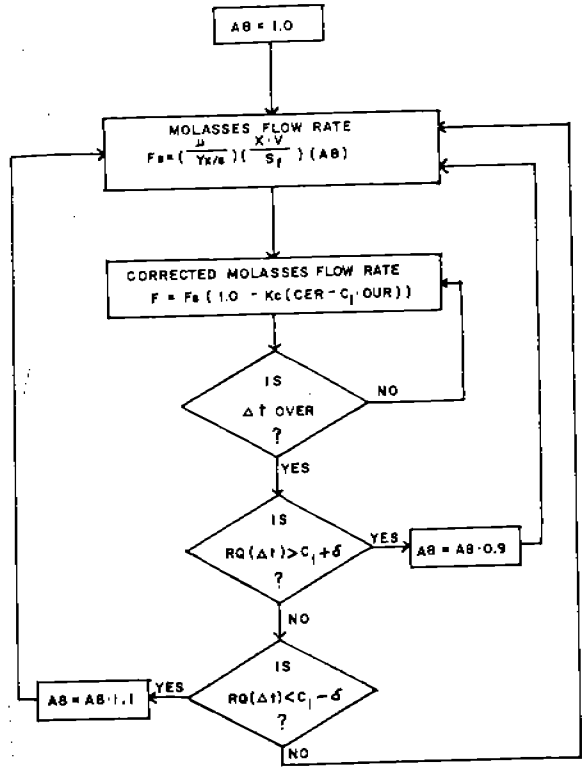

Fig. 5. Flow sheet to show the execution of the anticipatory feedback control system for control of molasses addition during yeast production. Details are described in the text.

The specific growth rate at low cell density can be present at a high value (e.g., > 0.25 hr^{-1}) to start the fermentation rapidly; however, as growth proceeds, the specific growth rate and, simultaneously, the correction factor (A8) gradually decrease. In order to minimize ethanol formation, the overall specific growth rate was found to adjust itself to about 0.2 hr^{-1}. Theoretically, the RQ set point (C_1) should have been around 1.0; however, the error in this measured variable was about ±5% because of drift in the gas analyzers over a time span of 24 hr. Therefore, the RQ set point was set between 1.05 and 0.95. A lower set point value can be used, but leads to a low overall specific growth rate. Three fermentations using different values of C_1 are compared in Figure 6.

We found by experimentation that a value of 1.01–1.02 liter-hr/

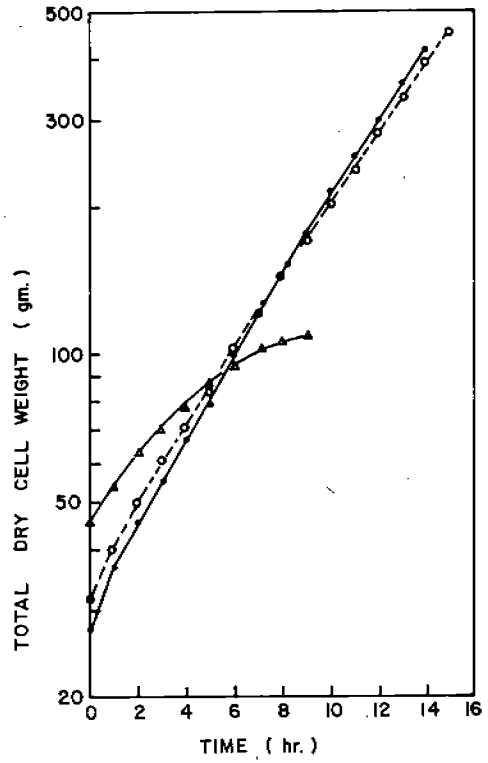

Fig. 6. Several yeast fermentations controlled with different RQ set points. (○) $C_1 = 1.05$, (●) $C_1 = 0.95$; (△) $C_1 = 0.90$; $\mu_0 = 0.20\ hr^{-1}$; $K_c = 0.01$.

mmol was quite satisfactory for our controller gain; larger values result in oscillations of the molasses flow and lower values cause constant offset. These phenomena are consistent with conventional control theory.

If the exit gas analyzers or other measuring devices used in this control scheme have poor sensitivity or slow response, then the time to readjust the molasses flow rate should be lengthened to avoid premature adjustment. A poor pH control system which allowed fluctuation in pH might lead to short-term errors in carbon dioxide evaluation, since CO_2 solubility is very pH dependent.

With the operating parameters identified, an experiment to test this control scheme was conducted and is described in Figure 7. The initial preset values for specific growth rate and cell yield were 0.25 hr^{-1} and 0.5 g cells/ g sugar, respectively. The dissolved oxygen

was maintained above 15% of air saturation to avoid oxygen limitation. Initially, the specific growth rate could be maintained above 0.25 hr^{-1} without significant ethanol production; however, as the fermentation progressed, the specific growth rate gradually decreased to a final value of 0.2 hr^{-1}. The RQ value at the end of fermentation was around 1.05, the limit value for the control system.

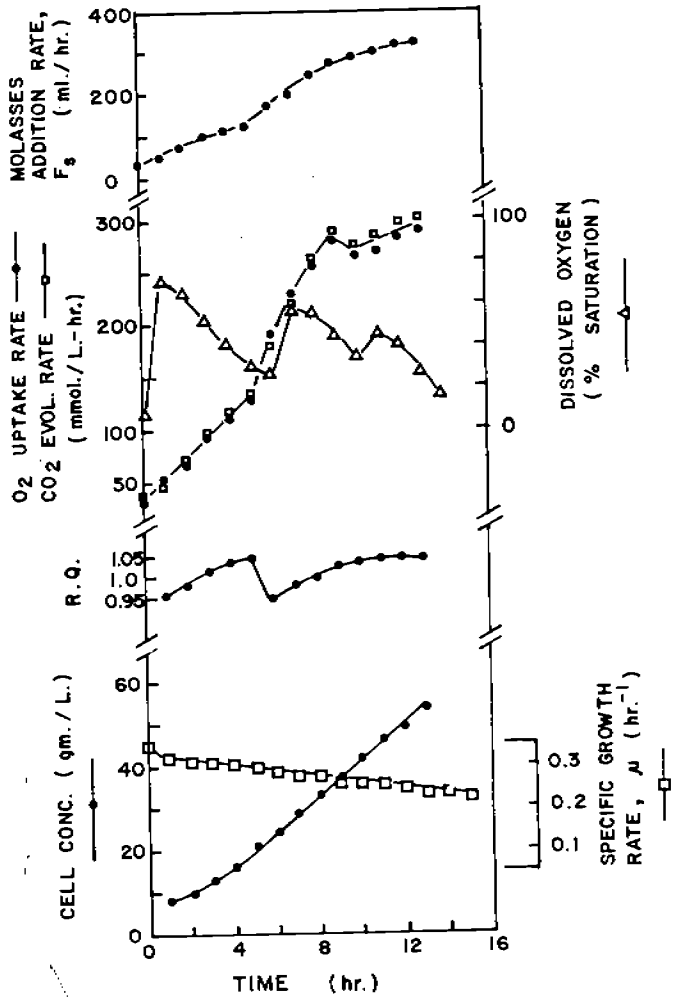

Fig. 7. Fermentation kinetics of bakers' yeast production under combined anticipatory and feedback control.

The overall cell yield was 0.5 g cells/ g sugar, showing that ethanol formation was avoided and the final cell concentration was 55 g/liter.

In order to demonstrate the general validity of the control system various process perturbations were designed to stimulate variations that may occur during commercial fermentations and to demonstrate the flexibility of the control scheme under these conditions.

One of the major constraints in a yeast fermentation is the oxygen

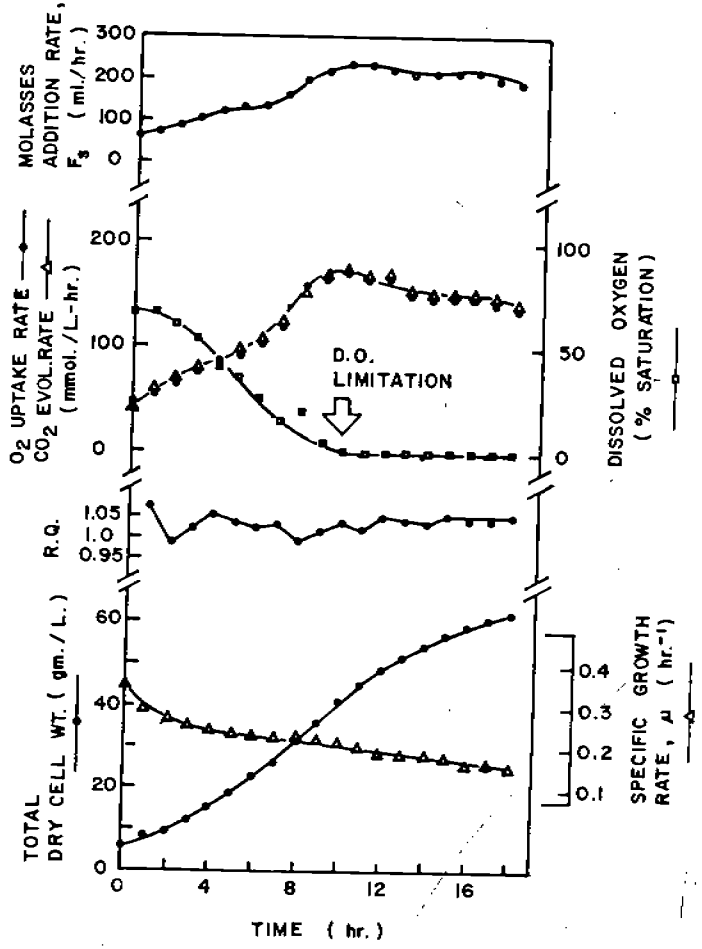

Fig. 8. Fermentation kinetics for a computer-controlled yeast fermentation allowed to become oxygen limited after the 9th hr.

transfer capacity of the fermentor. In typical commercial fermentations final yeast solids in the range of 40–45 g/liter are encountered.[15] In order to meet the oxygen-transfer limitations, it is often the practice in industry to maintain a minimal specific growth rate as low as 0.1 hr^{-1} during the last hours of fermentation. When dissolved oxygen falls to zero, the molasses addition rate must be readjusted because the specific growth rate then depends on the oxygen-transfer rate. In order to stimulate this situation, the maximum oxygen-transfer rate was fixed by maintaining the impeller speed and air flow rates constant at 1000 rpm and 5 liter/min, respectively. As shown in Figure 8, after 9 hr the dissolved oxygen approached zero; the molasses addition rate was immediately readjusted through computer control and therefore maintaining the RQ below 1.05.

In an industrial process, the inoculum must be prepared as aseptically as possible and precautions are taken to reduce contamination by storing the inoculum at low pH and temperature. As a consequence, the age and viability of the inoculum vary, and frequently ethanol formation is high during the first few hours of fermentation because the rate of molasses addition is greater than the demand. This problem can easily be solved. Two fermentations were run to

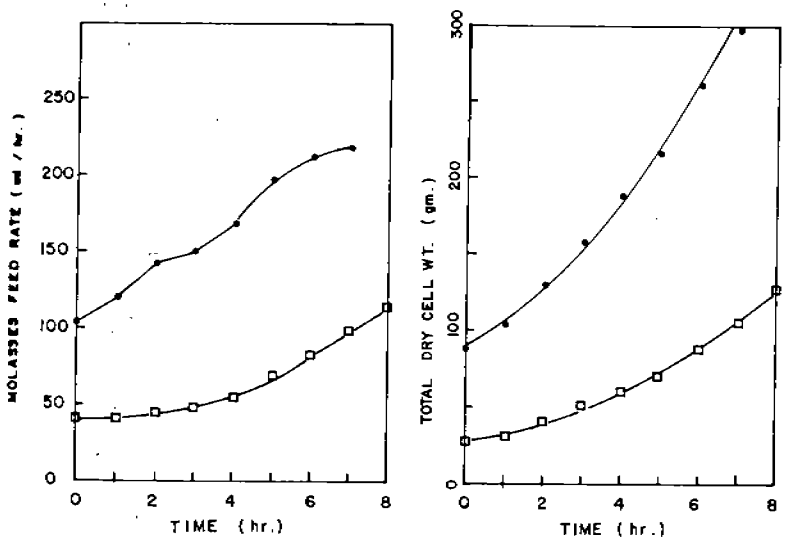

Fig. 9. Molasses flow rate and cell concentration for two different initial cell concentrations: (○) 12 g/liter; (□) 4.5 g/liter.

simulate variations in the biological activity of the inoculum, w using different inoculum concentrations (e.g., 4.5 and 12 g/liter). The results (Fig. 9) show that the molasses flow rate is adjusted t accommodate the different inocula.

The ultimate test of the control system was designed by using completely unknown source of molasses (obtained from a barrel of blackstrap molasses which had been stored in the Department of

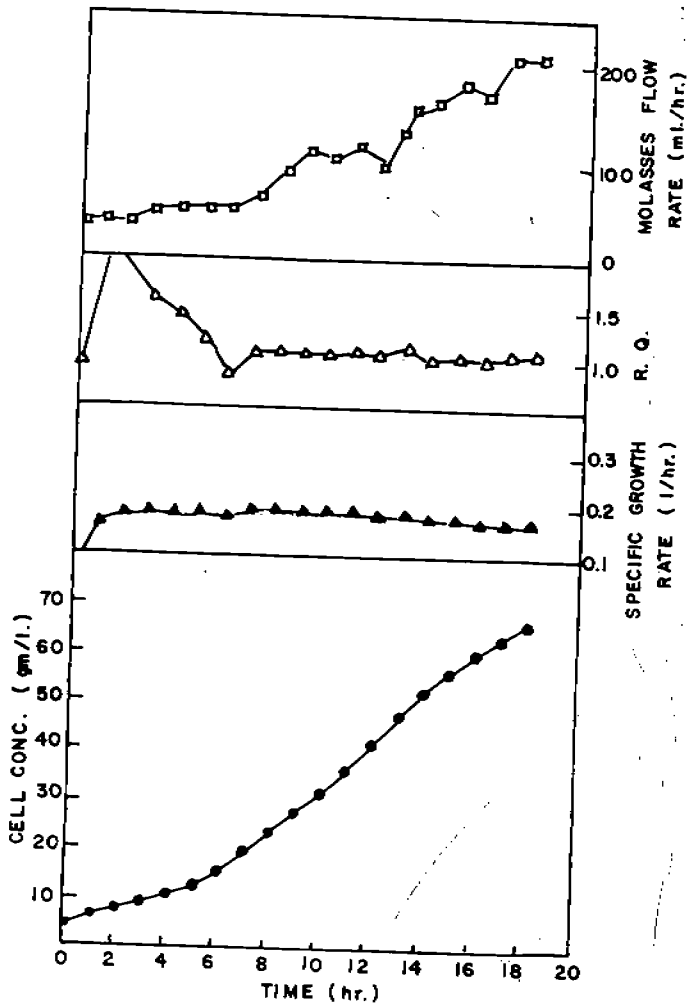

Fig. 10. Fermentation kinetics for a computer-controlled yeast fermentation with molasses of undefined quality and an aged inoculum. $Y_{x/s}$ = 0.48 g cell/g sugar.

TABLE II
Comparison of Computer-Controlled Yeast Fermentations with Different Initial Cell Concentrations

Initial cell conc (g/liter)	Initial fermentation volume (liter)	Final cell conc (g/liter)	Final fermentation volume (liter)	Fermentation time (hr)	Total molasses used (ml)	Overall cell yield (g cells/g sugar)	Volumetric productivity (g/liter-hr)
3.8	5.9	59.1	9.3	17.0	3156	0.49	3.3
4.9	5.8	52.0	8.6	16.0	2568	0.49	3.1
5.8	6.8	54.6	10.6	12.8	2998	0.50	4.0
8.9	6.4	54.1	9.0	14.0	2494	0.51	3.4
9.0	6.0	49.3	8.4	11.7	2136	0.49	3.7
14.4	5.8	63.2	8.6	10.2	2738	0.50	5.2

Nutrition and Food Science, M.I.T., for at least 10 years) and yeast inoculum that had been stored in the refrigerator (4°C) for more than two months. The experiment described in Figure 10 was started by setting the initial specific growth rate at 0.25 hr^{-1}. During the first few hours, the respiratory quotient reached values above 20 indicating ethanol production. Because the lowest molasses flow rate in our system is not zero but close to 10 ml/hr, the RQ value remained high for several hours before proper control action could be exercised. The control system allowed the RQ value to return to the set point of 1.0 and then maintained it around 1.05 throughout the remainder of the fermentation. The specific growth rate was maintained at around 0.2 hr^{-1} and the final cell yield was 0.48 g cells /g sugar.

The reproducibility of each batch is important in commercial fermentations. The control scheme presented here allows greater flexibility in operation than a fixed schedule. The consistency of this control is seen by comparing the results of a variety of fermentations in Table II. These experiments were performed with inoculum concentrations ranging from 3.8–14.4 g/liter. The overall cellular yield was consistently high at about 0.5 g cells/g sugar. Volumetric productivity varied from 3 to 5 g/liter-hr. These values are considerably higher than those reported in the literature.[15]

Nomenclature

A8	correction factor used in control strategy
C_1	value of RQ in the absence of ethanol production
CER	carbon dioxide evolution rate (mmol/liter-hr)
EPR	ethanol production rate (mmol/liter/hr)
$F_a(t)$	ammonia flow rate (liter/hr)
$F_n(t)$	nutrient flow rate (liter/hr)
$F_s(t)$	molasses flow rate (liter/hr)
F_{ss}	corrected molasses flow rate (liter/hr)
K_c	controller gain
OUR	oxygen uptake rate (mmol/liter-hr)
RQ	respiratory quotient (mmol CO_2/mmol O_2)
t	time (hr)
S_f	concentration of sugar in molasses (g/liter)
$X(t)$	cell concentration (g/liter)
$V(t)$	fermentor liquid volume (liter)
$Y(t)$	cell yield (g cell/ g sugar)
δ	deviation between calculated and desired value of C_1
μ	specific growth rate (hr^{-1})

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