Real-Time Estimation of Aerobic Batch Fermentation Biomass Concentration by Component Balancing

Real-time estimates of biomass concentration and growth rate in fermentation processes were obtained by performing a material balance on oxygen and employing a kinetic model for molecular oxygen utilization. A model containing yield and maintenance terms was satisfactory in fermentations where only the EMP and TCA pathways were utilized for glucose metabolism. However, model alterations based on metabolic energetics were required before accurate estimates were obtained for a metabolically complex fermentation producing bakers yeast.

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SCOPE

As economic pressures continue to build, research in process control is expanding rapidly to improve the efficiency of fermentation processes. A prerequisite to achieving closed loop control is the ability to perform real-time process variable measurements for feedback information to the control algorithms. This fact has inspired the recent development of on-line sensors suitable for fermentation process measurements including dissolved oxygen, glucose concentration, specific ion activities, redox potential, etc.

Biomass concentration and growth rate are two of the most fundamental fermentation process variables. However, efforts to design instruments capable of measuring these quantities on-line have generally been unsuccessful. Despite a broad variety of chemical, physical, and microbiological assays which are available (Herbert et al., 1971; Mallette, 1971) to measure these variables in the laboratory, the time delays per assay are too long to form the bases for on-line instruments sensitive to cell concentration and growth rate. The only class of techniques with adequate response times are ones which measure optical properties of the cell suspensions (Mallette, 1971; Wyatt, 1973). Unfortunately, these methods are not applicable to most practical fermentations owing to growth on the optical surfaces, low measurement sensitivity, mycelial culture morphology, nonhomogeneity of culture samples, and the high levels of coloration and suspended solids characteristic of industrial media.

In response to this need for on-line biomass concentration and growth rate data, attention has focused on procedures which provide estimates indirectly by obtaining process variable data which can be measured on-line and which are associated with culture growth. These include culture viscosity (Swartz et al., 1975), heat evolution (Cooney et al., 1968; Ericksson and Holme, 1973; Forrest, 1971), carbon dioxide evolution (Stouthamer, 1971), oxygen uptake (Jefferis and Humphrey, 1972; Jefferis et al., 1972), substrate consumption and product synthesis (Cooney et al., 1977; Wang et al., 1977; Minkevich and Eroshin, 1973). Accounts employing this principle are beginning to appear in the literature, but supporting experimental evidence remains a rarity.

Currently, there are two approaches for indirect estimation. The first is to stoichiometrically balance all of the principal chemical components which compose the biomass and products (C, H, O, N). The estimates are obtained by measuring enough of the components considered to effect closure of the system of stoichiometric equations (Cooney et al., 1977; Wang et al., 1977). The second approach, which is pursued in this project, is to consider only one chemical component and mathematially model the relationship of that species with culture growth (Zabriskie, 1976; Zabriskie et al., 1976).

The objective of this study was to provide indirect estimates of biomass concentration and growth rate of sufficient accuracy and response that the results would be applicable to on-line process control algorithms. Molecular oxygen was selected as the component to balance because of its close relationship to the energetics of growth and the ease with which it can be continuously measured in gaseous and aqueous phases. There are several practical advantages to this estimation procedure: results may be continuous and fully automatic; samples of the culture broth are not required; response is rapid, limited only by the response of the gas analyzers and calculation time; the approach is applicable to a broad spectrum of aerobic batch fermentations; and the required instrumentation is easily interfaced to industrial scale installations and introduces little risk to aseptic performance.

CONCLUSIONS AND SIGNIFICANCE

Correlations were established relating biomass concentration and growth rate with the molecular oxygen uptake and carbon dioxide evolution rate data. A numerical solution of the differential equation derived from a yield and maintenance term model for molecular oxygen consumption provided the basis for the correlations. Accurate estimates were obtained for aerobic fermentations that primarily produced metabolic energy by the reference sequence. The reference sequence designates the assimilation of glucose by the EMP (Embdem-Meyerhof-Parnas) pathway and the tricarboxylic acid (TCA) cycle to produce carbon dioxide, water and ATP (adenosine triphosphate) energy.

This model did not provide satisfactory correlations for

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experimental data from the aerobic bakers yeast fermentation. This was interpreted to reflect the greater complexity of yeast metabolism which produces partially oxidized intermediates by mechanisms which act simultaneously with the reference sequence to produce energy. Correlations of equivalent accuracy to the earlier cases were obtained by reexpressing the model parameters as functions. These functions were composed of a constant, defined for the reference sequence, and a metabolic correction function β used to account for variations away from reference sequence behavior. The form of β was determined from theoretical considerations of energy production rates. When we compensate for the effects of ethanol, β is a function of the respiratory quotient. Corrections beyond those for ethanol could not be statistically justified.

These procedures were extended to provide on-line estimates of biomass concentration and growth rate in a manner similar to the way they would be applied for real-time process control. Model parameters were used from the analysis of a previous experiment. Oxygen uptake rate and carbon dioxide evolution rate were measured during the experiment. The results were calculated essentially instantaneously using a real-time digital computer which was interfaced to the instrumentation.

The accuracy of both the correlation and real-time estimates was limited by the accuracy of the oxygen uptake rate data. Several options are available to minimize the source of these errors which will permit the reliable use of these estimates in the control of a variety of aerobic batch fermentations.

The estimation of biomass concentration and growth rate from the consumption and production of other components in the culture that can be readily measured is a classical technique in fermentation technology. The most frequently employed procedure follows from the elementary kinetic model:

$$-\frac{dS}{dt} = \frac{1}{Y^*_{X/i}} \frac{dX}{dt} \tag{1}$$

The integrated form of this equation is used to obtain the estimates for biomass concentration:

$$X = X_o + Y^*_{X/i}(S_o - S) \tag{2}$$

The accuracy of the estimates derived from Equation (2) are frequently low in practical fermentations owing to the apparent variability of global yield constant $Y^*_{X/i}$, especially during the latter phases of growth.

This observation, coupled with the fact that some substrate consumption or product formation could occur in the absence of growth, led to the introduction of another model (Marr et al., 1962; Pirt, 1965):

$$-\frac{dS}{dt} = M_{i/X}X + \frac{1}{Y_{X/i}} \frac{dX}{dt} \tag{3}$$

The interpretation of this equation for a substrate is that the utilization of the component may be conceptually partitioned into two parts. The first term refers to the substrate used to support cell viability, even in the absence of growth. These activities include cell motility, enzyme turnover, osmotic work, nutrient storage, and other processes referred to as maintenance functions. The second term reflects the additional substrate required to support reproduction and growth. During the course of a normal batch fermentation, the second or yield term dominates during the first stage of the cultivation when growth is at a maximum and the cell density is low. Toward the end of the fermentation, growth begins to slow as substrates deplete and inhibitory products accumulate. The diminishing growth rate and high cell density cause the maintenance term to dominate in this latter stage. This model accounts for the apparent variability of the global yield defined in Equation (1).

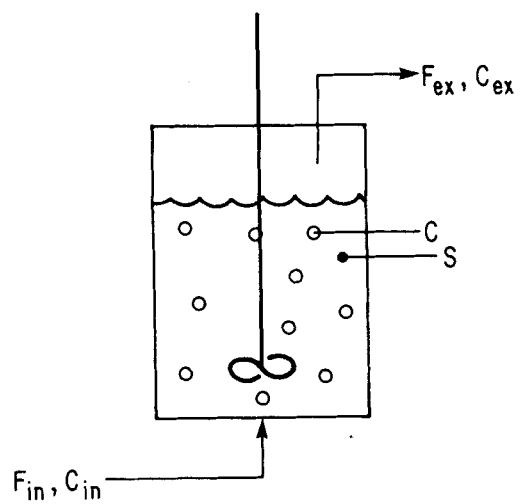

Fig. 1. Batch fermentor material balance of a component in the gas phase.

The application of the yield-maintenance model for predicting biomass concentration was first proposed by Jefferis and Humphrey (1972) using molecular oxygen as the balanced species. However, the absence of supporting assumptions, numerical procedures, and experimental data detracted from the importance of the work.

The foundation for the estimation of biomass concentration from substrate utilization or product synthesis data is the making of a component balance around the fermentor. Figure 1 is a schematic of the balance when the species of interest enters and leaves the vessel in the gas phase. The respective gas and aqueous phase component balances are

$$\frac{d}{dt}(V_G C) = F_{in} C_{in} - F_{ex} C_{ex} - J_i \tag{4}$$

and

$$\frac{d}{dt}(V_A S) = J_i - R_A \tag{5}$$

The common term expressing the rate of interfacial mass transfer J_i is eliminated by adding the Equations (4) and (5) to form

$$R_A = -\frac{d}{dt}(V_G C + V_A S) + (F_{in} C_{in} - F_{ex} C_{ex}) \tag{6}$$

The aqueous phase reaction rate term is modeled using Equation (3) to give

$$R_A = -V_A \frac{dS}{dt} = V_A \left[M_{i/X} X + \frac{1}{Y_{X/i}} \frac{dX}{dt} \right] \tag{7}$$

Substitution of Equation (7) into Equation (6) yields

$$-\frac{d}{dt}\left[\frac{V_G C + V_A S}{V_A}\right] + \left[\frac{F_{in}C_{in} - F_{ex}C_{ex}}{V_A}\right] = M_{i/X}X + \frac{1}{Y_{X/i}} \frac{dX}{dt} \tag{8}$$

When molecular oxygen is the species to be balanced, the

TABLE 1. SUMMARY OF EXPERIMENTAL CONDITIONS

Species	Morphology	Medium	Temp., °C	pH	Industrial products
Thermoactinomyces sp.	Mycelial	5% suspensions of cellulose—synthetic and defined	55	7.2	Single-cell protein
Streptomyces sp.	Mycelial	1% corn-steep liquor 2.3% glucose	30	6.0	Enzymes, antibiotics
Saccharomyces cerevisiae bakers yeast	Unicellular	2% corn-steep liquor glucose added on demand	30	5.0	Bakers yeast, ethanol, fine biochemicals, enzymes, vitamins, amino acids, CO_2

dynamic accumulation term [first term in brackets in Equation (8)] is insignificant under practical fermentation conditions and may be considered to be zero (Zabriskie, 1976). The remaining term involving oxygen concentration [second term in brackets in Equation (8)] is given a special name, the oxygen uptake rate or OUR, and is a quantity which is measured on-line. Therefore, Equation (8) reduces to

$$\text{OUR} = M_{O_2/X}X + \frac{1}{Y_{X/O_2}}\frac{dX}{dt} \tag{9}$$

The solution of Equation (9) is easily obtained by specifying the boundary condition at time zero when the initial biomass concentration (X_o) is known:

$$X(t) = \exp(-M_{O_2/X}\,Y_{X/O_2}\,t)\left[\int_o^t Y_{X/O_2}\exp(M_{O_2/X}\,Y_{X/O_2}\,t)\,\text{OUR}(t)\,dt + X_o\right] \tag{10}$$

Equation (10) forms the basis for estimating biomass concentration $X(t)$ from oxygen uptake rate data. The growth rate may be approximated using the estimates of $X(t)$ provided by Equation (10) and a rearrangement of Equation (9):

$$\frac{dX}{dt} = Y_{X/O_2}\,\text{OUR}(t) - M_{O_2/X}\,Y_{X/O_2}\,X(t) \tag{11}$$

CORRELATION PROCEDURES

The values of parameters Y_{X/O_2} and $M_{O_2/X}$ were obtained from off-line experiments in which oxygen uptake rate and biomass concentration data were available. A rearrangement of Equation (9) suggests one way in which the constants can be calculated:

$$Q_{O_2} = \frac{\text{OUR}}{X} = M_{O_2/X} + \frac{1}{Y_{X/O_2}}\left[\frac{1}{X}\frac{dX}{dt}\right] \tag{12}$$

A plot of the specific oxygen uptake rate Q_{O_2} vs. the specific growth rate $\frac{1}{X}\frac{dX}{dt}$ should give a straight line of slope $1/Y_{X/O_2}$ and intercept $M_{O_2/X}$ as suggested by Pirt (1965).

Difficulties arise, however, when we try to differentiate the relatively sparse biomass concentration data to obtain the growth rate. Therefore, an integrated form of Equation (12) has been used in this research (Zabriskie et al., 1976):

$$\frac{\int_o^t \text{OUR}(t)\,dt}{\int_o^t X(t)\,dt} = M_{O_2/X} + \frac{1}{Y_{X/O_2}}\left[\frac{X - X_o}{\int_o^t X(t)\,dt}\right] \tag{13}$$

Both integrations were performed using the method of trapezoids. This method is accurate in integrating the OUR data owing to a high data frequency (12 points/hr). This technique is less accurate in the integration of the biomass concentration data, although it is more accurate than an alternate method which numerically integrates polynomials derived from a curvilinear regression analysis. The slope and intercept values of the plot suggested by Equation (13) were obtained by a linear least-squares analysis.

The values of Y_{X/O_2} and $M_{O_2/X}$ obtained by this procedure were refined using a simplex optimization technique (Pierre, 1969) which minimized the sum of the deviations (error) squared between data and estimated values of $X(t)$ obtained by Equation (10).

The value of X_o arising from the boundary condition specification, was approximated by estimating the biomass concentration in the inoculum and accounting for the dilution which occurred when introducing the inoculum into the fermentor. The accuracy of the X_o estimate is not critical under most practical growth conditions in which the inoculum is less than 10% of the final cell density. Under these circumstances, the sensitivity of $X(t)$ to X_o diminishes rapidly after the first several hours of growth (Harima, 1977).

MATERIALS AND METHODS

Three aseptic fermentation types were studied to evaluate the degree of correlation between biomass concentration and oxygen uptake rate data. A summary of the microbial strain characteristics and fermentation conditions are shown in Table 1. These experiments were chosen to reflect conditions typical of industrial fermentations and to facilitate the making of a preliminary assessment pertaining to the generality of these procedures.

The batch cultivations were made using a 70 l aseptic fermentor. Culture environmental conditions were monitored and controlled by an on-line instrumentation package which was interfaced to a real-time digital minicomputer (PDP 11E10, Digital Equipment Co., Maynard, Mass.). Variables which were measured during these experiments included culture temperature, pH, dissolved oxygen tension, pressure, agitation speed, sparging gas flow rate, culture fluorescence, gaseous carbon dioxide and oxygen concentrations, and broth glucose concentration. Culture temperature and pH were controlled at the values indicated in Table 1. Dissolved oxygen tension was maintained above 3.0 ppm by varying the rates of agitation and sparging gas flow.

Of special importance to this project were the analyzers used in the measurement of oxygen and carbon dioxide in the gases entering and leaving the fermentor. Oxygen was measured by a paramagnetic wind analyzer (Mine Safety Appliances, Inc., Pittsburgh, Pa., model 802, range 15 to 25% accuracy specification ± 0.5% oxygen). Carbon dioxide was measured by the infrared absorption by the gas in a temperature controlled cell (Mine Safety Appliances, Inc., Pittsburgh, Pa., model 303, ranges 0 to 4% and 0 to 32%, accuracy specification ± 0.08% and ± 0.64% carbon dioxide, respectively).

Biomass concentration was determined in the laboratory using two procedures. The dry weight of cells from a sample of known volume was measured for the *Saccharomyces cerevisiae* and *Streptomyces sp.* fermentations. The presence of cellulose particulates in the *Thermoactinomyces sp.* experiments precluded the use of a dry weight procedure. In this case, biomass con-

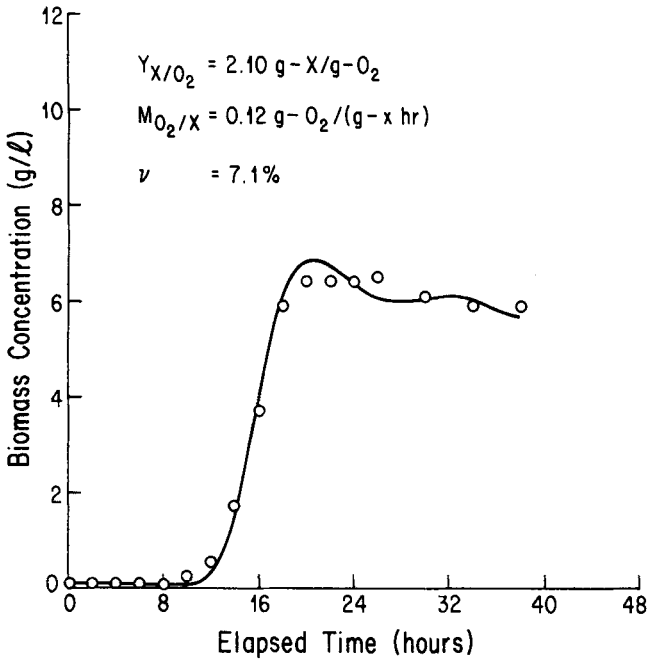

Fig. 2. *Thermoactinomyces sp.* biomass concentration data (○) and correlation results (continuous line).

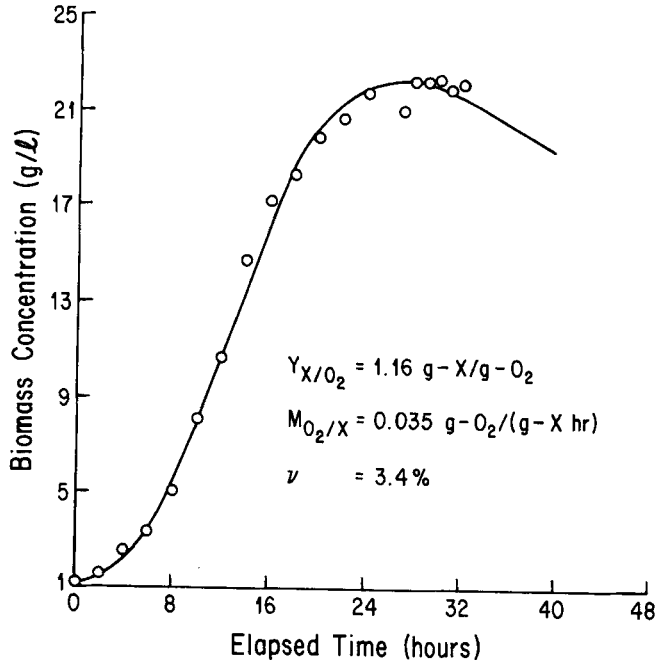

Fig. 3. *Streptomyces sp.* biomass concentration data (○) and correlation results (continuous line).

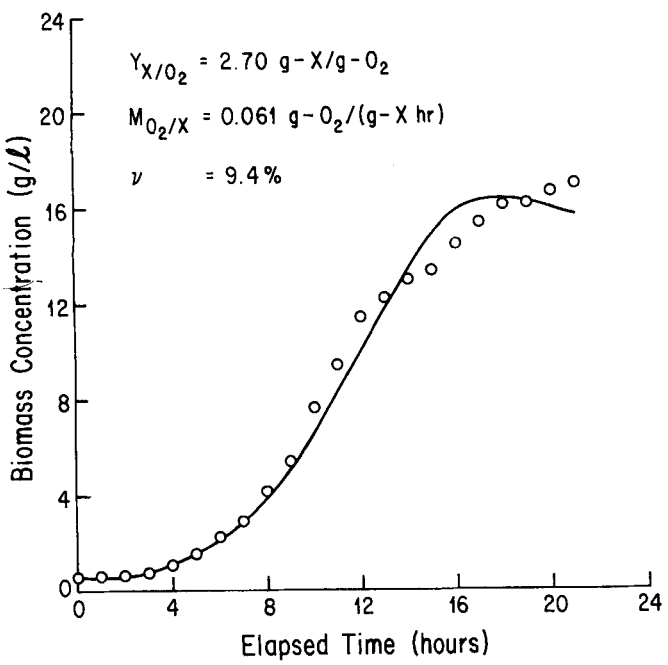

Fig. 4. *Saccharomyces cerevisiae* biomass concentration data (○) and correlation results (continuous line).

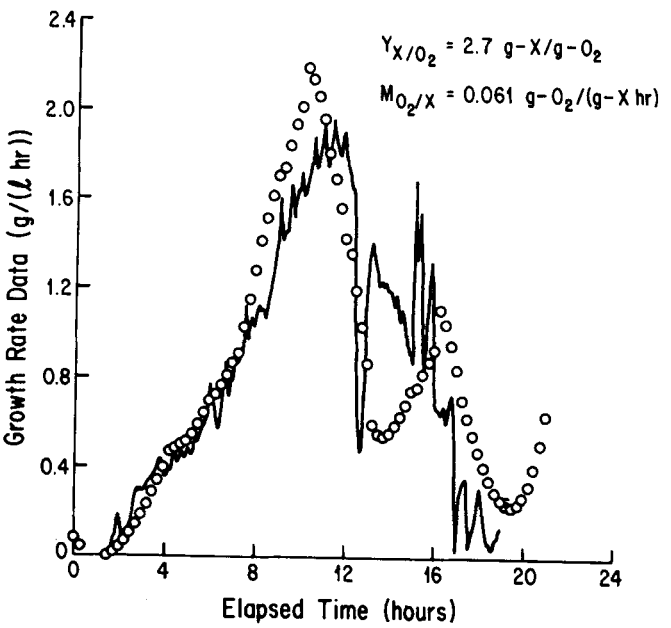

Fig. 5. *Saccharomyces cerevisiae* growth rate data (○) and correlation estimates (continuous line).

TABLE 2. STOICHIOMETRIC BALANCES OF THE PRINCIPAL METABOLIC ENERGY PRODUCING PATHWAYS OF YEAST GROWN AEROBICALLY ON GLUCOSE

Glucose assimilation period	Y^*_{ATP/O_2}
(a) Glucose + 6 O_2 + 36 ADP → 6 CO_2 + 6 H_2O + 36 ATP	6.0
(b) Glucose + 2 ADP → 2 CO_2 + 2 Ethanol + 2 ATP	∞
(c) Glucose + 2 O_2 + 10 ADP → 2 CO_2 + 2 Acetate + 2 H_2O + 10 ATP	5.0
Diauxic growth period	
(d) Ethanol + 3 O_2 + 14 ADP → 2 CO_2 + 3 H_2O + 14 ATP	4.7
(e) Acetate + 2 O_2 + 10 ATP → 2 CO_2 + 2 H_2O + 10 ATP	5.0

centration was determined by measuring the nitrogen content of the solids (cellulose is nitrogen free) using a micro Kjeldahl procedure. Further experimental details are available in Zabriskie (1976).

CORRELATION ANALYSIS OF EXPERIMENTAL DATA

The correlation analysis consisted of determining the parameters for each fermentation using the OUR data and biomass concentration data derived from laboratory procedures. This was followed by a comparison of the laboratory biomass concentration data with the estimates obtained using these parameter values, the OUR data, and Equation (10). Typical results are shown for *Thermoactinomyces sp.*, Figure 2, *Streptomyces sp.*, Figure 3, and *Saccharomyces cerevisiae*, Figure 4. The discrete points represent the laboratory data, and the continuous line is

the estimate provided by Equation (10). The success of the correlation procedure was expressed as a coefficient of variation, in percent, calculated as

$$v = \frac{100}{\overline{X}} \left[\frac{\sum_{i=1}^{n} [X_i - X_i']^2}{(n-1)} \right]^{1/2} \qquad (14)$$

These results show a general agreement between the biomass concentration data and the curves derived from the OUR correlation. Deviations between the data and correlation results were greatest for the yeast fermentation. Corresponding deviations were also found in the data and estimates for yeast growth rate, Figure 5. These observations suggested that a source of estimation error in the case of yeast was caused by the metabolic complexities associated with this organism.

VARIABILITY OF Y^*_{ATP/O_2} IN AN AEROBIC YEAST FERMENTATION

The stoichiometric equations for the principal pathways responsible for intercellular metabolic energy for bakers yeast are given in Table 2 (Lehninger, 1971; Mills, 1967). Equation (a) is the overall balance for the complete oxidation of glucose by the EMP pathway and the tricarboxylic acid (TCA) cycle. In the discussion to follow, this metabolic sequence will be referred to as the reference sequence since it operates in most aerobic prokaryotic and eukaryotic cells. In many circumstances, including the *Thermoactinomyces sp.* and *Streptomyces sp.* fermentations used in this research, the reference sequence is the only important pathway for producing metabolic energy from glucose. The global molar yield of energy, in ATP (adenosine triphosphate) equivalents, from molecular oxygen assimilated by the reference sequence is $Y^*_{ATP/O_2} = 6.0$ (alternatively expressed as a P/O ratio of 3).

The aerobic growth of yeast is complicated by the simultaneous operation of two additional metabolic energy producing sequences which compete with the reference sequence for the glucose substrate [Table 2, Equations (b) and (c)]. These pathways produce partially oxidized intermediates, ethanol and acetate, which accumulate in the culture medium. Equation (b) is derived from the pathway responsible for the anaerobic production of ethanol. Although it still functions under aerobic conditions, molecular oxygen is not consumed, and the Y^*_{ATP/O_2} becomes infinite. If Equation (b) is allowed to operate unrestricted, the ethanol will accumulate to concentrations that will repress further yeast growth. The metabolic sequence expressed in Equation (c) produces the acid which makes *p*H control necessary.

Two experimental procedures were used to minimize ethanol repression. First, glucose was metered to the fermentation in pulses. A pulse was added after a rapid decrease in exit gas carbon dioxide concentration was observed, which occurred when broth glucose became growth limiting. Each pulse added enough glucose to raise the broth concentration to a maximum of 1 g/l to reduce the repressive effects of high glucose concentration. Restriction of the amounts of glucose available to the culture helps to reduce the synthesis of the ethanol by-product. The second procedure allowed glucose to completely deplete in the medium after the period of maximum growth rate had passed. This initiated a diauxic or diphasic growth period in which the yeast consumed the ethanol and acetate intermediates for metabolic energy instead of glucose. When the removal of ethanol and acetate was completed, determined by monitoring exit gas carbon dioxide concentration, glucose additions were resumed for the duration of the fermentation.

During the diauxic growth period, Equations (d) and (e) operate simultaneously to produce metabolic energy. The respective energy yields are 4.7 and 5.0. Therefore, during the course of a fermentation, Y^*_{ATP/O_2} may vary from a minimum of 4.7 to a maximum of ∞ (anaerobic conditions). This variability forms the basis for restructuring the kinetic model for oxygen utilization.

RESTRUCTURING THE KINETIC MODEL FOR METABOLIC VARIATIONS

Molecular oxygen utilization by living aerobic cells is most closely associated with the production of energy to do metabolic work. Most of the molecular oxygen consumed forms water because of its role as the terminal electron acceptor in oxidative phosphorylation. In this regard, molecular oxygen utilization is somewhat of a unique substrate process, since it does not directly contribute to the mass of the cell. Oxygen which becomes part of the biomass is derived from the combined oxygen found in the culture nutrients.

The model, Equation (9), used to estimate biomass concentration relates molecular oxygen consumption directly with growth. A more accurate representation is made by coupling oxygen consumption to energy production and energy production with growth in a second step. Energy production and molecular oxygen consumption may be related by Equation (3):

$$\text{OUR} = M_{O_2/ATP}\, E + \frac{1}{Y_{ATP/O_2}} \frac{dE}{dt} \qquad (15)$$

The literature (Bauchop and Elsden, 1960; Stouthamer, 1971) suggests that the energy produced, in ATP equivalents, and growth can be expressed by a form of Equation (1):

$$\frac{dX}{dt} = Y^*_{X/ATP} \frac{dE}{dt} \qquad (16)$$

In this case, the global yield constant becomes the more familiar Y_{ATP}, which is relatively constant from organism to organism and under a variety of culture conditions. Substitution of Equation (16) in its integrated form (assuming $X_o = E_o = 0$) into Equation (15) yields

$$\text{OUR} = \frac{1}{Y^*_{X/ATP}} \left[M_{O_2/ATP}\, X + \frac{1}{Y_{ATP/O_2}} \frac{dX}{dt} \right] \qquad (17)$$

In the previous section, it was shown that the global yield of energy from molecular oxygen Y^*_{ATP/O_2} is a variable which depends on the metabolic sequences that are operating. This observation implies that $M_{O_2/ATP}$ and Y_{ATP/O_2} are also variables and therefore are reexpressed as functions:

$$M_{O_2/ATP} = \frac{M_{ref}}{\beta} \qquad (18)$$

$$P_{ATP/O_2} = \beta Y_{ref} \qquad (19)$$

Parameters Y_{ref} and M_{ref} are constants which pertain to the values of Y_{ATP/O_2} and $M_{O_2/ATP}$ that are obtained during the exclusive operation of the reference sequence for energy production. β is known as the metabolic correction function and is used to account for the effects of the other metabolic pathways operating in addition to the reference sequence. It appears inversely as a correction factor for Y_{ref} and M_{ref} due to the reciprocal nature of the units associated with each parameter.

Equation (17) may be rearranged to

TABLE 3. EFFECTS OF USING THE METABOLIC CORRECTION FUNCTION IN THE CORRELATION ANALYSIS

Metabolic factors corrected for	Metabolic correction function	Y_{X/O_2} g-X/g-O_2	$M_{O_2/x}$ g-O_2/(g − X hr)	ν (%)	F statistic
1—Uncorrected	$\beta = \beta_d = 1.0$	2.70	0.061	9.4	—
2—Ethanol production	$\beta = \beta_d = \frac{RQ + 5}{6}$	2.13	0.068	5.9	$F_{1,2} = 2.53$
3—Ethanol production, and diauxic growth on ethanol	$\beta = \frac{RQ + 5}{6}$, $\beta_d = 0.78$	2.21	0.064	3.1	$F_{2,3} = 3.55$
4—Ethanol production, and diauxic growth on ethanol and acetate	$\beta = \frac{RQ + 5}{6}$, $\beta_d = \frac{RQ + 4}{6}$	2.11	0.062	3.3	$F_{3,4} = 1.08$
5—Ethanol and Acetate production, and Diauxic growth on ethanol and acetate	$\beta = \frac{5 - \frac{A}{OUR} + RQ}{6}$, $\beta_d = \frac{RQ + 4}{6}$	2.18	0.061	3.6	$F_{4,5} = 1.23$

$$OUR = \frac{1}{Y^*_{X/ATP}}\left[\frac{M_{ref}}{\beta}X + \frac{1}{\beta Y_{ref}}\frac{dX}{dt}\right] \quad (20)$$

A collection of parameters $M_{O_2/X} = M_{ref}/Y^*_{X/ATP}$ and $Y_{X/O_2} = Y^*_{X/ATP}\,Y_{ref}$ yields

$$\beta \times OUR = M_{O_2/X}\,X + \frac{1}{Y_{X/O_2}}\frac{dX}{dt} \quad (21)$$

This is a convenient rearrangement since it is identical with Equation (9) except that the OUR is multiplied by β. Therefore, the same numerical procedures may be applied to obtain parameters Y_{X/O_2} and $M_{O_2/X}$, and estimates $X(t)$ and $dX(t)$, by replacing OUR with $(\beta \times OUR)$. The interpretation of the parameters is more specific now since they refer specifically to reference sequence conditions.

THE METABOLIC CORRECTION FUNCTION β

The β function is computed as the ratio of the actual global yield of ATP equivalents per mole of molecular oxygen consumed to the same yield defined for the reference sequence [$Y^*_{ref} = 6.0$ from Equation (a), Table 2]:

$$\beta = \frac{Y^*_{ATP/O_2}}{Y^*_{ref}} = \frac{1}{6}Y^*_{ATP/O_2} \quad (22)$$

The function Y^*_{ATP/O_2} is derived by considering substrates and products other than oxygen. This enables the approximation of the relative significance of the other metabolic pathways operating simultaneously with the reference sequence.

Glucose Assimilation Period

The stoichiometric balances which apply are obtained from Equations (a), (b), and (c), Table 2:

$$\left.\begin{aligned} G_a &= 1/6\ OUR_a \\ G_a &= 1/6\ CER_a \\ G_b &= 1/2\ CER_b \\ G_b &= 1/2\ OUR_c \\ G_c &= 1/2\ CER_c \\ G_c &= 1/2\ A \\ OUR &= OUR_a + OUR_c \\ CER &= CER_a + CER_b + CER_c \end{aligned}\right\} \quad (23)$$

where G and OUR are the respective molar rates of consumption of glucose and molecular oxygen, and A and CER are the respective molar rates of production of acetate and carbon dioxide. This system of equations is solved uniquely when OUR, CER, and A data are supplied. Under those circumstances, the relative molar rates for glucose assimilation by Equations (a), (b), and (c), respectively, are

$$\chi_a = \frac{G_a}{G_a + G_b + G_c} = \frac{OUR - A}{2A - 2OUR + 3CER} \quad (24)$$

$$\chi_b = \frac{G_b}{G_a + G_b + G_c} = \frac{3(CER - OUR)}{2A - 2OUR + 3CER} \quad (25)$$

$$\chi_c = 1 - \chi_a - \chi_b = \frac{3A}{2A - 2OUR + 3CER} \quad (26)$$

Therefore, Y^*_{ATP/O_2} may be calculated by

$$Y^*_{ATP/O_2} = \frac{36\chi_a + 2\chi_b + 10\chi_c}{6\chi_a + 2\chi_c} = 5 - \frac{A}{OUR} + \frac{CER}{OUR} \quad (27)$$

If we reexpress the CER/OUR ratio as the respiratory quotient RQ, the metabolic correction function becomes

$$\beta = \frac{5 - \frac{A}{OUR} + RQ}{6} \quad (28)$$

Diauxic Growth

Equations (d) and (e), Table 2, apply during the diauxic phase, yielding the following stoichiometric equations:

$$\left.\begin{aligned} Et &= -1/3\ OUR_d \\ Et &= -1/2\ CER_d \\ A &= -1/2\ OUR_e \\ A &= -1/2\ CER_e \\ CER &= CER_d + CER_e \\ OUR &= OUR_d + OUR_e \end{aligned}\right\} \quad (29)$$

where Et is the molar rate of ethanol production. This system of equations has a unique solution when OUR and CER data are available. Corresponding molar ratios for carbon utilization by mechanisms d and e, respectively, are

$$\chi_d = \frac{CER_d}{CER_d + CER_e} = \frac{2(OUR - CER)}{CER} \quad (30)$$

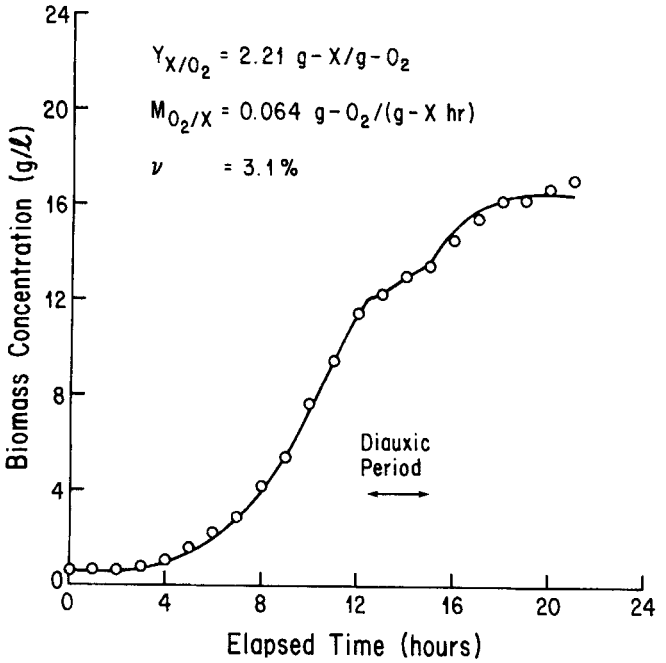

Fig. 6. *Saccharomyces cerevisiae* **biomass concentration data (○) and correlation results (continuous line) using the corrected kinetic model.**

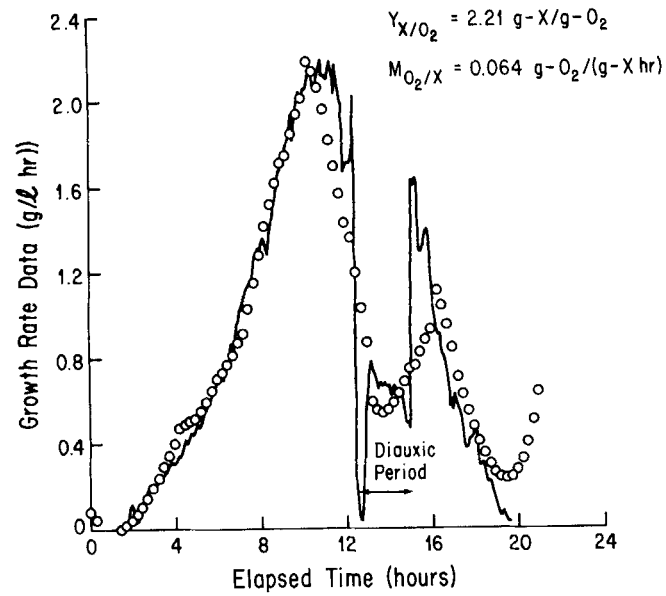

Fig. 7. *Saccharomyces cerevisiae* **growth rate data (○) and correlation estimates (continuous line) using the corrected kinetic model.**

$$\chi_e = 1 - \chi_d = \frac{3CER - 2OUR}{CER} \tag{31}$$

Y°_{ATP/O_2} is given by

$$Y^\circ_{ATP/O_2} = \frac{14\chi_d + 10\chi_e}{3\chi_d + 2\chi_e} = RQ + 4 \tag{32}$$

Therefore, the metabolic correction function relevant to the diauxic period is

$$\beta_D = \frac{RQ + 4}{6} \tag{33}$$

CORRELATION ANALYSIS USING CORRECTED MODEL

The effects on correlation accuracy, caused by the introduction of β in the kinetic model, were studied. Biomass concentration estimates were computed using the metabolic correction functions designated in Equations (28) and (33) and simplified variations of these equations. In this way, it was possible to study the improvement in estimation accuracy with increasing β complexity. The various forms of the metabolic correction function included compensation for ethanol production, ethanol production and diauxic growth on ethanol, ethanol production and diauxic growth on ethanol and acetate, and ethanol and acetate production and diauxic growth on ethanol and acetate. The effects of acetate were eliminated from β, Equation (28), by setting A = 0, or by setting RQ = 0.67 in β_D, Equation (33). The diauxic compensation was made by using β_D to replace β during the diauxic growth period. The results using each of the four model variations were expressed as coefficients of variation, defined by Equation (14), and are summarized in Table 3.

The accounting of the effects caused by the simultaneous operation of several metabolic pathways in the correlation model, Equation (21), has significantly improved the biomass concentration estimates. This is shown by the reductions in the coefficient of variation. However, the analysis of the estimation variances using the F statistic (Hamburg, 1970) indicates that the corrections beyond those for ethanol production and consumption cannot be statistically justified at the 5% level of significance ($F_{initial}$ = 2.12 at 5% level of significance and 20 deg of freedom). This is caused by the proximity of the Y°_{ATP/O_2} values for the reference sequence and the acetate production mechanism during glucose assimilation (6.0 and 5.0, respectively). This also is true of the diauxic growth period for the sequences for ethanol assimilation and acetate consumption (5.0 and 4.7, respectively). Therefore, only the metabolic correction functions compensating for ethanol production and uptake (Table 3, correction 3) will be considered further. Figures 6 and 7 present comparisons of the biomass concentration and growth rate data (discrete points), respectively, with the estimates provided by the correlation (continuous line) compensated for ethanol effects (compare with Figures 4 and 5, uncorrected estimates).

REAL-TIME BIOMASS CONCENTRATION ESTIMATION

Having developed satisfactory correlations relating biomass concentration and growth rate with OUR and RQ data, the next step was to use these methods to predict these quantities from on-line OUR and RQ data. In these experiments, X_o was estimated from the inoculation conditions, and Y_{X/O_2} and $M_{O_2/X}$ were supplied from the analysis of an earlier experiment. The calculations were performed using a real-time digital computer which supplied the results essentially instantaneously.

These experiments were performed for each of the three fermentation types using Equation (10) and the metabolic correction functions designated in Table 4. These results show greater errors, expressed as coefficients of variation, than the correlation analysis data. These increases are thought to be mostly due to the poor precision of the instruments used to measure the oxygen uptake rate.

DISCUSSION

Errors in the real-time biomass concentration estimates were systematic. They were characterized by a shifting of

TABLE 4. RESULTS OF REAL-TIME BIOMASS CONCENTRATION ESTIMATES

Fermentation	Metabolic correction function β	Y_{X/O_2} g-X/g-O_2	$M_{O_2/X}$ g-O_2/(g-X hr)	ν_1 correlation %	ν_2 real-time %
Thermoactinomyces sp.	$\beta = 1$	2.10	0.120	7.1	22.0
Streptomyces sp.	$\beta = 1$	1.34	0.041	3.4	8.1
Saccharomyces cerevisiae	$\beta = \frac{RQ + 5}{6}$ $\beta_d = 0.78$	2.21	0.064	3.1	6.7

the estimates away from the data while continuing to follow the trends established by the laboratory data. This is due to the integral in Equation (10) which accumulates the ($\beta \times$ OUR) data as well as instrumentation errors. The cumulative effect is most significant during the early stages of a fermentation in which the errors in the OUR data can be greater than the data values. This can be illustrated by considering the formula for computing the OUR:

$$\text{OUR} = \frac{F_{in} C_{in} - F_{ex} (C_{ex} \pm \delta)}{V_a} \tag{34}$$

The instrument error is expressed as δ. With the other parameters being specified as constants, the error in the OUR accounted for by the oxygen analyzer accuracy is

$$\epsilon = \pm \frac{F_{ex} \delta}{V_a} \tag{35}$$

Under typical experimental conditions, $F_{ex} = 2\,400$ l/hr, $V_A = 40$ l, and $\delta = 7.1$ mg//l, giving an error of $\epsilon = \pm$ 426 mg/(l hr). This error exceeds the value of the OUR data for the first 6 to 13 hr, depending on the fermentation type, and therefore can result in relative errors exceeding 100% during this early period.

Several options are available to maximize the accuracy of biomass concentration and growth rate estimates with errors in the OUR data. It is recommended that an oxygen analyzer with greater accuracy, precision, and stability be used. An analyzer that measures a reference gas simultaneously with the sample gas is particularly suited to this application. Secondly, low sparging gas flow rates should be used during the early phases of the fermentation to reduce the magnitude of ϵ and increase the difference between C_{in} and C_{out}. A third approach is to use the estimation procedure in conjunction with laboratory biomass concentration data. In this way, Equation (10) can be used to predict the biomass concentration data from the time of the last laboratory determination t_L to the current time by substituting $X(t_L)$ for X_o, and integrating the β and OUR data from t_L to the current time. This procedure breaks up the integration into parts so that the instrumentation errors from previous segments have no effect on the current estimates. Alternatively, the laboratory data can be used to recalculate or update Y_{X/O_2} and $M_{O_2/X}$ as the experiment proceeds.

Another important point in the accuracy of the OUR data is the determination of the exit gas flow rate F_{ex}. In the earlier work on estimation of biomass concentration using OUR data (Jefferis and Humphrey, 1972; Jefferis et al., 1972) F_{ex} was assumed to be equal to the inlet gas flow rate F_{in} which is measured. However, these rates are made unequal by the gas exchange caused by the respiring cells (Fiechter and Von Meyenburg, 1968). The correct value of F_{ex} is obtained from Equation (36) which is obtained by considering a material balance of the inert gas species (that is, the gas species except carbon dioxide and oxygen) in the gas streams entering and leaving the fermentor:

$$F_{in} (1 - \chi_{in,CO_2} - \chi_{in,O_2}) = F_{ex} (1 - \chi_{ex,CO_2} - \chi_{ex,O_2}) \tag{36}$$

Here χ is the mole fraction of carbon dioxide or oxygen in the gas streams. Substantial errors in computing OUR data result if F_{ex} is assumed to be equivalent to F_{in} (Zabriskie, 1976).

Biomass concentration estimates were not affected by errors in the CER data owing to the high accuracy of the carbon dioxide measurements using the 0 to 4% range. This fact suggested that a procedure balancing carbon dioxide might provide more accurate estimates than the oxygen balancing procedure. This approach was investigated, and the results of the estimates for all three fermentation types had substantially greater errors than the oxygen based estimates (Zabriskie, 1976). An explanation for this finding is that carbon dioxide is evolved as a consequence of many metabolic processes, including the conversion of pyruvate to Acetyl-CoA, the TCA cycle, pentose shunt, and amino acid metabolism. Only some of these processes are important in producing metabolic energy. Therefore, carbon dioxide evolution and growth are not as closely coupled as are growth and oxygen consumption. The resulting complexity is not adequately provided for in the models described, and hence greater estimation errors result.

A final comment may be made regarding the model for molecular oxygen consumption, Equation (21). A tacit assumption has been that X refers to viable cells, since metabolic energy production ceases in dead cells. This is a potentially significant advantage, since only the viable culture is producing product and is subject to environmental control. However, the same model has been used during the declining phase of growth when the maintenance requirement for molecular oxygen in the culture is not satisfied (OUR $< M_{O_2/X}X$) and a negative growth rate is predicted. This is conceptually correct when the death yield $Y_\dagger$ is numerically equivalent to Y_{X/O_2}. The accuracy of the estimates made in the declining phases of the fermentations evaluated in this project support this assumption, but this may not be valid under other circumstances.

It is concluded that these estimation procedures possess considerable potential for application in the control of the aerobic batch fermentation process owing to their accuracy and response characteristics. The results for the three fermentation types used in this study confirm the existence of this potential.

Nevertheless, one can anticipate some limitations to this approach. When we use the uncorrected model for oxygen consumption, any factor which causes the model parameters to vary will influence the estimation accuracy. Since these parameters are specific to the microorganism and source of carbon nutrition, applications involving mixed cultures or multiple carbon substrates may be re-

stricted. Model corrections for these effects, like those described for the growth of bakers yeast, are limited to those microorganisms in which metabolism energetics are well established. Estimates may also be influenced by severe changes in the microbial environment (for example, *p*H or temperature), although estimation tolerance to these disturbances may be substantial. Experimental evaluation of the importance of these considerations is recommended before implementation in the control of a specific fermentation process.

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NOTATION

A = acetate production rate, mole/(l hr)
C = concentration in gas phases, g/l
CER = carbon dioxide evolution rate, mole/(l hr) or g/(l hr)
E = energy in ATP equivalents, g/(l hr)
Et = ethanol production rate, mole/(l hr)
F = gas flow rate, l/hr
G = glucose consumption rate, mole/(l hr)
J = rate of interfacial mass transfer, g/hr
$M_{j/i}$ = maintenance coefficient, g-j/(g-i hr)
OUR = oxygen uptake rate, mole/(l hr), or g/(l hr)
Q_{O_2} = specific oxygen utilization rate, g-oxygen/(g-X hr)
R = reaction rate, g/hr
RQ = respiratory quotient, mole-carbon dioxide/mole-oxygen
S = concentration in aqueous phase, g/l
t = time, hr
V = volume, l
X = biomass concentration, g/l
$Y_{i/j}$ = yield coefficient, mole-i/mole-j or g-i/g-j

Greek Letters

β = metabolic correction function
δ = instrumentation accuracy limit, g/l
ϵ = computational accuracy limit, g/(l hr)
ν = coefficient of variation, %
χ = mole fraction

Subscripts

A = aqueous phase
a, b, c, d, e = metabolic reaction identification
ex = exit gas conditions
G = gas phase
i, j = specific chemical species
in = inlet gas conditions
o = initial condition
ref = refers to reference metabolic sequence
† = refers to cell death

Superscripts

* = global parameter
′ = laboratory measured value of X

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