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Modeling Batch Production of Single Cell Protein from Cheese Whey

III: Oxygen Utilization

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ABSTRACT

A mathematical model capable of describing the oxygen utilization by the yeast *Kluyveromyces fragilis* during the batch aerobic fermentation of cheese whey was developed. The model predicted the experimental results with R^2 of 0.97. The dissolved oxygen concentration in the fermenter was affected by the number of yeast cells present in the system. The dissolved oxygen curve displayed four distinct stages which corresponded to the lag, exponential, stationary, and death phases of the yeast growth curve. A steady state condition was observed during the stationary phase, during which the oxygen uptake rate by the yeast was equivalent to the oxygen added to the system by the aeration equipment. The mathematical model showed that the oxygen concentration would increase during the death phase and the initial dissolved oxygen concentration would be achieved after 80 h. The specific oxygen uptake rates for the lag, exponential, stationary and death phases were 0.3200×10^{-12}, 2.1400×10^{-12}, 0.5100×10^{-12}, and 0.0028×10^{-12} mg O_2 cell^{-1} h^{-1}, respectively.

Index Entries: Cheese whey; single cell protein; batch fermenter; yeast; modeling; oxygen.

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INTRODUCTION

The study of oxygen transfer from air bubbles, through the liquid medium, to microbial cells (Fig. 1) is of great importance to industrial aerobic, submerged culture fermentations. Irrespective of whether an aerobic microbiological process is operated in the batch, semicontinuous, or continuous flow mode, oxygen must be continuously supplied to the process if acceptable productivities are to be achieved. However, the oxygen requirement of an aerobic culture depends on the concentration of microbes in the reactor, their age and rate of growth, the appropriate yield coefficient, the carbon source, the storage of reserved food material, and the enzymatic complement of the microbes (*1*). However, in the vast majority of aerobic microbiological processes, air is used as the source of oxygen (*2–4*). Oxygen in the air is transferred from the gas phase to the liquid phase primarily by mechanical equipment that must be able to introduce dissolved oxygen as rapidly as it is utilized by the microorganisms.

In single cell protein processes, yeast production requires a large amount of oxygen than other microbiological processes (*5,6*). Wasserman et al. (*7*) reported a peak oxygen demand of the yeast *Kluyveromyces fragilis* of 5 $mMO_2\ L^{-1}\ min^{-1}$. An oxygen absorption rate of up to 4.5 $mMO_2\ L^{-1}\ min^{-1}$ was reported by Burgess (*8*). Maxon and Johnson (*1*) reported a peak oxygen demand of 0.25 $mMO_2\ L^{-1}\ min^{-1}$ under limited aeration and 5.7 $mMO_2\ L^{-1}\ min^{-1}$ under adequate aeration. Strohm et al. (*9*) reported a peak oxygen demand of 2.5 $mMO_2\ L^{-1}\ min^{-1}$ for baker's yeast. Vananuvat and Kinsella (*10*) and Knight et al. (*11*), in describing the optimum growth condition for *K. fragilis*, stated that one volume of air for each volume of medium/min would be sufficient. Wasserman et al. (*7*) used a much higher aeration rate of 4 VVM while using a much slower agitation speed than that used by Vananuvat and Kinsella (*10*).

During the aerobic fermentation cheese whey, lactose is utilized by *K. fragilis* for the synthesis of new yeast cells ($C_{13}H_{20}N_2O_7$) and the producduciton of energy according to the following equation (*12*).

(a) energy release or respiration

$$C_{12}H_{22}O_{11} + 12O_2 \xrightarrow{\text{yeast}} 12CO_2 + 11H_2O + Energy \quad (1)$$

(b) synthesis or growth

$$13C_{12}H_{22}O_{11} + 24NH_4 \xrightarrow{\text{yeast}} 12C_{13}H_{20}N_2O_7 + 59H_2O + 24H^+ \quad (2)$$

By combining Eqs. (1) and (2), the net reaction of the aerobic decomposition of lactose can be written as follows:

$$14C_{12}H_{22}O_{11} + 12O_2 + 24NH_4 \xrightarrow{\text{yeast}} 12C_{13}H_{20}N_2O_7 + 12CO_2 + 70H_2O + 24H^+ + Energy \quad (3)$$

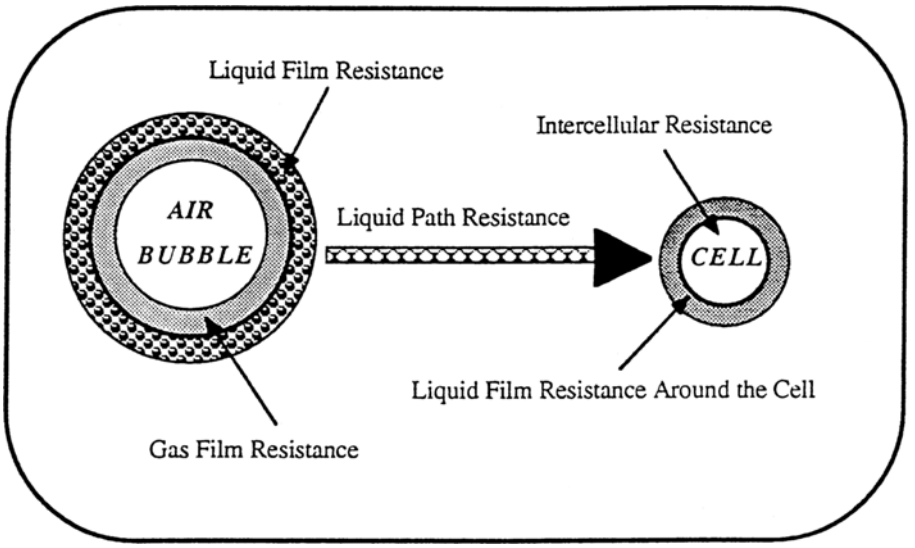

Fig. 1. Schematic representation of oxygen transfer from the air bubbles to the microbial cells.

According to Eq. (3), about 7% of the lactose must be oxidized by the *K. fragilis* to produce energy. The stoichiometric oxygen demand is 0.05 g O_2/g cell (0.04 g O_2/g lactose). Thus, limitation of oxygen renders the organism incapable of producing enough energy for growth and as a consequence, the yeast cells either cease to grow or use reductive metabolic pathway yielding ethanol in order to obtain the required energy.

The objective of this study was to develop a mathematical model capable of describing the changes in dissolved oxygen concentration and the oxygen uptake rate by *K. fragilis* during the aerobic batch fermentation of cheese whey for single cell protein production.

MATERIALS AND METHODS

The experimental appararus used in this study has been previously described by Mansour et al. (*13*). The whey used in this study had 6.4% total solids, 5% lactose, 1.7% total nitrogen, 0.9% minerals, and pH of 4.9. The cheese whey collection and sterilized, the inoculum preparation and the system operation were, also, previously described by Mansour et al. (*13*).

Samples were drawn from the fermenter at zero time and thereafter every two hours. The plate count was carried out on the samples according to the procedures described in the Standard Methods for the Examination of Dairy Products (*14*). The dissolved oxygen concentration in the fermenter was measured continuously.

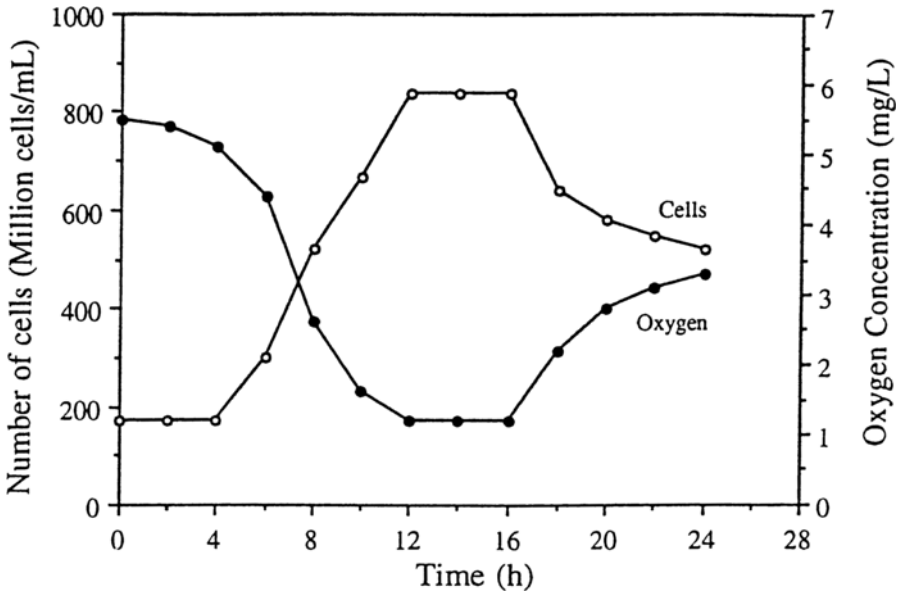

Fig. 2. Oxygen concentration and number of cells in the batch culture reactor.

RESULTS AND DISCUSSION

Initially, the whey was saturated with oxygen before the inoculum of the *K. fragilis* was introduced. The observed dissolved oxygen concentration at saturation (C_s) and the oxygen transfer coefficient (K_{La}) of a yeast free whey were 5.5 mg L^{-1} and 0.11 h^{-1}, respectively. The dissolved oxygen curve (virtually a mirror image of the yeast growth curve) displayed four distinct stages (Fig. 2). First, it decreased slowly, then decreased sharply until it reached a constant minimum value of 1.2 mg/L, remained constant at 1.2 mg/L for a period of time and finallyl increased with time. Generally, the concentration of dissolved oxygen in the reactor was affected by the number of yeast cells as shown in Fig. 3.

The characteristics of the four stages of the oxygen concentration curve can be described mathematically as shown in Fig. 4. The oxygen uptake of a given yeast mass in a continuously aerated system can be described by the following equation (*15–17*):

$$dC / dt = K_{La} (C_s - C) - \delta N \quad (4)$$

where: dC / dt is the oxygen uptake by yeast (mg cm^{-3} h^{-1}); K_{La} is the overall volumetric mass transfer coefficient (h^{-1}); C_s is the saturation concentration of oxygen in the liquid medium (mg cm^{-3}); C is the actual oxygen concentration in the liquid medium (mg cm^{-3}); N is the concentration of microorganisms in the liquid medium (cell cm^{-3}); δ is the specific oxygen uptake rate (mg $cell^{-1}$ h^{-1}).

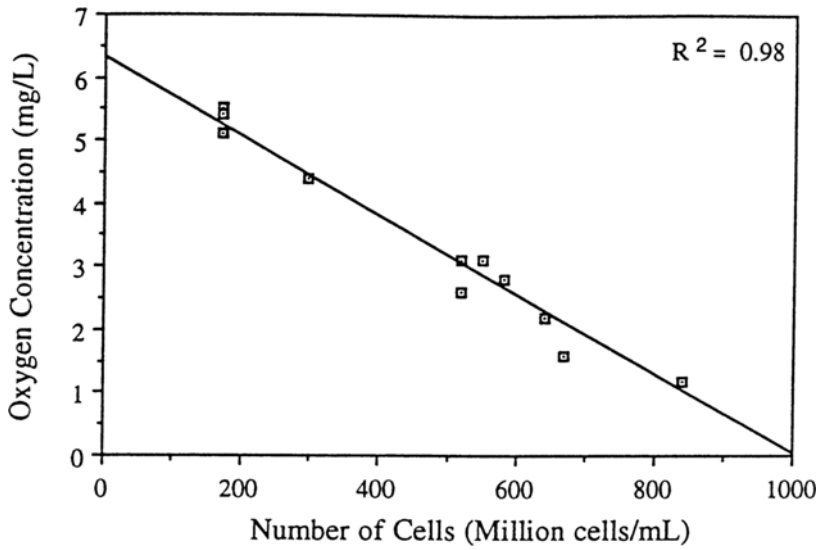

Fig. 3. Effect of yeast population size on the oxygen concentration in the batch culture reactor.

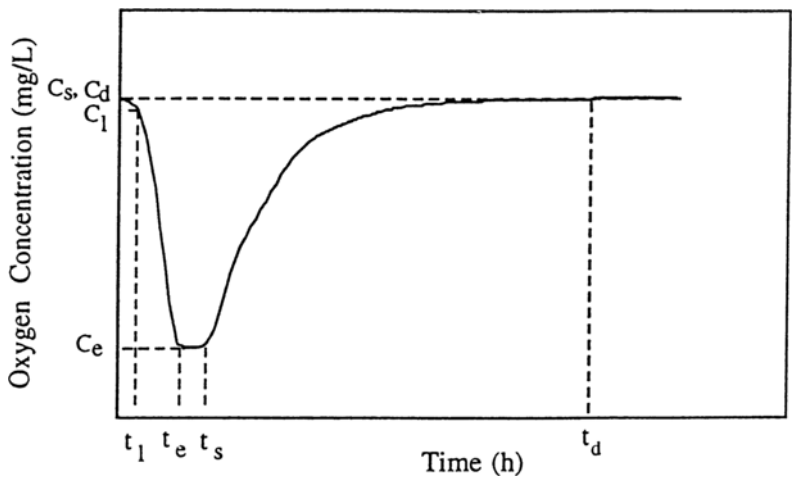

Fig. 4. Graphical representation of the four phases of the oxygen curve. t_l = end of the lag phase; t_e = end of exponential phase; t_s = end of stationary phase; t_d = end of death phase; C_s = saturated oxygen concentration; C_l = oxygen concentration at lag phase; C_e = oxygen concentration at exponential phase; C_d = oxygen concentration at death phase.

Lag Phase

During this period, the dissolved oxygen decreased slowly (almost linearly). The number of the yeast cells remained constant and the oxygen was mostly required for cell respiration as well as cell endogenous growth. The rate of change in oxygen can, thus, be described as follows:

$$dC / dt = K_{La} (C_s - C) - \delta_1 N_o \qquad , 0 < t < t_l \tag{5}$$

where: δ_1 is the specific oxygen uptake rate during the lag phase (mg cell^{-1} h^{-1}); N_o is the initial number of cells at time t (cell cm^{-3}); t_l is the end of the lag phase (h).

Equation (5) can be rewritten in the form of a first order differential equation as follows:

$$dC / dt + K_{La} C = K_{La} C_s - \delta_1 2 N_o \qquad , 0 < t < t_l \tag{6}$$

The solution of Eq. (6) depends on the following integration factor (IF):

$$IF = e^{\int K_{La} dt} = e^{(K_{La} t)} \tag{7}$$

Thus, Eq. (6) can be rewritten as follows:

$$d / dt (C e^{(K_{La} t)} = (K_{La} C_s - \delta_1 N_o) e^{(K_{La} t)} \tag{8}$$

Integrating both sides of Eq. (8) yields the following equation:

$$C e^{(K_{La} t)} = (C_s - \delta_1 N_o / K_{La}) e^{-K_{La} t} + A_1 \tag{9}$$

Thus,

$$C_t = C_s - \delta_1 N_o / K_{La} + A_1 e^{-(K_{La} t)} \qquad , 0 < t < t_l \tag{10}$$

where: C_t is the oxygen concentration at time t (mg cm^{-3}).

The constant A_1 can be obtained from the initial condition of the lag phase (i.e., $C = C_s$) as follows:

$$C_s = C_s - \delta_1 N_o / K_{La} + A_1 \tag{11}$$

Thus,

$$A_1 = \delta_1 N_o / K_{La} \tag{12}$$

Equation (10) can be rewritten in nondimensional form by dividing by C_s as follows:

$$C_t / C_s = 1 - \delta_1 N_o / K_{La} C_s (1 - e^{-(K_{La} t)}) \qquad , 0 < t < t_1 \tag{13}$$

The oxygen concentration at the end of the lag phase (C_l) can be calculated from the following equation:

$$C_l = C_s - \delta_1 N_o / K_{La} (1 - e^{(K_{La} t_l)}) \tag{14}$$

By using the least squares method to solve Eq. (14), the value of δ_1 was found to be 0.32×10^{-12} mg oxygen/cell/hour.

Exponential Phase

During this phase, the cell number increased exponentially as shown in the following equation (13):

$$N_t = N_o e^{\mu (t - t_l)} \qquad , t_l < t < t_e \tag{15}$$

where: N_t is the number of cells at time t (cell cm^{-3}); μ is the specific growth rate (h^{-1}).

Thus, Eq. (5) can be rewritten as follows:

$$dC / dt = K_{La} (C_s - C) - \delta_2 N_o e^{\mu(t - t_l)} \qquad , t_l < t < t_e \tag{16}$$

where: δ_2 is the specific oxygen uptake rate during the exponential phase (mg cell^{-1} h^{-1}); t_e is the exponential growth phase (h).

Equation (16) can be solved using the integration factor (IF) given in Eq. (7). Thus, Eq. (16) can be rewritten as follows:

$$dC / dt (C e^{(K_{La} t)} = K_{La} C_s e^{K_{La}} - \delta_2 N_o e^{-\mu t_l} e^{(\mu + K_{La})t} \qquad , t_l < t < t_e \tag{17}$$

Integrating both sides of Eq. (17) yields the following equation:

$$C_t e^{K_{La} t} = C_s e^{(K_{La} t)} - [\delta_2 N_o / (\mu + K_{La})] e^{\mu(t - t_l)} e^{K_{La} t} + A_2 \tag{18}$$

The oxygen concentration at the beginning of the exponential phase (C_e) can be calculated as follows:

$$C_e = C_s - [\delta_2 N_o / (\mu + K_{La})] e^{\mu(t - t_l)} + A_2 e^{-(K_{La} t)} \tag{19}$$

where: C_e is the oxygen concentration during the exponential phase (mg cm^{-3}).

At the end of the lag phase and the beginning of the exponential phase $C_1 = C_e$ and Eqs. (14) and (19) can, therefore, be combined as follows:

$$\begin{aligned} &C_S - \delta_1 N_o / K_{La} (1 - e^{K_{La} t_l}) = \\ &C_s - [\delta_2 N_o / (\mu + K_{La})] e^{\mu(t_e - t_l)} + A_2 e^{-K_{La} t_l} \end{aligned} \tag{20}$$

Thus, the constant A_2 can be determined as follows:

$$A_2 = [\delta_2 N_o / (\mu + K_{La})] e^{-(K_{La} t_l)} - [\delta_1 N_o / K_{La}] (e^{-(K_{La} t_l)} - 1) \tag{21}$$

By substituting Eq. (21) in Eq. (18), the oxygen concentration during the exponential phase can be expressed as follows:

$$\begin{aligned} C_t = C_s &- [\delta_1 N_o / K_{La}] (1 - e^{-(K_{La} t_l)}) e^{-K_{La} (t - t_l)} \\ &- (\delta_2 N_o / \mu + K_{La}) [e^{\mu(t - t_l)} - e^{-K_{La}(t - t_l)}] \qquad , t_1 < t < t_e \end{aligned} \tag{22}$$

By using the least squares method to solve Eq. (22), the value of δ_2 was found to be 2.14×10^{-12} mg oxygen/cell/hour, which is about 6.7 times the value of δ_1.

Stationary Phase

The yeast population (N_m) remained constant and as a result the oxygen concentration in the reactor remained constant. Thus, as temporary steady state condition was achieved during which the amount of oxygen added to the system by the aeration equipment was equivalent to that consumed by the yeast. Equation (5) can, therefore, be rewritten as follows:

$$dC / dt = K_{La} (C_s + C) - \delta_3 N_m \qquad , t_e < t < t_s \tag{23}$$

where: δ_3 is the specific oxygen uptake rate during the stationary phase (mg cell^{-1} h^{-1}); N_m is the maximum number of yeast cells achieved in the system (cell cm^{-3}).

At the steady state condition $dC/dt = 0$ and $C_t = C_e$. Thus, Eq. (23) can be rewritten as follows:

$$C = C_e = C_s - \delta_3 N_m / K_{La}, \qquad t_e < t < t_s \tag{24}$$

The value of δ_3 can be calculated as follows:

$$\delta_3 = K_{La} (C_s - C_e) / N_m \tag{25}$$

The value of δ_3 was found to be 0.51×10^{-12} mg oxygen/cell/hour. It is interesting to note that the specific oxygen uptake rate of the stationary phase was slightly higher (1.6 times) than that of the lag phase and about one fourth of that of the exponential phase.

Death Phase

In this period, the number of yeast cells decreased with time according to the following equation (*13*).

$$N_t = \tau N_m / [1 - (1 - \tau) e^{\mu \tau (t - t_s)}] \qquad , t < t_s \tag{26}$$

where: τ is the survival constant, the final number of cells (N_f) divided by the maximum number of cells (N_m) achieved in the system = 0.55 (−).

As a result of the decline of yeast population, the oxygen concentration in the system was continously increasing. Equation (5) can, therefore, be rewritten as follows:

$$dC / dt = K_{La} (C_s - C) - \delta_4 \tau N_m / [1 - (1 - \tau) e^{\mu \tau (t - t_s)}] \qquad , t < t_s \tag{27}$$

where: δ_4 is the specific oxygen uptake rate during the death phase (mg cell^{-1} h^{-1}).

Equation (27) can be solved using the integration factor (IF) given in Eq. (7).

$$d / dt (C e^{(K_{La} t)}) = K_{La} C_s e^{(K_{La} t)} - \delta_4 \tau N_m e^{(K_{La} t)} / [1 - (1 - \tau) e^{-\tau \mu (t - t_s)}] \tag{28}$$

Integrating both sides yields the following equation:

$$C e^{(K_{La} t)} = C_s e^{(K_{La} t)} - \delta_4 \tau N_m \int e^{(K_{La} t)} [1 - (1 - \tau) e^{-\tau \mu (t - t_s)}]^{-1} dt \tag{29}$$

The bracket $[1 - (1 - \tau) e^{-\tau \mu (t - t_s)}]^{-1}$ is similar to $1/(1-x)$ where $x < 1$ and thus, can be expressed in terms of Taylor's series $(1 + x + x^2 + x^3 + ...)$ as follows:

$$\int e^{(K_{La} t)} [1 - (1 - \tau) e^{-\tau \mu (t - t_s)} - 1] dt$$

$$= \int [e^{(K_{La} t)} + (1 - \tau) e^{(\tau \mu t_s)} e^{(K_{La} - \tau \mu) t} + (1 - \tau)^2 e^{(2 \tau \mu t_s)} e^{(K_{La} - 2 \tau \mu) t}$$

$$+ (1 - \tau)^3 e^{(3\tau\mu t_s)} e^{(K_{La} - 3\tau\mu)t} + \ldots\ldots] dt \quad (30)$$

$$= [1 / k_{L_a} + [(1 - \tau) / (K_{La} - \tau\mu)] e^{-\tau\mu(t - t_s)} + [(1 - \tau)^2 / (K_{La} - 2\tau\mu)] e^{-2\tau\mu(t - t_s)}$$

$$+ \ldots\ldots] e^{(K_{La} t)}$$

$$= [1 / K_{La} + \sum_{n=1}^{\infty} \{[(1 - \tau)^n / (K_{La} - n\tau\mu)] e^{-n\tau\mu(t - t_s)}\} e^{(K_{La} t)}$$

Therefore, the oxygen concentration C_t at time $t > t_s$ can be expressed as follows:

$$C_t = C_s - \delta_4 \tau N_m \{1 / K_{La} + \sum_{n=1}^{\infty} [(1 - \tau)^n / (K_{La} - n\tau\mu)] e^{-n\tau\mu(t - t_s)}\}$$

$$+ A_4 e^{-(K_{La} t)}, t > t_S \quad (31)$$

The constant A_4 can be determined from the initial conditions of the stationary phase ($C_e = C_d = 1.2$ mg/L) as follows:

$$A_4 = \delta_4 \tau N_m [1 / K_{La} + \sum_{n=1}^{\infty} (1 - \tau)^n / (K_{La} - n\tau\mu)] e^{(K_{La} t_s)}$$

$$- (\delta_1 N_o / K_{La}) (1 - e^{-(K_{La} t_l)}) e^{-K_{La}(t_s + t_e - t_l)} \quad (32)$$

$$- (\delta_2 N_o / \mu + K_{La}) [e^{\mu(t_e - t_l)} e^{-K_{La}(t_e - t_l)} e^{(K_{La} t_s)}]$$

Using the least squares method to solve Eq. (32), the value of δ_4 was found to be 0.0028×10^{-12} mg oxygen/cell/hour. Although, this is very small (compared to 0.88%, 0.13%, and 0.55% for δ_1, δ_2, and δ_3, respectively) it indicated that some of the yeast cells were still utilizing oxygen either for energy or growth. Loehr (*20*) reported that in a nutrient deficient system some cells will die and lyse and the constituents of their bodies will be utilized as food by still existing cells.

It is interesting to note that in Eq. (31) as t --> ∞, the oxygen concentration approaches the C_s value (after approx 52 h). This was calculated as follows:

$$C_\infty = C_s - \delta_4 \tau N_m / K_{La}$$

$$= 5.5 - [(0.0028 \times 10^{-12} \times 0.55 \times 840 \times 10^6) / 0.10] \quad (33)$$

$$= 5.49 \text{ mg/L}$$

The oxygen uptake equation and the integral form for each phase of yeast cell growth are presented in Table 1.

The predicted and measured oxygen concentration values are shown in Fig. 5. The model showed good predictions with R^2 value of 0.97. The values of the specific oxygen uptake rate for the lag, exponential, stationary, and death phases (δ_1, δ_2, δ_3, and δ_4) are presented in Table 2. Gancedo and Serrano (*18*) stated that the oxygen consumption rate of the great majority of yeast ranges from 5 to 250 μMO_2/g cell/min. The oxygen uptake rate calculated in this study is within this range.

Table 1
Oxygen Uptake Rate Equation and Integral Form of Each Phase of the Yeast Cell Growth

Phase	Uptake equation	Integral form	Limit
Lag	$dC / dt = K_{La} (C_s - C) - \delta_1 N_o$	$C_t = C_s - (\delta_1 N_o / K_{La}) (1 - e^{(K_{La} t_l)}$	$t_o < t < t_e$
Exponential	$dC / dt = K_{La} N (C_s - C) - \delta_2 N_o e^{\mu(t - t_l)}$	$C_t = C_s - (\delta_1 N_o / K_{La}) (1 - e^{-(K_{La} t_l)}) e^{-K_{La}(t - t_l)}$ $- (\delta_2 N_o / \mu + K_{La}) [e^{\mu(t - t_l)} e^{-K_{La}(t - t_l)}]$	$t_l < t < t_e$
Stationary	$dC / dt = K_{La} (C_s - C) - \delta_3 N_m$	$C_t = C_s - (\delta_3 N_m / K_{La})$	$t_e < t < t_s$
Death	$dC / dt = K_{La} (C_s - C) - \delta_4 (\tau N_m) / [1 - (1 - \tau) e^{\mu \tau (t - t_s)}]$	$C_t = C_s - \delta_4 \tau N_m \{I / K_{La} + \sum_{n=1}^{\infty} [(1 - \tau)^n / (K_{La} - n \tau \mu)] e^{-n \tau \mu (t - t_s)}\}$ $+ e^{-(K_{La} t_l)} e^{(K_{La} t_s)} [\delta_4 \tau N_m (1 / K_{La} + \sum_{n=1}^{\infty} (1 - \tau)^n / (K_{la} \ n \tau \mu))]$ $- e^{-(K_{La} t)} e^{-K_{La}(t_s + t_e + t_l)} [(\delta_1 N_o / K_{La}) (1 - e^{-(K_{La} t_l)})]$ $- e^{-(K_{La} t_l)} e^{(K_{La} t_s)} e^{\mu(t_e - t_l)} e^{-K_{La}(t_e - t_l)}$	$t > t_s$

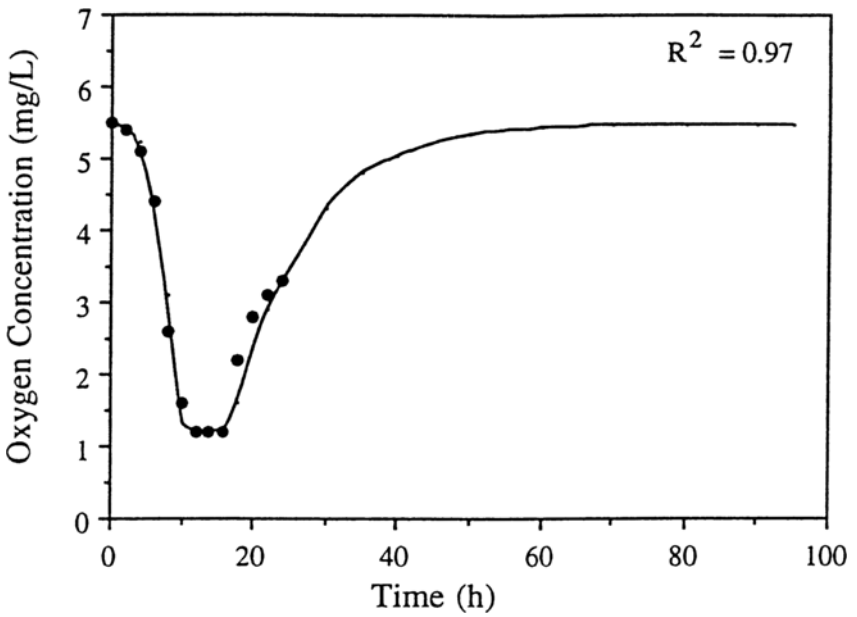

Fig. 5. The measured and predicted values of the dissolved oxygen concentration.

Table 2
The Specific Oxygen Uptake Rate
by *K. fragilis* During Batch Culture Operation

Phase	δ, mg O_2/cell/h
Lag growth phase	0.3200×10^{-12}
Exponential growth phase	2.1400×10^{-12}
Stationary growth phase	0.5100×10^{-12}
Death phase	0.0028×10^{-12}

CONCLUSION

The dissolved oxygen curve displayed four distinct stages that corresponded to the lag, exponential growth, stationary and death phases of the yeast. Also, the dissolved oxygen concentration was affected by the number of yeast cells present in the system. A dissolved oxygen steady state condition was observed during the stationary phase during which the oxygen uptake rate by the yeast was equivalent to the oxygen added to the sytem by the aeration equipment. A model describing the change in dissolved oxygen concentration during the batch operation as a function of number of yeast cells was developed. The model predicted the experimental results with R^2 of 0.97. The specific oxygen uptake rates of *K. fragilis* during the lag, exponential, stationary, and death phases were found to be 0.32×10^{-12}, 2.14×10^{-12}, 2.14×10^{-12}, 0.51×10^{-12}, and 0.003×10^{-12} mg O_2 $cell^{-1}$ h^{-1}. The oxygen concentration increased during the death

phase and the mathematical model showed that the initial dissolved oxygen concentration would be achieved after 52 h.

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