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Modeling Batch Production of Single Cell Protein from Cheese Whey

I: *Kluyveromyces fragilis* Growth

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ABSTRACT

A mathematical model was developed to describe the growth and death of the yeast *Kluyveromyces fragilis* in cheese whey under aerobic batch fermentation. The model is capable of determining the lengths of the lag, exponential, stationary, and death phases as well as the number of yeast cells during these phases. It predicted the experimental results with R^2 value of 0.99. The total number of yeast cells synthesized in the fermenter was reduced to 55% after 52 h (46 h from the beginning of the death phase). The high percentage of survival was attributed to the fact that the yeast *K. fragilis* reproduced by sporulation (each ascus had 8–10 spores) during the death phase owing to the lack of nutrient. The spores protected the yeast from dying. The maximum specific growth rate (during exponential phase) was 0.23 h^{-1}, whereas the maximum specific death rate (during the death phase) was $-0.32\ h^{-1}$.

Index Entries: Cheese whey; single cell protein; batch fermenter; yeast; modeling; growth; death; spores.

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INTRODUCTION

Microbial growth is the orderly increase in the quantity of all components of the microbial cell. After a microbial cell has doubled in size, and in the amount of each of its components, it divides into two daughter cells. The time required for one cell to grow and divide into two cells is referred to as the doubling time (generation time), which depends on the cell growth characteristics as well as the environmental conditions. Consequently, the growth of microorganisms is measured in terms of the increase in the number of cells, rather than the increase in the size of a single organism.

The growth rate of microorganisms is generally believed to follow Monod's first order equation, which relates the growth rate to the cell mass as follows:

$$dX / dt \quad \mu X \tag{1}$$

where: dX / dt is the growth rate (g mL^{-1} h^{-1}); X is the cell concentration (g mL^{-1}); μ is the specific growth rate (h^{-1}).

The relationship between the specific growth rate and the limiting substrate can be described by the Michaelis-Menton's equation that was developed for enzymatic reactions:

$$\mu = \mu_m S / K_s + S \tag{2}$$

where: μ_m is the maximum specific growth rate (h^{-1}); K_s is the saturation constant (g L^{-1}); S is the substrate concentration (g L^{-1}).

The above equation only describes the growth during the exponential phase. Furthermore, it cannot fully correspond to real courses of microbial cell growth under batch operations since the yield of cell mass, based on the limiting substrate, is not constant. Also, the equation does not take into consideration the exact characteristics of all microbial cell growth. The growth of microbial population is normally limited either by the exhaustion of available nutrients or by the accumulation of toxic metabolic end products. Since these changes in the environment are produced by the microorganisms themselves, the growth and development of microbial population is self-limiting. After the population maximum has been attained, the growth ceases and the population starts to decline as a result of the death of its individual members. These factors determine the shape of the microbial growth curve in batch culture (*1,2*).

The objective of this study was to develop a mathematical model capable of predicting the growth and survival/death rates of the yeast *Kluyveromyces fragilis* during the aerobic batch fermentation of cheese whey for the production of single cell protein (SCP).

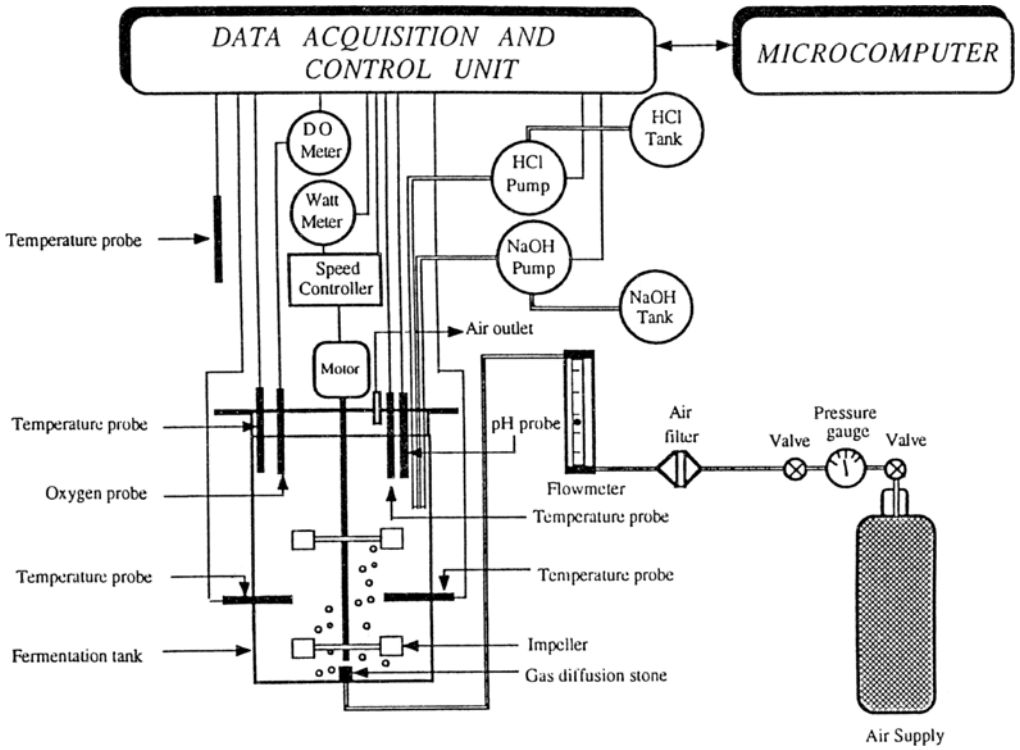

Fig. 1. Experimental setup.

EXPERIMENTAL APPARATUS

The experimental apparatus used in this study is shown in Fig. 1. The fermenter provided a liquid capacity of 4.8 L and was designed to be completely mixed. The mixing system consisted of a steel mixing shaft, two flat-bladed turbine impellers, and a variable speed electric motor (Dayton Electric MFG Co., Model 4Z142). Four baffles were used in the fermenter to reduce vortexing and to improve the top-to-bottom turnover. Compressed air (Medigas Atlantic Ltd, Cat. No. T100172), composed of 78.084% N_2, 20.996% O_2, 0.033% CO_2, and 0.937% other gases, was supplied to the fermenter through a flowmeter with a high-resolution valve using tygon tubing of 10 mm diameter. A microfilter (Cole-Parmer, Cat. No. L-29701-00) was used to reduce the risk of cross contamination. The air was introduced from the bottom of the fermenter through a gas diffusion stone (Fisher Scientific Cat. No. 11-139B) 26 mm height and 22 mm diameter. Dissolved oxygen was monitored using an oxygen electrode probe

Table 1
Some Characteristics of the Raw Cheese Whey Used in the Study

Characteristics	Measured value	Unit
Total solids	63840	mg/L
Ash	9100	mg/L
Ammonium nitrogen	270	mg/L
Organic nitrogen	1420	mg/L
Lactose	5.0	% w/v
pH	4.9	

(YSI 5739, Fisher Scientific, Cat. No. 13-299-43) connected to a digital dissolved oxygen meter (YSI Model 58, Fisher Scientific, Cat. No. 13-298-58). The pH was measured using a pH probe (Cole-Parmer, Cat. No. J-5990-40) connected to a pH control system (3).

EXPERIMENTAL PROCEDURE

The whey was obtained from the Farmer's Cooperative Dairy Plant in Truro, Nova Scotia. It was pumped from the plant cheese whey storage tank into 60 L plastic containers. The containers were subsequently sealed and transported to the Cold Storage Facility of the Biotechnology Laboratory at the Technical University of Nova Scotia, where they were stored at −25°C until used. Some characteristics of the cheese whey used in this study are presented in Table 1. These analyses were performed according to the procedures described in the Standard Methods for the Examination of Water and Wastewater (4). Prior to placing the whey into the fermenter, it was allowed to completely thaw at room temperature for 48 h. Raw whey was first pasteurized in several 250 mL Erlenmeyer flasks by heating the whey to 70°C for 45 min and then cooling it to 1°C for 30 min. The process of heating and cooling was repeated three times.

Freeze dried pellets of *K. fragilis* (NRS 5790) culture were obtained from the Division of Biological Sciences, the National Research Council, Ottawa, Canada. The inoculum was prepared according to the procedure described by Ghaly et al. (5). The fermenter was chemically sterilized using a 2% potassium meta-bisulfite solution and washed with hot distilled-deionized water several times before starting the experiment in order to remove any chemical traces. The fermenter was filled with pasteurized cheese whey and immediately inoculated using 1 L of inoculum. The air flow (2 VVM) and turbine drives (350 RPM) were immediately initiated and dissolved oxygen, pH, and temperature were continuously monitored. Samples were drawn from the fermenter at zero time and thereafter every two

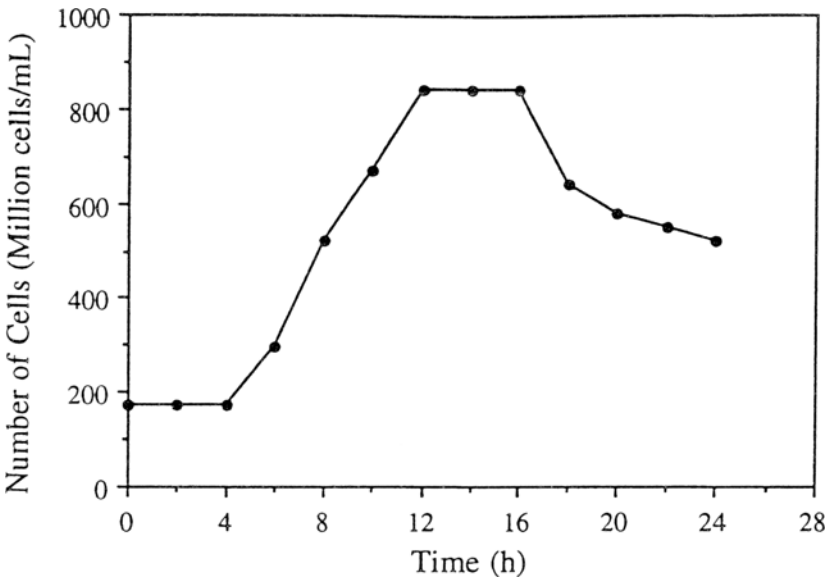

Fig. 2. Batch culture yeast population.

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 (*6*).

RESULTS AND DISCUSSION

Experimental Results

The growth curve is presented in Fig. 2. The pH of the medium was maintained at 4.4±2 (by the addition of 1*N* HCl solution), which is the optimum range for the growth of *K. fragilis* as reported by several authors (*7–11*).

The four principal phases encountered in the history of a microbial culture grown under a batch operation were clearly recognized in the growth curve of *K. fragilis*. These were:

1. The lag phase, which represented the time for the yeast cells to acclimatize themselves to the new environment;
2. The exponential growth phase, during which the growth rate had a constant maximum value;
3. The stationary phase, during which the growth rate was zero; and
4. The death phase, during which the yeast cells died faster than new cells were produced.

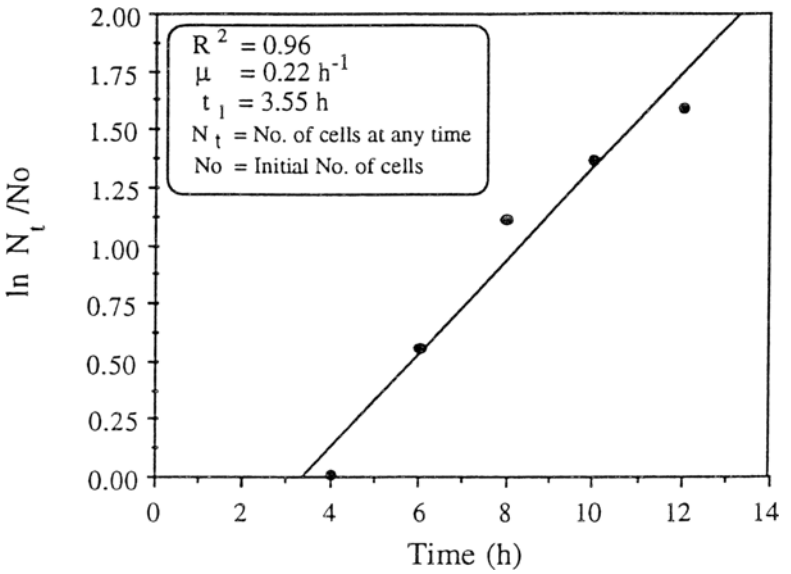

Fig. 3. Determination of specific growth rate and the lag period.

The duration of the lag phase (t_l) and the specific growth rate (μ) were calculated graphically (Fig. 3) and found to be 3.55 h and 0.22 h^{-1}, respectively. Vananuvat and Kinsella (*12*) studied the production of yeast protein from lactose using *K. fragilis* and observed a lag phase of about 4.0–5.0 h. Garibay et al. (*13*) used *K. fragilis* grown in whey with pectin under aerobic conditions to produce single cell protein and observed a lag phase of about 5.0–6.0 h. Ghaly et al. (*2*) stated that the length of the lag phase usually depends on the extent to which the new media and environmental factors such as dissolved oxygen, pH, and temperature are different from those under which the inoculum was prepared. The specific growth rate found in the present study was, also, in agreement with that reported by several authors (*9,14–16*).

The mean doubling time, or generating time, (g) is defined as the time required for all components of the culture to increase by factor of two (*1*). The relationship between μ and g is as follows:

$$\mu = (\ln 2) / g = 0.693 / g \quad (3)$$

In this study, the doubling time for *K. fragilis* was found to be 3 h. Bernstein et al. (*9*) reported a mean doubling time of 2.0 h for *K. fragilis* grown in cheese whey. Hough (*15*) reported a mean doubling time of 2.62 h for brewery yeast. However, Litchfield (*17*) stated that yeasts used for single cell protein production have a generation time in the range of 2–3 h.

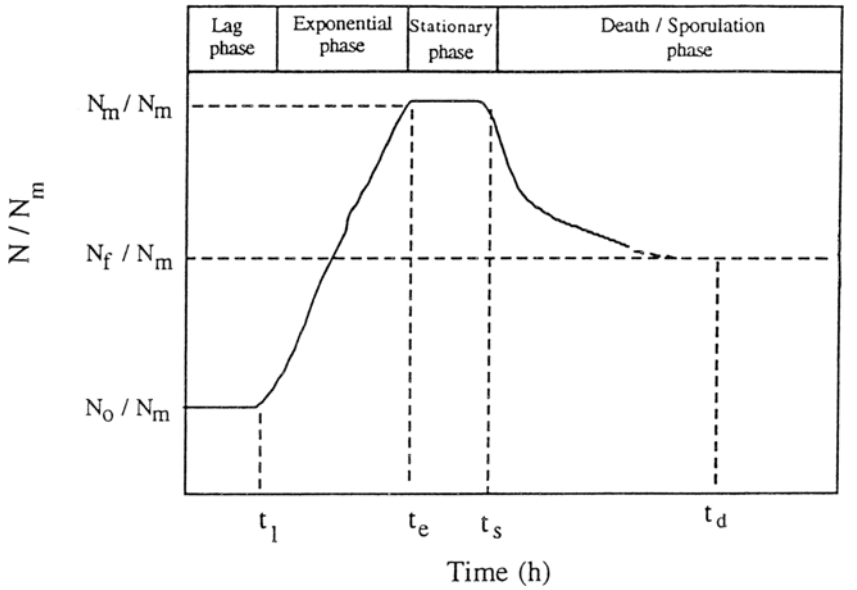

Fig. 4. Graphical representation of the four phases of the growth curve. t_l = end of the lag phase; t_e = end of exponential phase; t_s = end of stationary phase; t_d = end of death phase; N_o = initial number of cells; N_m = maximum number of cells; N_f = final number of cells.

Model Development

The duration and characteristics of the four yeast growth phases can be described graphically as shown in Fig. 4. Thus, Eq. (2) can be rewritten in terms of cell number as follows:

$$dN / dt = \mu N \tag{4}$$

where: dN / dt is the change in cell number (cell mL^{-1} h^{-1}); N is the number of microorganisms (cell mL^{-1}).

Lag Phase

This time (t_l) was necessary for the yeast cells in the inoculum to adjust to the new environment. Thus, the number of cells remained constant during this phase (i.e., $dN/dt = 0$ at $0 < t < t_l$; or $\mu = 0$). The lag phase can be described in nondimensional form as follows:

$$N_t / N_m = N_o / N_m \qquad , 0 < t < t_l \tag{5}$$

where: t is the time (h); t_l is the end of the lag phase (h); N_o is the initial number of cells (cells mL^{-1}); N_m is the maximum number of cells (cells mL^{-1}); N_t is the number of cells at time t (cells mL^{-1}).

Exponential Phase

The growth rate of microorganisms during the exponential growth phase is generally believed to follow the first order kinetic model (*14,18,19*). The growth rate is proportional to the microbial cell number as described by Eq. (4). On integration, Eq. (4) can be rewritten as follows:

$$\int_{N_o}^{N_t} dN / N = \mu \int_{t_l}^{t} dt \tag{6}$$

which yields the following equation:

$$N_t = N_o \cdot e^{\mu(t - t_l)} \qquad , t_l < t < t_e \tag{7}$$

where: t_e is the end of the exponential growth phase (h).

The exponential growth phase can be described in nondimensional form as follows:

$$N_t / N_m = N_o / N_m \cdot e^{\mu(t - t_l)} \qquad , t_l < t < t_e \tag{8}$$

Equation (6) has the form of the following linear equation:

$$Y = a \cdot t + b \tag{9}$$

where: $Y = \ln N_t/N_o$; $a = \mu$; $b = \mu\, t_l$.

The least squares method was used to determine the values of the constants "*a*" and "*b*". Then, the values of "μ" and "t_l" were calculated from Eq. (8). The lag phase (t_l) and the specific growth rate (μ) were found to be 3.72 h and 0.23 h^{-1}, respectively. These values are in good agreement with those obtained graphically from Fig. 3. Letting $N_t/N_m = 1$ at $t = t_e$ in Eq. (8) resulted in a t_e value of 10.6 h. The duration of the exponential growth phase is, therefore, 6.88 h (from 3.72 to 10.60).

Stationary Phase

During the stationary phase, the growth of yeast cells slows down because of the lack of nutrient (lactose), and the number of newly produced cells is equivalent to that of dying cells. The stationary phase can, thus, be considered as a temporary steady state during which N remains constant (i.e., $dN/dt = 0$ at $t_e < t < t_s$). It can be described in nondimensional forms as follows:

$$N_t / N_m = 1 \qquad , t_e < t < t_s \tag{10}$$

where: t_s is the end of the stationary phase (h).

According to Loehr (*20*), microbial cells may die and lyze to provide the constituents of their bodies as nutrients for still-existing cells, a process that may increase the length of stationary phase. However, Ghaly et al. (2) reported that the length of the stationary growth phase will depend on the size of the microbial population and the concentration of nutrient in the medium. In this study, the duration of the stationary growth phase was approx 5.4 h (from 10.6 to 16.0), which is longer than that of 3 h reported by Mickle et al. (*21*).

Death Phase

A decrease in the total number of viable cells in the population signals the onset of the death phase. In the death phase, the microbial cells held in a nongrowing state eventually die. Death results from a number of factors such as the depletion of viable nutrients or accumulation of toxic metabolic end products. However, the death rate of microorganisms is highly variable, being dependent on the environment as well as on the type of organisms (*1,18,20*). In this study, the decrease in the number of viable *K. fragilis* cells appears to fit a nonlinear model. The maximum number of cells synthesized in the system will continue to decrease with time until it reaches a final value (N_f), which can be defined as follows:

$$N_f / N_m = \tau \tag{11}$$

where: τ is the survival constant (−); N_f is the final number of cells (cell mL^{-1}).

The rate at which N decreases can be described in nondimensional form as follows:

$$d / dt\,(N / N_m) = -K_d\,(N / N_m)\,(N / N_m - \tau) \tag{12}$$

where: k_d is the specific death rate (h^{-1}).

On integration, Eq. (12) can be rewritten as follows:

$$\int_1^{N/N_m} d\,(N/N_m) / N/N_m\,(N/N_m - \tau) = -k_d \int_{t_s}^{t} dt \tag{13}$$

Which yields the following equation:

$$N_t / N_m = \tau / [\,1 - (1-\tau)\,e^{-k_d \tau\,(t - t_s)}] \qquad , t > t_s \tag{14}$$

Equation (14) indicates that the number of yeast cells will approach a certain value (N_f) as t approaches ∞. By solving Eq. (14) numerically the values of τ, k_d, and t_s were found to be 0.55 cell mL^{-1}, $-0.31\ h^{-1}$, and 16 h, respectively. The model suggests that a final cell number (N_f) of 462×10^6 cell mL^{-1} will be obtained after approx 52 h from the start of the experiment (46 h from the start of the death phase).

Loehr (*20*) stated that the synthesis and endogenous metabolism occur simultaneously in biological systems with synthesis predominating when there is an excess of exogenous food and endogenous metabolism predominating when the exogenous food supply is small or nonexistent. He also pointed out that the microbial mass would not be reduced to zero, even with long endogenous metabolism periods, and a residue of about 20–25% of the total synthesized mass would remain in the system. The present results indicated that the total microbial mass synthesized in the fermenter was reduced to 55% of its size after 46 h from the start of the death phase. Close observation of the morphology of the yeast cells under the light microscope indicated that the yeast culture obtained from the fermenter during the exponential growth phase had only vegetative cells (oval to elongated) of the yeast *K. fragilis* (Fig. 5A) and those cells were

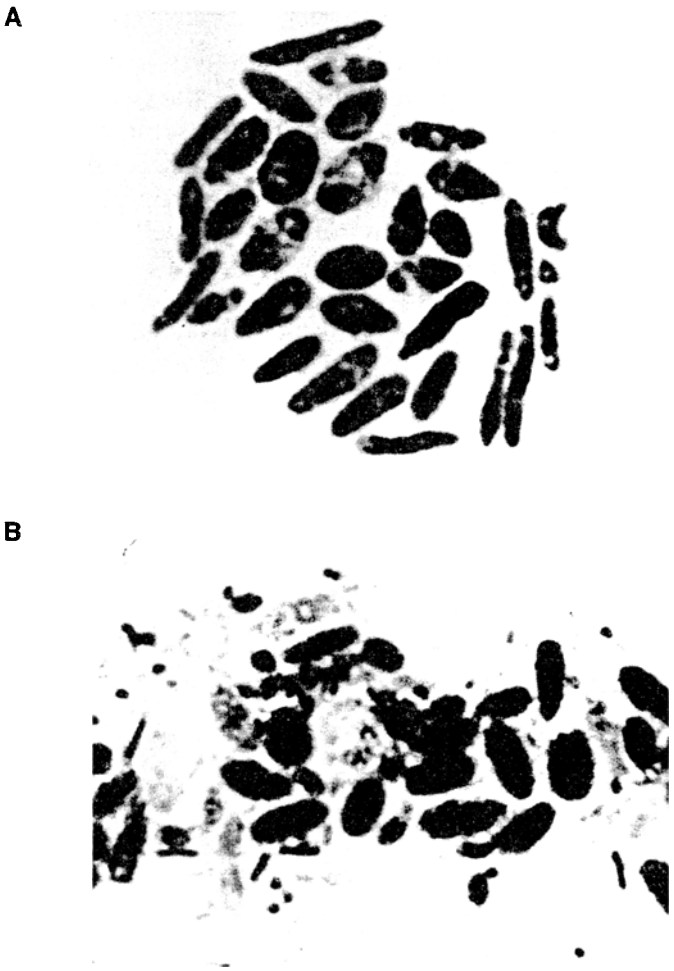

Fig. 5. Microscopic observations of yeast cells. A. Elongated cells (exponential growth phase). B. Vegetative cells and ascopores observed (death phase).

reproducing by budding. In contrast, the majority of cells in the yeast culture obtained from the fermenter during the death phase were spores with 8–10 spores per ascus (Fig. 5B). It is very clear from these observations that when the nutrient source became limited some of the cells died, whereas others formed the kidney shaped ascospore. When these spores were transferred into cheese whey under favorable environmental conditions, germination occurred and the spores ruptured and developed into new vegetative cells.

The growth rate equation and the integral form for each phase of yeast cell growth are presented in Table 2. The model parameters are presented in Table 3. The predicted and experimented cell number are shown in Fig. 6. The coefficient of correlation (R^2) was 0.99.

Table 2
The Growth Rate Equation and Integral Form
of Each Phase of the Yeast Cell Growth

Phase	Uptake equation	Integral form	Limit
Lag	$dN / dt = 0$	$N_t = N_o$	$0 < t < t_l$
Exponential	$dN / dt = \mu N$	$N_t = N_o \cdot e^{\mu(t - t_l)}$	$t_l < t < t_e$
Stationary	$dN / dt = 0$	$N_t = N_m$	$t_e < t < t_s$
Death	$dN / dt = K_d N(N / N_m - \tau)$	$N_t = \tau N_m / [1 - (1 - \tau) e^{-k_d \tau (t - t_s)}]$	$t < t_s$

Table 3
Model Parameters

Parameters	Value	Unit
N_o	210×10^6	cell mL^{-1}
N_f	462×10^6	cell mL^{-1}
N_m	840×10^6	cell mL^{-1}
t_l	3.72	h
t_e	10.60	h
t_s	16.00	h
t_d	46.00	h
μ	0.23	h^{-1}
K	−0.31	h^{-1}
τ	0.55	

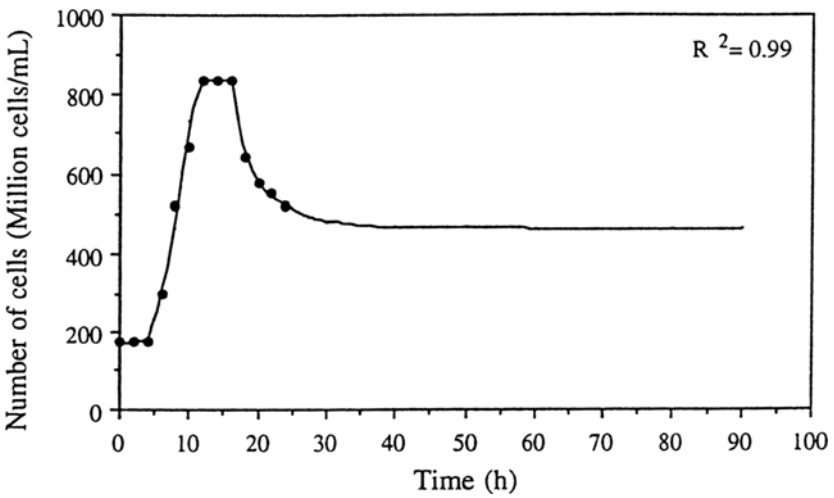

Fig. 6. Measured and predicted values of the cell number.

CONCLUSIONS

A mathematical model was developed to describe the four phases of the yeast growth curve. The model predicted the experimental results with R^2 value of 0.99. The model is capable of determining the lengths of the lag, exponential, stationary and death phases as well as the size of the yeast population during these phases. The maximum specific growth rate of the yeast *K. fragilis* used in this study was 0.23 h^{-1}. The death rate was −0.31 h^{-1} and the total microbial mass synthesized in the fermenter was reduced to 55% after 46 h from the start of the death phase. The relatively high percentage of survival can be attributed to the fact that the yeast *K. fragilis* reproduced by sporulation during the death phase owing to the lack of nutrient. The number of spores formed were 8–10/ascus.

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