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Modeling Batch Production of Single Cell Protein from Cheese Whey

II: Lactose Metabolism

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ABSTRACT

A mathematical model capable of describing lactose metabolism 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 0.99. The lactose curve displayed three distinct stages that corresponded to the lag, exponential, and stationary growth phases of the yeast. The reduction of the lactose concentration was affected by the number of yeast cells present in the system. Only 4% of the lactose was utilized during the lag phase, 85% was utilized during the exponential phase, and 9% was utilized during the stationary phase. The lactose utilization rates for the lag, exponential and stationary phases were 0.292×10^{-12}, 1.475×10^{-12}, and 0.286×10^{-12} g cell^{-1} h^{-1}, respectively. The lactose consumed during the lag and stationary phases was metabolized for cell endogenous growth and cell maintenance, whereas that consumed during the exponential phase was metabolized for cell endogenous growth, cell maintenance, and cell multiplication.

Index Entries: Cheese whey; single cell protein; batch fermenter; yeast; modeling; lactose; multiplication; endogenous; growth; maintenance.

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INTRODUCTION

Growing microorganisms must obtain from their surroundings all the nutrients necessary for synthesizing and maintaining their cell substance. They require inorganic salts as well as sources of carbon, nitrogen, and energy. Most microbes can synthesize their nitrogenous materials from ammonium or nitrate and obtain their carbon source and energy requirements from organic compounds such as sugars (*1*). Lactose ($C_{12}H_{22}O_{11}$) is the only commercial sugar that comes from mammalian sources and its use is expanding in the food industry. Lactose can be found in a variety of food sources such as milk, cream, nonfatty dry milk, whey solids, modified whey products, and refined lactose (*2*). Lactose in cheese whey has been used by Mansour et al. (*3–8*) for the production of single cell protein (SCP) using the yeast *Kluyveromyces fragilis*. Cheese whey is a byproduct fluid (greenish yellow liquid) of the cheese industry. It contains approx 5% lactose, 1% nitrogen compounds, 0.8% minerals, and small amount of vitamins. The aerobic decomposition of lactose is a process that provides energy for growth and supplies nutrient for synthesis of new microbial protoplasm. The net reaction is as follows:

$$14C_{12}H_{22}O_{11} + 12O_2 + 24NH_4 \xrightarrow{\text{yeast}} 12C_{13}H_{20}N_2O_7 + 12CO_2 + 70H_2O + 24H^+ + Energy \quad (1)$$

The objective of this study was to develop a mathematical model capable of describing the lactose utilization rate by *K. fragilis* during various growth phases of aerobic batch fermentation of cheese whey for single cell protein production.

MATERIALS AND METHODS

The experimental apparatus used in this study has been previously described by Mansour et al. (*8*). The whey used in the study had 6.4% total solids, 5% lactose, 1.7% total nitrogen, 0.9% minerals, and a pH of 4.9. The cheese whey collection and sterilization, the inoculum preparation, and the system operation were previously described by Mansour et al. (*8*).

Samples were drawn from the fermenter at zero time and thereafter every two hours. For each sample, the lactose concentration and plate count tests were conducted. The lactose analysis was performed using a sugar analyzer (YSI Model 27, Fisher Scientific Cat. No. 14-660). The plate count was carried out according to the procedures described in the Standard Methods for the Examination of Dairy Products (*10*).

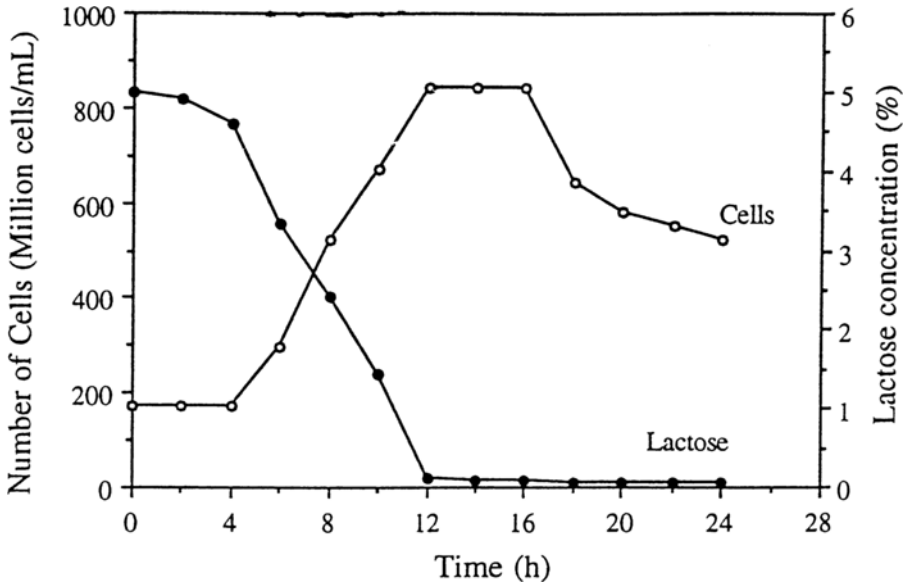

Fig. 1. Batch culture population and lactose concentration.

RESULTS AND DISCUSSION

The lactose concentration and cell number results are presented in Fig. 1. 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 are:

1. The lag phase, which represented the time (3.55 h) required for the yeast cells to acclimatize themselves to the new environment;
2. The exponential growth phase, which represented the time (6.88 h) during which the growth rate (μ) had a constant maximum value of 0.23 h^{-1};
3. The stationary phase, which represented the time (5.40 h) during which the growth rate was zero; and
4. The death phase, during which the death rate was -0.31 h^{-1}.

The initial cell number (N_o) was 171×10^6 cell mL^{-1}, which increased during the exponential phase to a maximum value (N_m) of 840×10^6 cell mL^{-1} and then declined during the death phase to a final value (N_f) of 462×10^6 cell mL^{-1}.

The initial value of lactose in the fermenter was 240 g (5.0%), which declined to 2.4 g (0.05%) after 12 h. This resulted in a reduction of about 99.0% of lactose. The reduction of lactose displayed three distinct stages

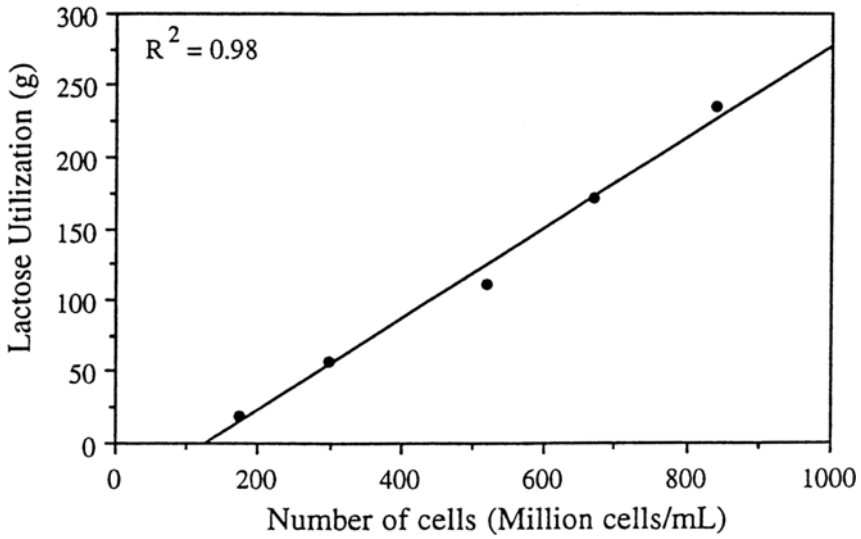

Fig. 2. Effect of number of cells on the lactose utilized during the exponential growth phase of the batch fermentation.

that corresponded to the lag, exponential, and stationary phases of the yeast growth curve. There was a slow reduction in the lactose concentration in the first stage followed by a period of rapid lactose reduction. In the third stage, the concentration of lactose in the fermenter was very low, 5.76–2.4 g (0.12–0.05%) and, thus, an insignificant reduction of lactose was achieved during this period. The lactose utilization was affected by the number of yeast cells present in the system, as shown in Fig. 2. The lactose utilization efficiency was 95.6% and the yeast yield was 0.78 g cell/g lactose. The stoichiometric yield (Eq. 1) is 0.79 g cell/g lactose.

The duration and characteristics of the three phases of lactose concentration are described mathematically in Fig. 3.

Lag Phase

In this stage, about 4% of the lactose was metabolized by the yeast cells. This was utilized mainly for cell respiration (maintenance) and cell endogenous growth (individual cell growth). Since the number of cells remained constant during this phase, the change in lactose concentration in the reactor can be described as follows:

$$dL / dt = -\eta_1 N_o \qquad ,0 < t < t_l \tag{2}$$

where: dL / dt is the change in lactose concentration (g L^{-1} h^{-1}); η_1 is the specific lactose utilization rate during the lag phase (g $cell^{-1}$ h^{-1}); N_o is the initital number of cell (cell mL^{-1}); t_l is the end of the lag phase (h); and t is the time (h).

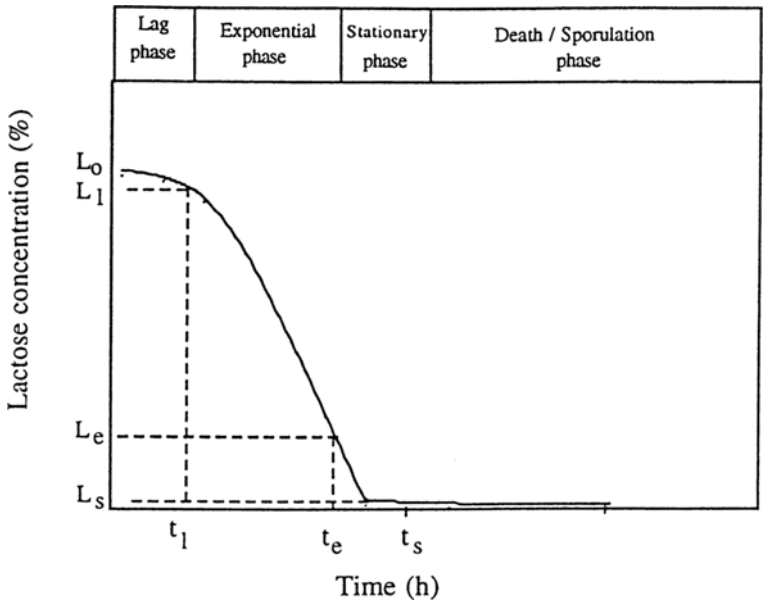

Fig. 3. Graphical representation of the four phases of lactose curve. t_l = end of the lag phase; t_e = end of exponential phase; t_s = end of stationary phase; L_o = initial lactose concentration; L_l = lactose concentration at the end of the lag phase; L_e = lactose concentration at the end of exponential phase; L_s = lactose concentration at the end of stationary phase.

On intregation, Eq. (2) can be rewritten as follows:

$$\int_{L_o}^{L} = -\eta_1 N_o \int_o^t dt \tag{3}$$

which yields the following equation:

$$L_t = L_o - \eta_1 N_o t \qquad ,0 < t < t_l \tag{4}$$

where: L_o is the initial lactose concentration (g); L_t is the lactose concentration at time t (g); and t is the time (h).

By using the least squares method to solve Eq. (4), the value η_1 was found to be 0.292×10^{-12} g lactose cell^{-1} h^{-1}. The model suggests that the lactose concentration at the end of the lag phase and the beginning of the exponential phase (i.e., $t = 3.55$ h) would be 4.82%.

Exponential Phase

During this phase, about 85% of the lactose was removed from the system. This was utilized by the yeast for endogenous cell growth (cellular growth), cell mass growth (cell multiplication), and cell respiration (maintenance). During this phase, the number of cells increased exponentially as shown in the following equation (*8*).

$$N_t = N_o e^{\mu(t - t_l)} \qquad , t_l < t < t_e \tag{5}$$

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

Thus, the rate of change in lactose concentration in the reactor can be described as follows:

$$dL / dt = - \eta_2 N_o e^{\mu(t - t_l)} \qquad , t_l < t < t_e \tag{6}$$

where η_2 is the specific lactose consumption rate during the exponential phase (g $cell^{-1}$ h^{-1}); and t_e is the end of the exponential phase (h).

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

$$\int_{L_l}^{L} dL / dt = - \eta_2 N_o \int_{t_l}^{t} e^{\mu(t - t_l)} dt \tag{7}$$

which yields the following equation:

$$L_t = L_o - \eta_1 N_o t_l - \eta_2 N_o / \mu \, [e^{\mu(t - t_l)} - 1] \qquad , t_l < t < t_e \tag{8}$$

By using the least squares method to solve Eq. (8), the value of η_2 was found to be 1.475×10^{-12} g lactose $cell^{-1}$ h^{-1}, which is about 5.1 times the value of η_1. The model also suggests that about 89% of the initial lactose in the system would be utilized by the end of the exponential phase (i.e., at $t = 10.6$ h), of which 85.4% was utilized during the exponential phase.

Stationary Phase

In the third stage, which lasted approx 5.6 h, only 9.0% of initial lactose was removed from the system. The concentration of the lactose during this phase was very low (0.55–0.08%). As a result, the microbial growth was kept stationary owing to the limited substrate. According to Tuite (*17*), yeast cells enter the stationary (nongrowing) phase when deprived of essential nutrients such as a carbon source. Entry into the stationary phase is accompanied by a significant drop in the overall rate of protein synthesis, to less than 10% of that observed in actively growing logarithmic phase cells. Lactose uptake during this period was, therefore, considered to be a function of the concentration of the lactose in the system. Thus, the term (L/L_{te}) was used in the equation describing the rate of change of lactose as follows:

$$dL / dt = \eta_3 N_m L / L_e \qquad , t_e < t < t_s \tag{9}$$

where: η_3 is the specific lactose consumption rate during the stationary phase (g $cell^{-1}$ h^{-1}); t_s is the end of the stationary phase (h); N_m is the maximum cell number (cell mL^{-3}); and L_e is the lactose concentration at the end of the exponential phase (g).

On integration, Eq. (9) can be rewritten as follows:

$$\int_{L_e}^{L} dL / L = \eta_3 N_m / L_e \int_{t_e}^{t} dt \tag{10}$$

which yields the following equation:

$$L_t = L_e e^{-(\eta_3 N_m / L_{te})(t - t_e)} \quad , t_e < t < t_s \tag{11}$$

By using the least square method to solve Eq. (11), the value of η_3 was found to be 0.286×10^{-12} g lactose cell^{-1} h^{-1}, which is equivalent to η_1.

Death Phase

The concentration of lactose during this phase was almost zero, which resulted in the death and/or the sporulation of the vegetative cells of the yeast as previously described (*8*). The lactose uptake equation and the integral form for each phase of yeast cell growth are presented in Table 1.

The values of the specific lactose utilization rate for the lag, exponential, stationary, and death phases (η_1, η_2, η_3, and η_4) are presented in Table 2. The predicted and measured lactose concentrations are shown in Fig. 4. The model predicted the experimental results with R^2 value of 0.99. The results indicated that the specific lactose uptake rate of the yeast *K. fragilis* was much higher in the exponential phase than those of the lag and stationary phase. The specific lactose uptake during the lag phase was similar to that of the stationary phase. However, the lag phase was a period of endogenous cellular growth, whereas the stationary phase was a period of a limited substrate.

About 99% of the initial lactose was utilized in 12 h in this study, of which 4%, 85%, and 9% were utilized during the lag, exponential, and stationary growth phases, respectively. Bernstein et al. (*3*) reported that in batch fermentation of cheese whey by *K. fragilis*, most of the lactose was utilized in 8 h for the production of single cell protein. Barraquio et al. (*18*) reported 0.92% lactose removal in 13 h. The stoichiometric cell yield (Eq. 1) is 0.79 g cell/g lactose. The yeast cell yield found in this study was 0.78 g cell/g lactose. This was much higher than those of 0.45 and 0.43 g cell/g lactose reported by Harju et al. (*19*) and Wasserman et al. (*20*) for *K. fragilis*, respectively.

CONCLUSIONS

The lactose curve displayed three distinct stages that corresponded to the lag, exponential, and stationary growth phases of the yeast. Only 4.0% of the lactose was utilized during the lag phase, 85.0% was utilized during the exponential phase, and 9.0% was utilized during the stationary phase. The reduction of the lactose concentration was affected by the number of yeast cells present in the system. The lactose consumed during the lag and stationary phases was used for cell endogenous growth as well as respiration, whereas that consumed during the exponential phase was used for cell multiplication as well as energy release.

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

Phase	Uptake equation	Integral form	Limit
Lag	$dL / dt = -\eta_1 N_o$	$L_t = L_o - \eta_1 N_o t_e$	$t_o < t < t_l$
Exponential	$dL / dt = -\eta_2 N_o$	$L_t = L_o - \eta_1 N_o t_l - \eta_2 N_o / \mu [e^{\mu(t - t_l)} - 1]$	$t_l < t < t_e$
Stationary	$dL / dt = -\eta_3 N_m$	$L_t = L_e e^{-(\eta_3 N_m / L_{t_e})(t - t_e)}$	$t_e < t < t_s$
Death	$dL / dt = 0$	$L_t = 0$	$t < t_s$

Table 2
The Specific Lactose Utilization Rate

Phase	η (g/cell/h)
Lag growth phase	0.292×10^{-12}
Exponential growth phase	1.475×10^{-12}
Stationary growth phase	0.286×10^{-12}
Death phase	0.000×10^{-12}

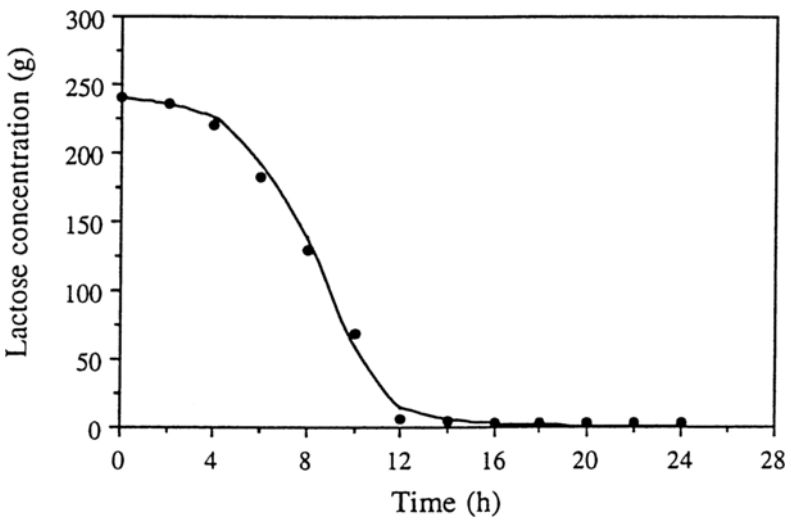

Fig. 4. The measured and predicted values of the lactose concentration.

A model describing the lactose concentration during the batch operation as a function of the number of yeast cells was developed. The model predicted the experimental results with R^2 of 0.99. The specific lactose uptake rates of *K. fragilis* during the lag, exponential, stationary, and death phase were found to be 0.292×10^{-12}, 1.475×10^{-12}, 0.286×10^{-12}, and 0.000 g lactose cell^{-1} h^{-1}, respectively.

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