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Metabolism of Cheese Whey Lactose by *Kluyveromyces fragilis* for Energy and Growth Under Batch Condition

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ABSTRACT

The fermentation of lactose by *Kluyveromyces fragilis* is an exothermic reaction in which heat is released, resulting in a rise in reactor temperature. A heat balance was performed on a 5-L batch reactor used for single cell protein (SCP) production to determine the portions of cheese whey lactose used for energy and growth. On the average, about 88% of lactose was used for growth, and 12% was used for energy. The lactose consumed during the lag and stationary phases was used mostly for cell endogenous growth and respiration, whereas that consumed during the exponential growth phase was used for cell multiplication and energy. The heat released varied from 6.5 to 8.9 kJ/g cell. Because of the proper design of the fermenter, the temperature of the medium did not rise above 33°C; thus, no cooling system was needed.

Index Entries: Single cell protein; yeast; lactose; batch fermenter; growth; energy; heat; temperature; pH; oxygen; respiration.

INTRODUCTION

Cheese whey is a greenish-yellow watery byproduct of cheese-making. When whole milk is used to produce natural and processed cheeses such as cheddar, the resultant fluid is called *sweet whey* and has a pH in the

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range of 6.0–7.0. When skim milk is used to produce cottage cheese, the by-product fluid is called *acid whey*, which has a pH in the range of 4.0–5.0 as a result of the acid developed (or employed) during the coagulation process. Generally, about 10 kg of whey are produced for every 1 kg of cheese (*1*). The composition of cheese whey is about 93% water, 5% lactose, 0.9% protein, 0.3% fat, 0.2% lactic acid, and small amounts of vitamins (*2,3*). Modification of whey via fermentation has intrigued scientists for some time now. The most popular processes have been the production of ethyl alcohol, nonalcoholic and alcoholic beverages, and SCP (*4,5*). However, only a few species of microorganisms metabolize lactose. Some of these species are *Kluyveromyces fragilis, Trichosporon cutaneum, Morchella crassipes,* and *Marchella hortensis* (*6*).

Microorganisms are made of many small fractions of more than 100 elements, four of which (carbon, hydrogen, oxygen, and nitrogen) comprise about 98.0% by wt of the atoms of cells. Phosphorus and sulfur make up an additional 1.0%, and all of the remaining elements account for less than 0.5%. According to Nester et al. (*7*) and Luria (*8*), this composition may differ in some specialized cells in which certain elements are associated with a specific structure or function. On the average, a yeast cell contains 47% carbon, 6% hydrogen, 32.5% oxygen, 8.5% nitrogen, and 6% ash. Thus, the empirical formula of the yeast is $C_{13}H_{20}N_2O_7$.

Under aerobic conditions, yeast metabolize cheese whey lactose to produce more SCP. The aerobic decomposition of cheese whey lactose is a process that provides energy for growth and supplies nutrient for synthesis of new microbial protoplasm. The process can be illustrated as follows:

(a) energy release (respiration)

$$C_{12}H_{22}O_{11} + 12O_2 \xrightarrow{\text{Yeast}} 12CO_2 + 11H_2O + \text{Energy} \quad [1]$$

(b) synthesis (growth)

$$13\,C_{12}H_{22}O_{11} + 24NH_4 \xrightarrow{\text{Yeast}} 12C_{13}H_{20}N_2O_7 + 59H_2O + 24H^+ \quad [2]$$

Combining Eq. 1 and 2, a typical net reaction of the aerobic decomposition of lactose can be written as follows:

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

The fermentation of lactose by the yeast *K. fragilis* is an exothermic reaction in which heat is released, thereby causing a rise in the reactor temperature. Reisman et al. (*9*) reported that a value of 4 kcal/g (16.7 kJ/g) of substrate is generally accepted for heat of reaction of carbohydrates. This was also confirmed by Weast (*10*), who reported that the heat of reaction of lactose ($C_{12}H_{22}O_{11}$) is equal to 5650 kJ/g of mol wt.

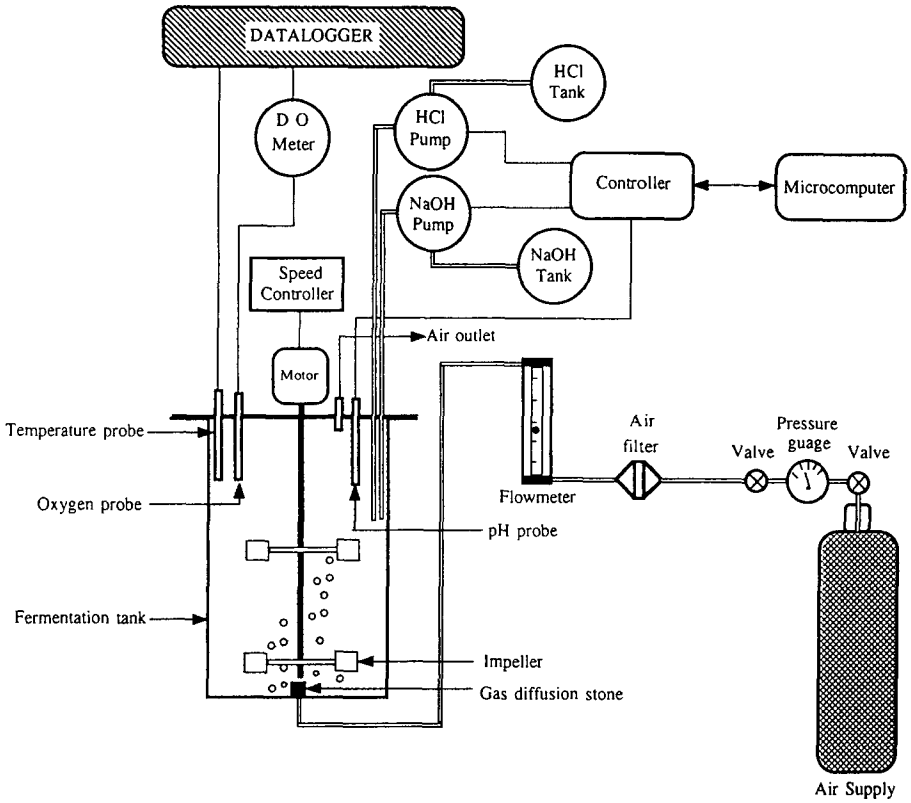

Fig. 1. Experimental apparatus.

OBJECTIVES

The aim of this study was to investigate the production of SCP from cheese whey using *K. fragilis* under batch conditions. The specific objectives were:

1. To conduct heat balance on a cheese whey batch fermentation system used for the production of SCP, and
2. To calculate the portions of cheese whey lactose used for energy and growth of *K. fragilis* during batch fermentation.

EXPERIMENTAL APPARATUS

The experimental apparatus used in this study is shown in Fig. 1. The fermenter shown in Fig. 2 provided a liquid capacity of 4.8 L when the mixing device was submerged. Three holes were drilled and tapped through the lid: one hole for the inlet pipe for cheese whey loading, another for the oxygen probe, and the third for the pH probe.

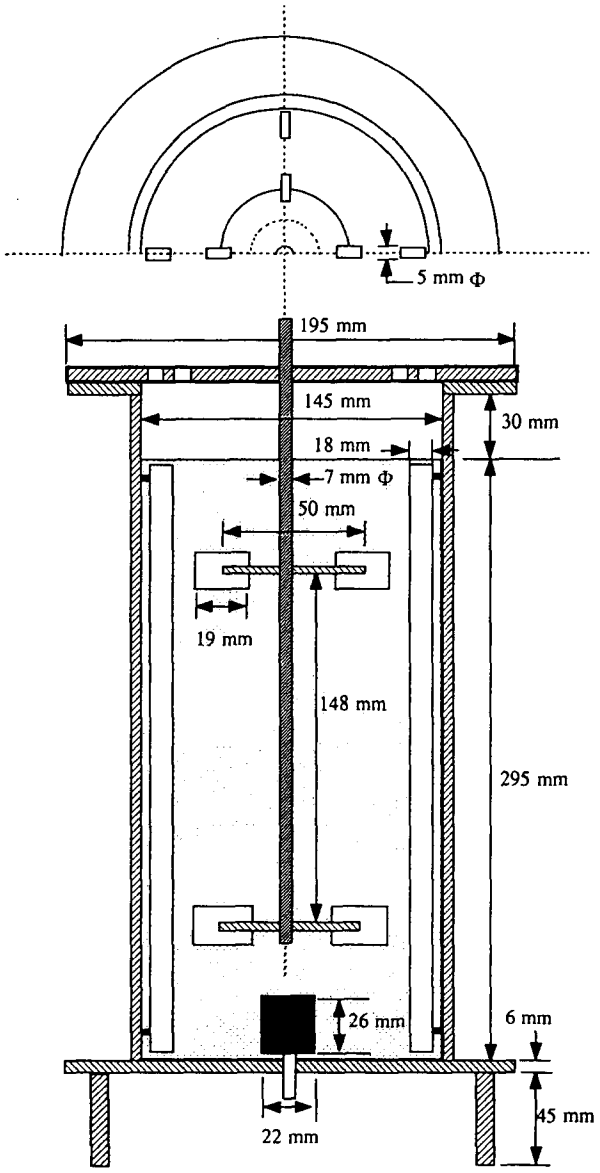

Fig. 2. A vertical cross-section of the fermenter.

The fermenter was designed to be completely mixed, and hence, a steel mixing shaft of 7 mm diameter and 400 mm length was installed through the center of the lid. Two flat-bladed turbine impellers of 75 mm diameter were used to mix the fermenter content. The first impeller was positioned at the end of the mixing shaft at about 30 mm from the fermenter floor, and the other impeller was positioned at 148 mm from the first impeller (or 178 mm from the fermenter floor). The mixing shaft

was driven by a variable speed electric motor (Dayton Electric MFG Co., Chicago, IL, Model 4Z142). Four baffles were used in the fermenter to reduce vortexing and to improve the top-to-bottom turnover. Standard design recommended for baffles, given by *Perry's Chemical Engineer's Handbook* (*11*), was used.

Compressed air (Medigas Atlantic Limited, Halifax, N.S., Cat. No. T100172) was supplied to the fermenter through a flowmeter with high-resolution valve using tygon tubing of 10 mm diameter. The air was made of 788.084% N_2, 20.996% O_2, 0.033% CO_2, and 0.937% other gases. The moisture and impurities in the air were less than 10 and 2 ppm, respectively. A microfilter (Cole-Parmer, Chicago, IL, 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, Ottawa, Ont., Cat. No. 11-139B), 26 mm height and 22 mm diameter.

Dissolved oxygen was monitored using an oxygen electrode probe (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). A copper-constantan thermocouple inserted in the fermenter and connected to a data logger (Cole-Parmer Cat. No. L-08360-14) was used to measure the temperature. The pH was measured using a pH probe (Cole-Parmer Cat. No. J-5990-40) connected to a pH control system. The pH control system included a microcomputer (TRS-80 Model 100), a data acquisition unit, signal conditioning circuit, and two pumps. More elaborate discussion on the pH control system can be found in Ben-Hassan et al. (*12*).

EXPERIMENTAL PROCEDURE

The cheese whey was obtained from the Farmer's Cooperative Dairy Plant in Truro, Nova Scotia. The cheese whey 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 in a large freezer at −25°C until required. 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 *Standard Methods for the Examination of Water and Wastewater* (*13*). Prior to placing the cheese whey into the fermenter, it was allowed to completely thaw at room temperature for 48 h. Raw cheese whey was first pasteurized in several 250-mL erlenmeyer flasks. The pasteurization process involved heating the whey to 70°C for 45 min and then cooling it to 1°C for 30 min. The processes 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, National Research Council,

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

Characteristics	Measured value	Unit
Total solids	63835.00	mg/L
Fixed solids	9100.00	mg/L
Volatile solids	54735.00	mg/L
Percent volatile solids	85.74	%
Percent fixed solids	14.26	%
Suspended solids	22150.00	mg/L
Fixed solids	185.00	mg/L
Volatile solids	21965.00	mg/L
Percent volatile solids	99.16	%
Percent fixed solids	0.84	%
Total Kjeldhal nitrogen	1690.00	mg/L
Ammonium nitrogen	270.00	mg/L
Organic nitrogen	1420.00	mg/L
Percent organic nitrogen	84.02	%
Percent ammonium nitrogen	15.98	%
Total chemical oxygen demand	74220.00	mg/L
Soluble chemical oxygen demand	59640.00	mg/L
Insoluble chemical oxygen demand	14580.00	mg/L
Percent soluble chemical oxygen demand	80.36	%
Percent insoluble chemical oxygen demand	19.64	%
Lactose	5.00	%
pH	4.90	—

Ottawa, Canada. A pellet of *K. fragilis* was dissolved in 5 mL sterilized growth medium containing 1% yeast extract, 2% peptone, and 2% dextrose. A loop of this solution was streaked on an agar medium containing 1% yeast extract, 2% dextrose, 2% peptone, and 2% agar in a petri dish. The petri dish was then placed in a controlled environment incubator at 35°C and left until visual growth appeared on the petri dish (after about 72 h). The yeast was transferred from the petri dish to the pasteurized cheese whey in the 250-mL sterilized erlenmeyer flasks. Two petri dishes of pure culture of *K. fragilis* were added to each flask containing 100 mL pasteurized cheese whey in order to start with a large seed size and a high cell count. The erlenmeyer flasks were capped with nonabsorbent cotton plugs and mounted on a controlled environment reciprocating shaker. The shaker was operated at a speed of 250 rpm for 48 h.

The fermenter was chemically sterilized using a 2% potassium metabisulfite 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 imme-

diately inoculated using separate flasks of inoculum (950 mL). The air flow (2 vum) and turbine drives (350 rpm) were started immediately, and dissolved oxygen, pH, and temperature were monitored continuously. The ambient temperature remained at 21 ± 1°C during the experiment.

At 0 h, samples were drawn from the fermenter immediately. Then, samples were drawn every 2 h. For each sample, the percentage of lactose 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 plant count was carried out according to the procedures described by Messer et al. (*14*) in *Standard Methods for the Examination of Dairy Products.*

RESULTS AND DISCUSSION

Ghaly et al. (*15*) reported that maintaining the pH between 4.0 and 5.0 is very essential for the growth and survival of *K. fragilis*. It also has been recognized by Singh and Ghaly (*2*), Bernstein et al. (*16*), Bernstein and Everson (*17*), and Wasserman (*18*) that keeping the pH at about 4.5 eliminates possible contamination by lethal bacteria that grow at pH levels above 6.0. In this study, the fermenter was operated under batch condition for approximately 28 h. The pH of the medium was maintained at 4.4 ± 0.2 by the addition of 1*N* HCl solution. The cell number and lactose concentration were measured every 2 h, and the oxygen concentration and temperature were continuously monitored. The results are presented in Table 2 and Fig. 3.

Microbial Growth

The growth curve of *K. fragilis* is shown in Fig. 3. The important feature of the four principal phases encountered in the history of a microbial culture grown under a batch operation can be clearly recognized. These are:

1. The lag phase, which represents the time for the yeast cells to acclimatize themselves to the new environment;
2. The exponential growth phase, during which the growth rate has a constant maximum value;
3. The stationary phase, during which the growth rate is zero; and
4. The death phase, during which yeast cells die faster than new cells are produced.

The yeast grow exponentially between 4.0 and 12.0 h. The specific growth rate (μ) of *K. fragilis* population was about 0.2 h^{-1} during the exponential growth phase. Alvarez and Ricano (*19*) reported that the specific growth rate of *K. fragilis* using the batch culture fermentation for producing SCP was about 0.21 h^{-1} at 37°C and pH of 4.5. The duration of

Table 2
Measured Values of Cell Number, Lactose Concentration, Dissolved Oxygen, and Temperature at 2-h Time Intervals

Time, h	Cell number, 10^6 cells/mL	Lactose concentration (%)	Lactose concentration (g)	Lactose consumed (g)	Lactose consumed (%)*	Oxygen, mg/L	Temperature °C
0	171	5.00	240.00	0.00	0.00	5.50	22.0
2	171	4.90	235.20	4.00	2.00	5.40	22.5
4	172	4.60	220.86	19.14	8.00	5.10	23.0
6	298	3.82	183.36	56.64	23.60	4.40	25.2
8	520	2.70	129.60	110.40	46.00	2.60	28.0
10	670	1.43	68.84	171.36	71.40	1.60	31.7
12	840	0.12	5.76	234.24	97.60	1.20	33.0
14	840	0.10	4.80	235.20	98.00	1.20	33.0
16	840	0.08	3.84	236.16	98.40	1.20	33.0
18	640	0.06	2.88	237.17	98.80	2.20	31.5
20	580	0.05	2.40	237.60	99.00	2.80	28.5
22	550	0.05	2.40	237.60	99.00	3.10	25.5
24	520	0.05	2.40	237.60	99.00	3.20	22.5

Fermenter vol = 4.8 L.
*Percent of the original value.

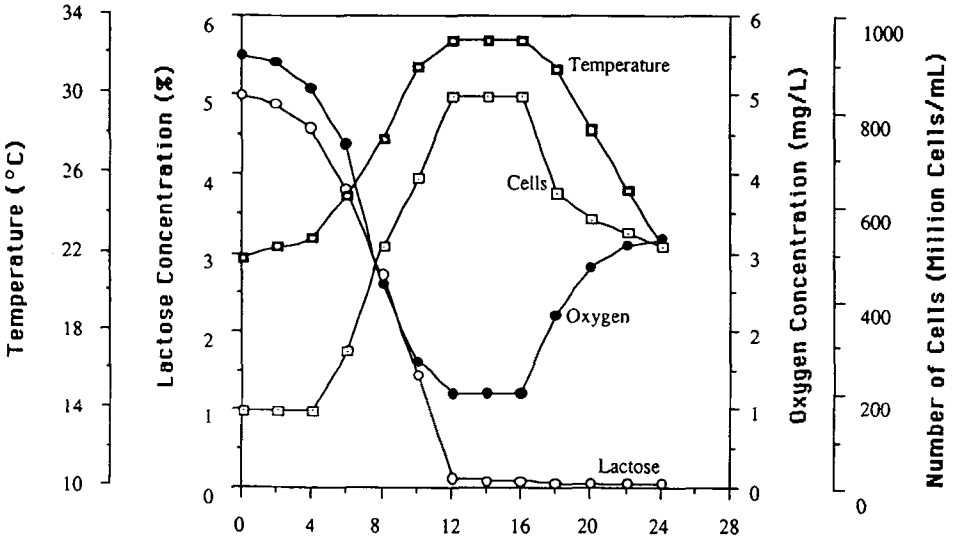

Fig. 3. Batch culture yeast population, lactose concentration, dissolved oxygen concentration, and temperature.

the lag phase was found to be approx 4.0 h. Vananuvat and Kinsella (*20*) studied the production of yeast protein from lactose using *K. fragilis* and observed a lag phase of about 4.0–5.0 h.

Lactose Utilization

The initial value of lactose was 5.0% (which is equal to 50 g/L). Since the liquid vol was 4.8 L, the total amount of lactose in the fermenter at zero time was 240.0 g. The lactose was reduced from 5.0 to 0.05% after 20 h. This resulted in a reduction of about 99.0% of the initial lactose in the fermenter. The reduction of lactose displayed three distinct stages that corresponded to the lag, exponential growth, and stationary phases of the yeast growth curve. During the first stage, there was a slow reduction in the lactose concentration. This was utilized mainly for cell respiration and cell endogenous growth (individual cell growth). The second stage, was a period of rapid lactose reduction. This was utilized by the yeast for both endogenous cell growth (cellular cell growth) and the cell mass growth (cell multiplication) as well as cell respiration. In the third stage, the concentration of lactose was very low, and thus, an insignificant reduction of lactose was achieved during this period. As a result, the microbial growth was kept stationary because of the lack of substrate. This resulted in the death and/or sporulation of the vegetative cells of the yeast.

Mohmoud and Kosikowski (*21*) studied the rate of lactose utilization by different yeasts under aerobic and anaerobic conditions. They found that the rate of lactose utilization was faster under aerobic than anaerobic conditions for all yeasts. The highest percentage of lactose utilized by *K. fragilis* was 99.7% aerobically and 60.9% anaerobically. Burgess (*22*) reported that the lactose consumption under batch condition using a laboratory-scale tower fermenter was fairly low in the first 2 h (approx less than 10%) while the yeast was adapting to the new substrate. After 2 h, lactose consumption increased rapidly. The lactose concentration in the medium at the end of exponential growth was 1.75% and was almost totally depleted by the end of the experiment.

Dissolved Oxygen Concentration

The cheese whey was saturated with oxygen before the inoculum of *K. fragilis* was introduced. The observed dissolved oxygen concentration (at saturation) of the yeast-free cheese whey was 5.5 mg/L. During the fermentation process, the dissolved oxygen displayed four distinct stages. The first stage corresponded to the lag phase of the yeast growth curve. During this period, the dissolved oxygen decreased slowly (almost linearly) as the number of yeast cells was kept constant and oxygen was mostly required for cell respiration as well as endogenous cell growth.

The second stage corresponded to the exponential growth phase of yeast growth curve. In this phase, the cells increased exponentially and as a result the oxygen concentration in the fermenter, decreased rapidly until it reached a constant minimum value of 1.2 mg/L. The third stage corresponded to the stationary growth phase of the yeast population, during which the number of cells was constant. The oxygen concentration in the reactor remained constant as a temporary steady-state condition was achieved. During this steady-state period, the amount of oxygen added to the system by the aeration equipment was equivalent to that consumed by the yeast. The fourth stage corresponded to the death phase of the yeast growth curve. In this period, the number of cells was decreasing with time and as a result the oxygen concentration in the system was continuously increasing.

The minimum dissolved oxygen values observed in this study appeared to be higher than that reported by several authors. Porges (*23*), Longmuir (*24*), Winzler (*25*), and Ghaly et al. (*26*) reported 0.3 mg/L as being the critical value below which the oxygen uptake rate by microorganisms is dependent on the oxygen concentration of the medium. Maxon and Johnson (*27*) and Hixon and Gaden (*28*) reported peak oxygen demands of 0.17–0.25 mmol/L of medium each min (m*M* O_2/L/min) in yeast grown in poor media or under limiting condition. Maxon and Johnson (*27*), however, showed that in a rich medium with adequate aeration, the peak oxygen demand was 4.8–5.7 m*M* O_2/L/min. Strohm et al. (*29*) reported that the critical concentration of dissolved oxygen required by a baker's type yeast culture for an adequate yield is about 1/25 that at saturation or 0.2 ppm. Wasserman (*18*) reported that the oxygen demand of *K. fragilis* growing in a cheese whey medium is approx 110 mg O_2/L/min (5.0 m*M* O_2/L/min). This also was confirmed by Litchfield (*6*), who reported that the peak oxygen demand of the *K. fragilis* was 5.0 m*M* O_2/L/min. An oxygen absorption rate of up to 4.5 m*M* O_2/L/min also was used in fermentation studies by Burgess (*22*)

Temperature

The temperature curve displayed four distinct stages similar to those of the growth curve as shown in Fig. 3. These stages corresponded to the lag, exponential growth, stationary, and death phases of the yeast growth curve. The first stage corresponded to the lag phase of the growth curve. During this stage, the temperature increased slowly by 1°C (from 22.0 to 23.0°C) over a 4-h period. The second stage corresponded to the exponential growth phase of the growth curve. During this stage, the temperature increased sharply by 10°C (from 23.0 to 33.0°C) over a period of 8 h. The third stage corresponded to the stationary growth phase of the growth curve. Since the number of the yeast cells remained constant during this phase, the temperature leveled off and remained constant during this

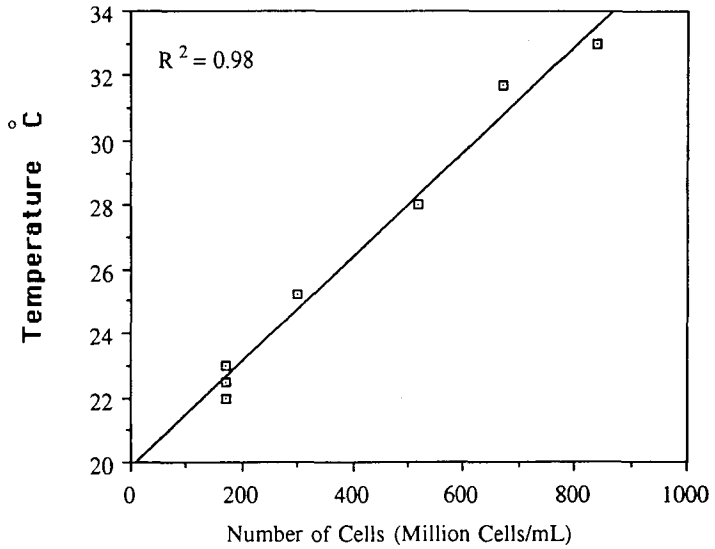

Fig. 4. Effect of cell number on temperature.

stage at a value of 33.0°C for about 4 h. The fourth stage corresponded to the death phase. During this stage, the temperature decreased as the number of the yeast cells decreased continuously, and thus, the temperature decreased rapidly by almost more than 10.0°C (from 33.0 to 22.0°C) over a period of 8 h. The rate of heat production (increase of the temperature) during the metabolism of cheese whey lactose was found to correlate with cell number in the fermenter as shown in Fig. 4.

The optimum temperature for *K. fragilis* propagation is in the range of 30–35°C (*20,30,31*). Ghaly and Singh (*32*) reported average temperatures for batch and continuous operations of 35 and 38°C, respectively. Berstein et al. (*16*) suggested that the temperature of the fermenter medium must be maintained at 35°C by running a low level of water through jacketed fermenter. In this experiment, cooling was not found necessary. The temperature of the cheese whey in the fermenter rose quickly in the first 3 h and then reached a maximum value of 33°C after 5–6 h.

Heat Balance

In order to determine the portion of lactose used for energy as a percentage of the total lactose utilized, a heat balance was performed on the fermentation system as shown in Fig. 5. The heat balance on the entire system includes: (1) the heat generated by the metabolism of lactose, (2) heat generated by mixing, (3) the heat required to raise the temperature of the medium, (4) the heat losses through the fermenter lid, (5) the heat losses through the fermenter wall, (6) the heat losses through the fermenter floor, and (7) the heat losses with the exhaust gas,

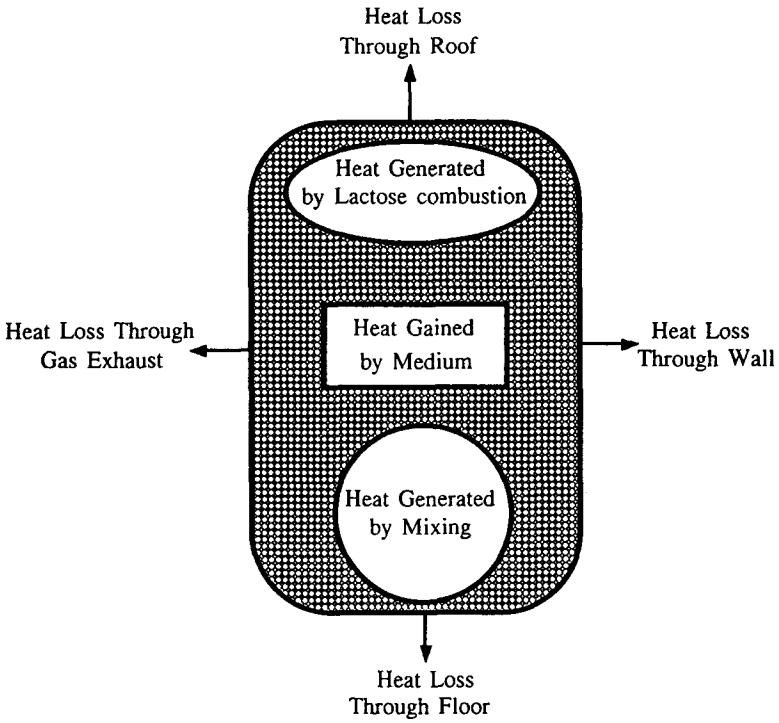

Fig. 5. Heat balance of the entire system.

$$q_y + q_m = q_l + q_t + q_w + q_b + q_a \quad [4]$$

where

q_y is the heat generated by the lactose metabolism (kJ h^{-1}).
q_m is the heat generated by mixing (kJ h^{-1}).
q_l is the heat required to raise the temperature of the liquid medium (kJ h^{-1}).
q_t is the heat lost through the fermenter lid (kJ h^{-1}).
q_w is the heat lost through the fementer wall (kJ h^{-1}).
q_b is the heat lost through the fementer floor (kJ h^{-1}).
q_a is the heat lost with exhaust gas (kJ h^{-1}).

The heat generated by mixing is usually a very small amount and can be neglected. Therefore, Eq. (4) can be rewritten as follows:

$$q_y = q_l + q_t + q_w + q_b + q_a \quad [5]$$

With reference to Fig. 6, the values of q_l, q_t, q_w, q_b, and q_a can be calculated from the following equations:

$$q_l = M_l C_{pl} (T_f - T_i) / \Delta t \quad [6]$$

$$q_t = U_t A_t (T_v - T_a) \quad [7]$$

$$q_w = U_w A_w (T_v - T_a) \quad [8]$$

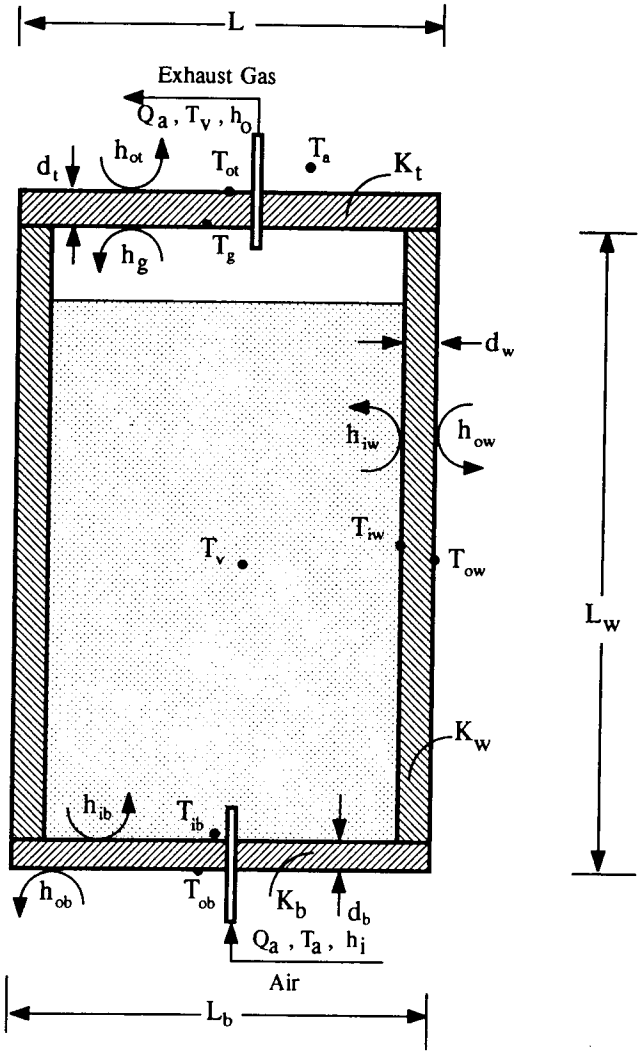

Fig. 6. Heat transfer in the entire system.

$$q_b = U_b A_b (T_v - T_a) \quad [9]$$

$$q_a = M_a C_{pa} (T_v - T_a) \quad [10]$$

where

A_b is the surface area of the fermenter floor (m^2).
A_t is the surface area of the fermenter lid (m^2).
A_w is the surface area of the fermenter wall (m^2).
C_{pa} is the specific heat of the air (kJ kg^{-1} K^{-1}).
C_{pl} is the specific heat of the liquid medium (kJ kg^{-1} K^{-1}).
M_a is the mass flow of the air (kg h^{-1}).
M_l is the mass of the liquid medium (kg).
T_a is the air ambient temperature (K).

T_f is the final temperature of liquid medium at the end of a time interval (K).
T_i is the initial temperature of liquid medium at the beginning of a time interval (K).
T_v is the average temperature of liquid medium within a time interval (K).
Δt is the time interval (h).
U_b is the overall heat loss coefficient of the fermenter floor (kJ m^{-2} h^{-1} K^{-1}).
U_t is the overall heat loss coefficient through the fermenter lid (kJ m^{-2} h^{-1} K^{-1}).
U_w is the overall heat transfer coefficient of the fermenter wall (kJ m^{-2} h^{-1} K^{-1}).

The overall heat transfer coefficient of the floor (U_b) can be calculated as follows:

$$U_b = \frac{1}{(d_b/K_b) + (1/h_{ob}) + (1/h_{ib})} \quad [11]$$

where:

d_b is the thickness of the floor (m).
h_{ob} is the convective heat transfer coefficient between the floor and the ambient air (kJ m^{-2} h^{-1} K^{-1}).
h_{ib} is the convective heat transfer coefficient between the medium and the fermenter floor (kJ m^{-2} h^{-1} K^{-1}).
K_b is the thermal conductivity of the floor material (kJ m^{-1} h^{-1} K^{-1}).

The heat transfer from the medium to the inside wall of the fermenter is by forced convection since the medium is stirred. The heat transfer on the outside wall is by natural convection. Since the heat transfer coefficeint caused by forced convection is very large compared to that of the outside wall, Eq. 11 can be rewritten as follows:

$$U_b = \frac{1}{(d_b/K_b) + (1/h_{ob})} \quad [12]$$

The convective heat transfer coefficients (h_{ob}) can be calculated as follows:

$$h_{ob} = 0.59 \left(\frac{T_{ob} - T_a}{L_b} \right)^{0.25} \quad [13]$$

where

L_b is the characteristic length, diameter for disc (m).
T_{ob} is the temperature of the outside surface of the fermenter floor (K).

The overall heat transfer coefficient of the wall (U_w) based on the outside area of the cylinder can be calculated as follows:

$$U_w = \frac{1}{(A_{ow}/A_{iw})(1/h_{iw}) + A_{ow}\ln(r_o/r_i)/(2\pi K_w L_w) + (1/h_{ow})} \quad [14]$$

where

A_{ow} is the surface area of the outside wall of the fermenter (m^2).
A_{iw} is the surface area of inside wall of the fermenter (m^2).
r_i is the inner radius of the fermenter (m).
r_o is the outer radius of the fermenter (m).
h_{iw} is the convective heat transfer coefficient between the medium and the fermenter wall (kJ m^{-2} h^{-1} K^{-1}).
h_{ow} is the convective heat transfer coefficient between the fermenter wall and the ambient air (kJ m^{-2} h^{-1} K^{-1}).
K_w is the thermal conductivity of the wall material (kJ m^{-1} h^{-1} K^{-1}).
L_w is the characteristic length, height for vertical cylinder (m).

As with U_b, $(1/h_{iw}) << (1/h_{ow})$. Therefore, Eq. 14 can be rewritten as follows:

$$U_w = \frac{1}{(A_{ow}\ln(r_o/r_i)/2\pi K_w L_w) + (1/h_{ow})} \quad [15]$$

For an average film temperature of 300 K, a maximum temperature difference of 11°C and a characteristic length of 325 mm, the calculated Rayleigh number is 3.55×10^7. The flow is therefore liminar, and the convective heat transfer coefficients (h_{ow}) can be calculated as follows:

$$h_{ow} = 1.42 \left(\frac{T_{ow} - T_a}{L_w}\right)^{0.25} \quad [16]$$

where

L_w is the characteristic length, height for vertical cylinder (m).
T_{ow} is the temperature of the outside of the fermenter wall (K).

The overall heat transfer coefficient of the fermenter lid (U_t) can be calculated as follows:

$$U_t = \frac{1}{(d_t/K_t) + (1/h_{ot}) + (1/h_g)} \quad [17]$$

where:

d_t is the thickness of the fermenter lid (m).
h_{ot} is the convective heat transfer coefficient between the fermenter lid and the ambient air (kJ m^{-2} h^{-1} K^{-1}).

Table 3
Calculated Heat Values for Batch Culture Operation

Period, h	q_l, kJ	q_t, kJ	q_w, kJ	q_b, kJ	q_a, kJ	q_y, kJ
00–02	10.056	0.246	0.933	0.061	2.266	13.562
02–04	10.056	0.318	1.205	0.079	2.769	14.428
04–06	44.247	0.530	1.879	0.123	4.129	50.908
06–08	56.313	0.973	3.467	0.228	6.646	67.626
08–10	74.415	1.620	5.755	0.378	9.919	92.087
10–12	26.145	2.160	7.929	0.520	12.436	49.192
12–14	00.000	2.306	8.567	0.562	13.091	24.527
14–16	00.000	2.306	8.567	0.562	13.091	24.527
16–18	−30.168	2.138	8.074	0.530	12.336	− 7.090
18–20	−60.336	1.651	6.396	0.420	10.070	−41.799
20–22	−60.336	1.048	4.156	0.273	7.049	−47.810
22–24	−60.336	0.513	2.133	0.140	4.028	−53.522

q_y = Heat generated by lactose metabolism.
q_l = Heat required to raise the temperature of the medium.
q_t = Heat lost through the fermenter lid.
q_w = Heat lost through the fermenter wall.
q_b = Heat lost through the fermenter floor.
q_a = Heat lost with exhaust gas.

h_g is the convective heat transfer coefficient between inside surface of the fermenter lid and the gas (kJ m^{-2} h^{-1} K^{-1}).
K_t is the thermal conductivity of the fermenter lid material (kJ m^{-1} h^{-1} K^{-1}).

As with U_b, $h_g >> h_{ot}$. Therefore, the term $(1/h_g)$ in Eq. 17 can be neglected. The convective heat transfer coefficient (h_{ot}) can be calculated as follows:

$$h_{ot} = 1.32 \left(\frac{T_{ot} - T_a}{L_t} \right)^{0.25} \quad [18]$$

where:

L_t is the characteristic length (m).
T_{ot} is the temperature of the outside surface of the fermenter lid (K).

The heat transfer coefficients in Eq. 11–18 were taken from ASHRAE (33) and Holman (34).

A Fortran® computer program was written to perform the heat balance on the system. The results are shown in Table 3. The amount of lactose used for energy was calculated from q_y. The amount of lactose used for growth was then calculated by subtracting the amount of lactose used for energy from the total amount of lactose consumed by the yeast. These

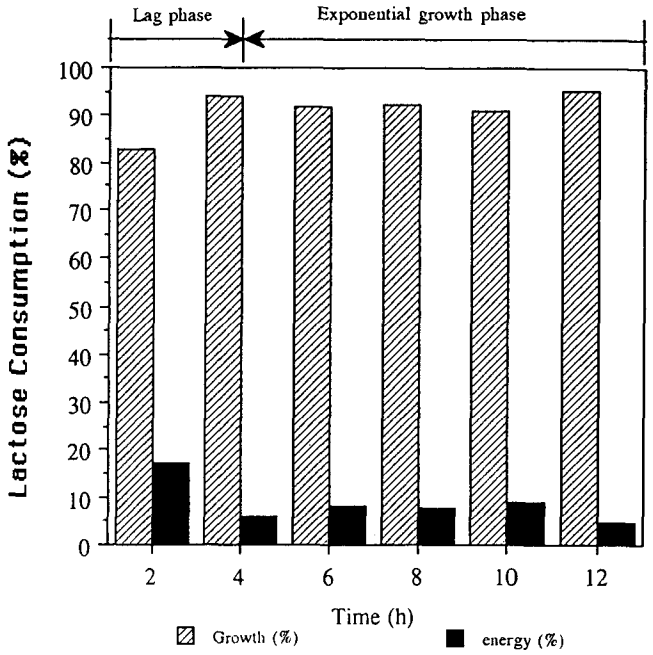

Fig. 7. Portions of lactose used for growth and energy during the fermentation process.

values are presented in Fig. 7. In the lag phase, the average values of lactose used for growth and energy were 88.5 and 11.5%, respectively. In the exponential phase, the average values of lactose used for growth and energy were 92.6 and 7.4%, respectively. The high percentage of lactose was used for the growth, because during this phase, about 85% of the lactose was removed from the system. This was utilized by *K. fragilis* for both endogenous cell growth (individual cell growth) and cell mass growth (cell multiplication). Using soluble chemical oxygen demand technique, Ghaly and Singh (*32*) reported that approx 59 and 55% of the soluble material of cheese whey were converted to microbial cells for batch and continuous operations, respectively.

Stockar and Birou (*35*) reported that the heat released per unit biomass formed is highest when growth occurs fully aerobically (11–13 kJ/g cell). It decreases in a hyperbolic function of decreasing oxygen supply and reaches a much lower value (around 4 kJ/g) for completely fermentative growth. In this study, the heat released varied from 6.5 to 8.9 kJ/g cell.

As a relatively low percentage of lactose was used for energy compared to that used for growth, the temperature of the fermenter did not rise above 33°C (35°C is the critical temperature for *K. fragilis* growth). No cooling system was needed in this study because of the proper design of the fermenter. However, if the heat transfer problem is not properly addressed in the design of large-scale fermenters, the use of expensive refrigeration techniques may be required to keep the industrial process of fermentation at its optimum temperature.

CONCLUSION

The reduction of lactose concentration was affected by the number of yeast cells present in the system. The lactose curve displayed three distinct stages that corresponded to the lag, exponential, and stationary growth phases of the yeast growth curve. 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 growth phase was used for cell multiplication and energy release.

The temperature curve displayed four distinct stages that corresponded to the lag, exponential, stationary, and death phases of the yeast growth curve. The maximum temperature observed in the system was 33°C. This indicated that a cooling unit for a single cell protein system is not needed. On the average, about 88% of the lactose was utilized for growth, and 12% was used for energy release. The heat released varied from 6.5 to 8.9 kJ/g cell.

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