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Utilization of Cheese Whey Lactose by *Kluyveromyces fragilis* for Energy and Growth Under Continuous Fermentation

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ABSTRACT

A heat balance was performed on a 25 L jacketed continuous stirred tank reactor used for the production of single cell protein from cheese whey using *Kluyveromyces fragilis* under three levels of retention time (12, 18, and 24 h), two levels of air flow rate (1 and 3 VVM), and three levels of mixing speed (200, 400, and 600 RPM) to determine the heat of reaction and the portions of lactose used for energy and growth as well as to assess the need for the cooling system. The yeast population size, oxygen concentration, and lactose concentration in the reactor as well as the portions of lactose used for energy and growth were all affected by the hydraulic retention time, mixing speed, and air flow rate. About 8–14% of lactose was utilized for energy and 86–92% was utilized for growth. The highest cell number was obtained at the 12 h retention time, 3 VVM air flow rate, and 600 RPM mixing speed. Under these conditions, the lactose removal efficiency was 95.6% and the yeast yield was 0.78 g cell/g lactose removed.

Index Entries: Single cell protein; yeast; lactose; continuous fermentation; growth; energy; heat; temperature; retention time; air flow rate; mixing speed.

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INTRODUCTION

The continuous culture system has been defined as a mixed system of cell growth to which the substrate is continuously added and from which the spent culture media is continuously withdrawn (*1*). At steady state, the viable cell population is kept constant as the removal of dead and viable cells from the system is being replaced by new cell growth. The continuous flow stirred tank reactor (CFSTR) is an example of the continuous culture system and has been used by Ghaly and Singh (*2*) and Ben-Hassan (*3*) for the production of single cell protein (SCP) from cheese whey using the yeast *K. fragilis*.

During aerobic fermentation of cheese whey, lactose is utilized by *K. fragilis* for the synthesis of new microbial cells and production of energy. 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 Eqs (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 production of SCP is an exothermic process in which heat is released causing a rise in the fermenter temperature. A value of 16.7 kJ/g of substrate is generally accepted for heat of reaction of carbohydrates (*4–6*). Because temperature above 35°C will retard the growth and metabolism of yeast, Bernstein et al. (*7*) recommended the use of jacketed fermenters that can be cooled by running cooling water through the jacket. On the other hand, Ghaly and Singh (*2*) reported that the cooling process could be eliminated if the reactor is properly designed.

OBJECTIVES

The specific objectives of this study were:

1. To perform heat balance on a CFSTR used for the production of SCP at 3 retention times, 2 air flow rates and 3 mixing speeds taking into consideration:
   a. heat generated by metabolism of the lactose,
   b. heat losses through the wall, bottom and lid of the fermenter,
   c. heat losses with the exhaust gas,
   d. heat losses with the effluent material, and
   e. heat losses with the coolant water;
2. To determine the portions of cheese whey lactose used for energy and growth of *K. fragilis* during the continuous fermentation; and
3. To determine the optimum condition for SCP production and evaluate the need for a cooling system.

EXPERIMENTAL APPARATUS

The experimental apparatus used in the study (Fig. 1) consists of a CFSTR, an air supply system and a whey feeding and effluent removal system.

CFSTR

A 25L working volume, upright cylindrical fermenter (Fig. 2) was constructed of 6.35 mm thick stainless steel material. The fermenter was designed with a water jacket for temperature control. Two considerations were taken into account in choosing the capacity of the fermenter: since the cheese whey required for the entire experiment was collected, mixed and stored in a freezer, there was a logistical reason for designing a small capacity fermenter, and the capacity of the fermenter was to be large enough in order to allow for extrapolation of the results obtained from the laboratory study for scale up purpose. Lyons (*8*) recommended a minimum fermenter capacity of 15 L for bench scale type fermenters if results are to be scaled up. The size of the fermenter used in this study was 67% larger than the recommended size. The ratio of the diameter to height chosen for the final design of the fermenter was about 1:2.

The fermenter was designed to be completely mixed and hence a stainless steel mixing shaft of 10.0 mm diameter and 700.0 mm length was installed through the center of the lid. Three, six-vaned flow disc impellers were used to ensure adequate mixing in the vertical direction. A heavy duty laoratory stirred motor (G. K. Heller Corp., Model No. 99P46-18) with a gear head reducer was mounted on the lid of the fermenter to drive the mixing shaft and impellers. The electric motor was connected to a mixing speed controller (Cole-Parmer Instrument Company, Cat. No. J-004407-00). Four baffles were used in the fermenter to reduce vortexing

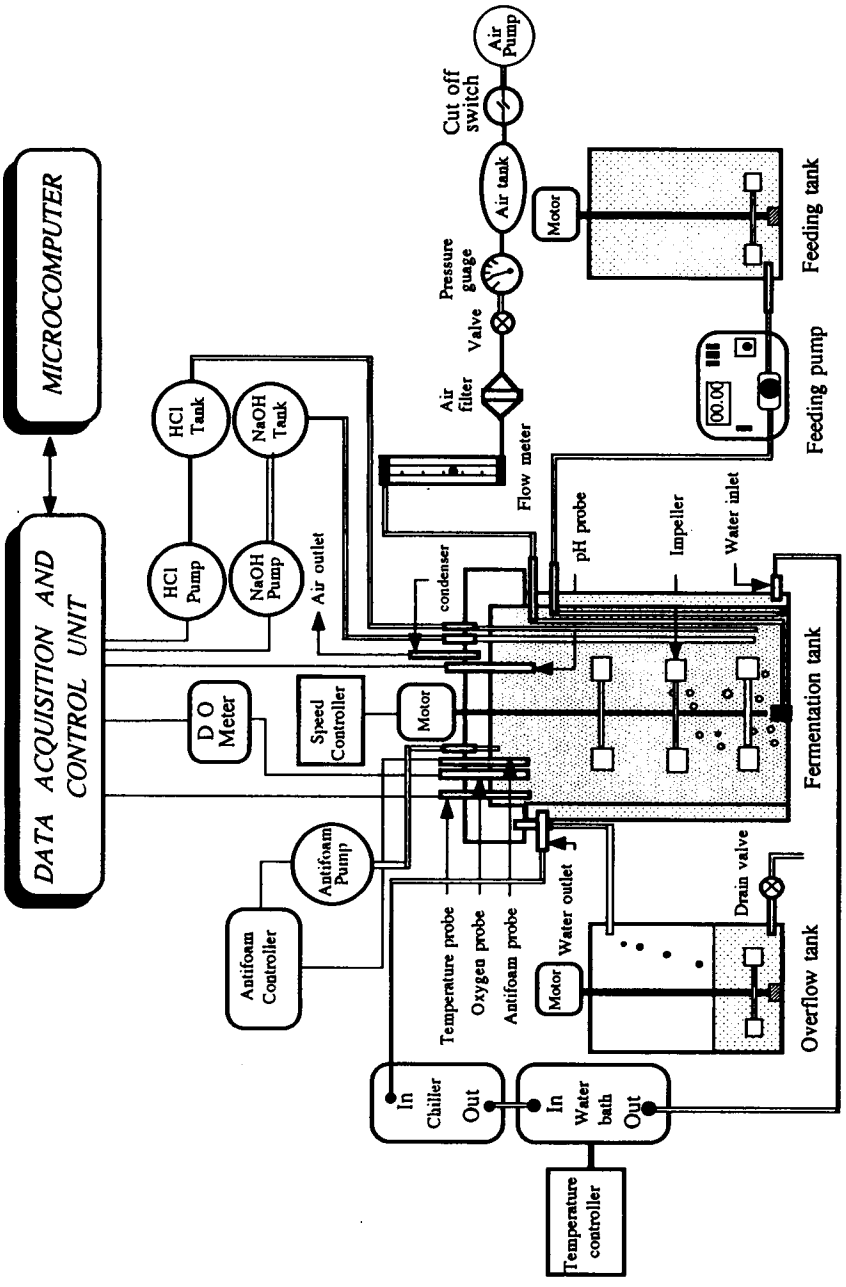

Fig. 1. Experimental apparatus.

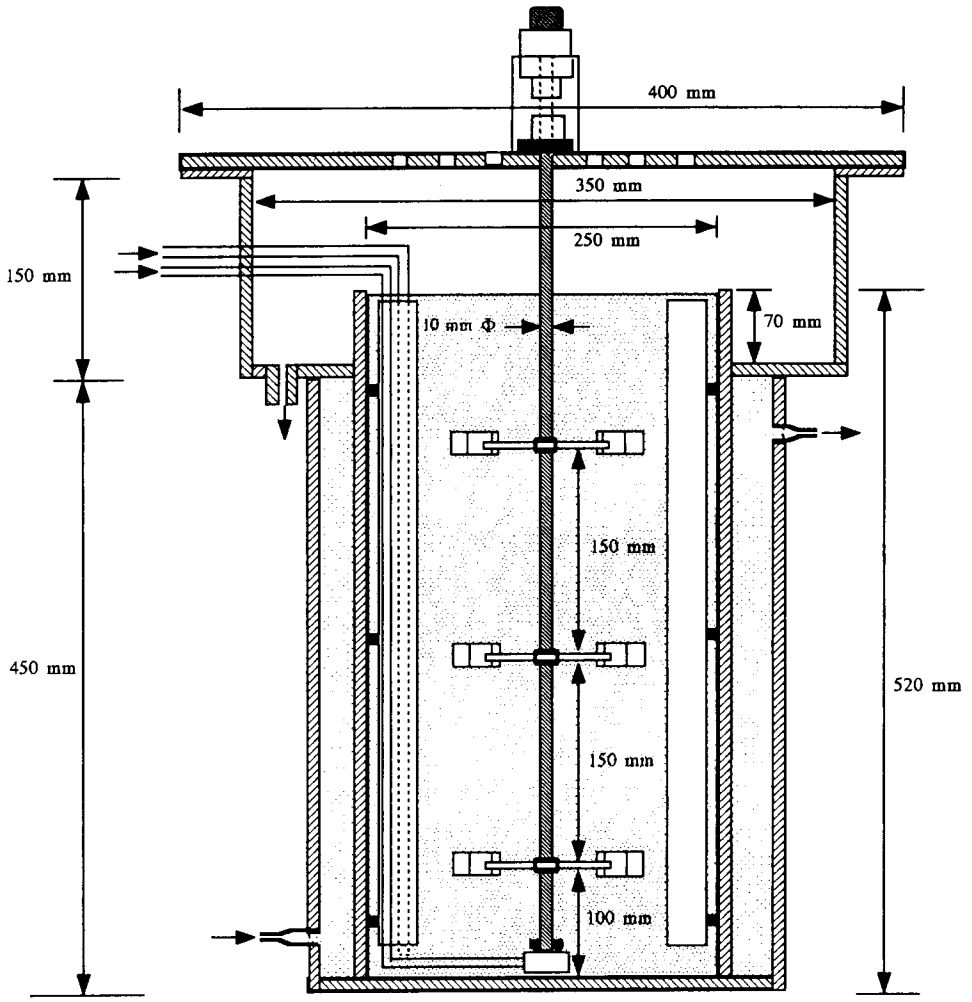

Fig. 2. Cross-sectional elevation of the fermenter.

and to improve the top-to-bottom turnover. The standard design recommended for baffles, given by Perry and Green (1984), was followed. They were positioned every 90° on the inside of the fermenter wall. More information on the fermenter design can be found elsewhere (3).

Air Supply System

The air supply system consisted of an air pump (Miniature oil-less air compressor with a maximum pressure of 700 kPa, Cole-Parmer Instrument Company, Cat. No. J-7053), an air tank (equipped with an automatic pressure switch, a check valve, a pressure gage, a shutoff valve at the tank outlet, manual tank drain, and a tank safety valve), a pressure regulator, an air filter, and a flowmeter. The air is introduced to the fermenter through its bottom, passing through an air diffuser.

A glass condenser was used as a gas vent system. The condensation coil was made from a glass tube of 7.0 mm diameter fitted inside a large glass cylinder of 155 mm diameter and 285 mm length. Cold water entered the coil from the water inlet located on the side of the lower end of the outer cylinder and left through the water outlet located on the side of the upper end of the outer cylinder. As the exhaust gas entered the outer glass cylinder through the gas inlet and came in contact with the cool inner coil, the moisture in the exhaust air was condensed and the water was returned to the fermenter through the condensate drain.

Whey Feeding and Effluent Collection System

The whey feeding and effluent removal system included: a cheese whey feeding tank, a feeding pump, and an effluent collection tank. The cheese whey feeding tank was constructed of a PVC cylinder of 6.0 mm thickness, 298.0 diameter and 560.0 mm height. The lid was fabricated from a plexiglass material. An electric motor (Franklin Electric, Model No. 6105121401) with a speed-reducing gear arrangement was mounted on the lid of the tank to drive the mixing shaft and a flat-bladed turbine impeller of 150.0 mm diameter.

A pump with a variable speed motor (1-100 rpm) and a precision optical tachometer (Cole-Parmer Instrument Company, DIGI-STALTIC Digital Flow Controller Cat. No. N-07525-30) was used to feed the cheese whey in to the reactor. LED display gave readout for flow rate (mL/min), motor speed (rpm), and cumulative volume (mL). The cumulative volume resolution ranged from 0.01 mL to 1.0 mL, whereas the motor speed resolution was 1.0 rpm.

The effluent collection tank was constructed from PVC material. The thickness, diameter and height of tank were 6.0, 298.0, and 463.0 mm, respectively. A plastic tube of about 20 mm diameter connected the fermenter outlet to the lid of the overflow collection tank.

EXPERIMENTAL PROCEDURE

Whey Collection, Storage, and Preparation

The cheese whey was obtained from Farmer's Cooperative Dairy Plant in Truro, Nova Scotia. It was pumped from the plant storage tank into 60 L plastic containers. The containers were 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 the Standard Methods for the Examination of Water and Wastewater (*9*). Prior to placing the cheese whey into the fermenter, it was allowed to completely thaw at room temperature for 24 h.

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

Characteristics	Measured value	Unit
Total solids	63835	mg/L
Fixed solids	9100	mg/L
Volatile solids	54738	mg/L
Percent volatile solids	85.74	%
Percent fixed solids	14.26	%
Suspended solids	22150	mg/L
Fixed solids	185	mg/L
Volatile solids	21965	mg/L
Percent volatile solids	99.16	%
Percent fixed solids	0.84	%
Total Kjeldahl nitrogen	1690	mg/L
Ammonium nitrogen	270	mg/L
Organic nitrogen	1420	mg/L
Percent organic nitrogen	84.02	%
Percent ammonium nitrogen	15.98	%
Total chemical oxygen demand	74220	mg/L
Soluble chemical oxygen demand	59640	mg/L
Insoluble chemical oxygen demand	14580	mg/L
Percent soluble chemical oxygen demand	80.36	%
Percent insoluble chemical oxygen demand	19.64	%
Lactose	5.0	%
pH	4.9	

Inoculum Preparation

Fifteen liters of raw cheese whey were first pasteurized in several 4 L reagent bottles by heating the whey to 70°C for 45 min, cooling it suddenly to 1°C for 30 min and then heating it to room temperature (20°C) for 10 min. The processes of heating and cooling were repeated three times. The pasteurized cheese whey was transferred to several 250 mL sterilized Erlenmeyer flasks (150 mL/flask). The yeast culture was then transferred from the stock culture to the pasteurized cheese whey in the sterilized Erlenmeyer flasks (two Petri dishes of pure culure of *K. fragilis* were added to each flask containing 150 mL pasteurized cheese whey). 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. Following the 48 h growth period, 15000 mL of the yeast cultures were collected from the flasks and transferred to a large container and then mixed thoroughly. The yeast culture was then divided into three equal parts of 5000 mL each and stored in the refrigerator at 4°C until needed.

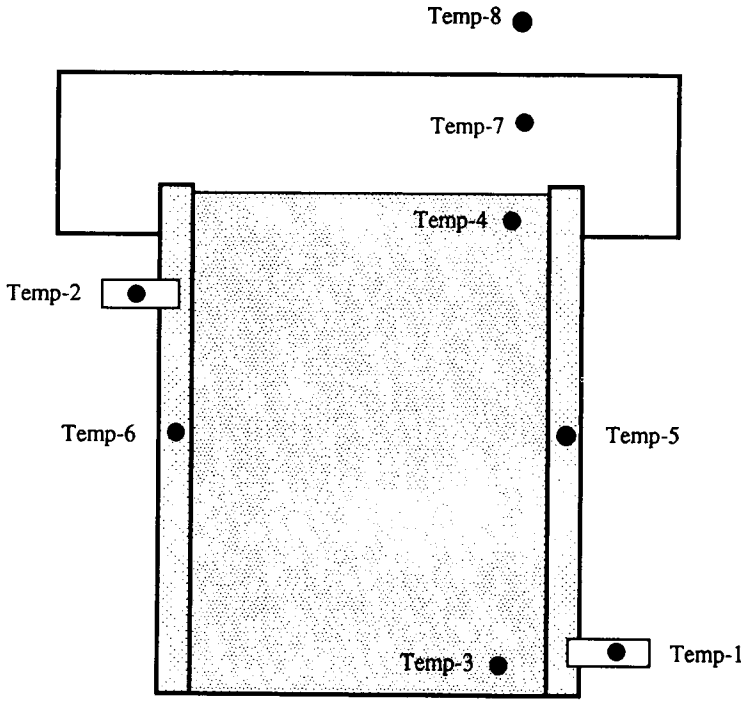

Fig. 3. Positions of the temperature probes.

Instrumentation and Measurements

The temperature of the fermentation medium was maintained at 33±2°C by circulating a cooling water through the fermenter jacket using a temperature controlled water bath. Temperature transducers (AD590) were used to measure the temperature. The temperatures were measured at 8 different position as shown in Fig. 3. These were:

1. The inlet water temperature;
2. The outlet water temperature;
3. The temperature at the bottom of the fermenter;
4. The temperature at the top of the fermenter;
5. The temperature of the water at mid height on the right side of the jacket;
6. The temperature of the water at mid height on the left side of the jacket;
7. The temperature of the fermenter head space; and
8. The laboratory ambient temperature.

Dissolved oxygen concentration was continuously monitored. The pH was maintained at 4.5±0.2 with the aid of a computer based pH measurement-and-control system developed by Ben-Hassan et al. (*10*), which added HCl or NaOH as needed.

Experimental Protocol

The fermenter and all accessories (mixing system, tubing, and feeding tank) were chemically sterilized using 2% potassium metabisulfite solution, and then washed with hot water several times before starting the experiment in order to remove any chemical traces. The fermenter was filled to two thirds of the working volume (about 16.0 L) with cheese whey and then inoculated with 5.0 L of inoculum. The air flow (1VVM), the turbine drive (200 RPM), the temperature controller (33°C), the computer, and the data acquisition and control unit were started immediately. The remaining 4 L (to a full capacity) were made up with a continuous addition of cheese whey at a hydraulic retention time of 24 h (a flow rate of 1.04 L/h). When the fermenter reached the steady state condition (constant lactose and cell concentrations in the effluent), samples were collected and analyzed ever 12 h for six d during the steady state operation. The culture composition was checked every 12 h during the start-up and the steady-state periods to determine if there were any contaminations in the medium.

The mixing speed, hydraulic retention time, and air flow rate were changed until all the treatment combinations were carried out. The same procedure was used for samples collection and analysis during the steady state condition of all the hydraulic retention time—air flow rate—mixing speed combinations.

RESULTS AND DISCUSSION

Reactor Operating Parameters

The initial pH of the cheese whey lactose was 4.30. In this experiment, the pH of the medium was maintained at 4.5 ± 0.2 with the aid of a computer based pH measurement and control system. The samples taken from the reactor during the start-up and steady-state periods were plated on agar yeast peptone growth medium. The results showed that maintaining the pH around 4.5 eliminated possible contamination by other microorganisms. The colonies developed from the samples taken from the reactor exhibited the typical elevated concave, smooth appearance and creamy color of *K. fragilis.* Staining specimens with crystal violet showed elongated clustered yeast cells. Also, the gram reaction showed that yeast cells multiplied vegatively by budding.

The temperature and dissolved oxygen measurements were taken every 2 h, whereas the number of active cells of *K. fragilis* and the lactose concentration were determined for the sample taken every 12 h from the effluent during the steady state operation. For each experimental run the mean, standard deviation, and coefficient of variation were calculated. The results are presented in Tables 2 and 3. The lower coefficients of variation of the cell number (0.41–4.41%), lactose concentration (0.27–8.00%),

Table 2
Measured Temperature at Various Hydraulic Retention Times, Air Flow rates, and Mixing Speeds

Retention Time (h)	Air flow Rate (VVM)	Mixing Speed (RPM)		Temp-1 (°C)	Temp-2 (°C)	Temp-3 (°C)	Temp-4 (°C)	Temp-5 (°C)	Temp-6 (°C)	Temp-7 (°C)	Temp-8 (°C)
12	1	200	Mean	29.8	31.9	34.1	33.9	31.9	31.9	33.6	29.3
			STD	0.3	0.1	0.1	0.2	0.1	0.1	0.2	0.3
			CV	0.9	0.4	0.3	0.5	0.2	0.3	0.6	1.0
		400	Mean	29.8	31.9	34.1	34.0	31.9	31.9	33.6	29.3
			STD	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.3
			CV	0.8	0.4	0.3	0.5	0.2	0.3	0.6	1.1
		600	Mean	29.0	32.1	34.7	34.5	32.3	32.3	32.2	29.2
			STD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
			CV	0.3	0.2	0.2	0.4	0.3	0.2	0.2	0.3
	3	200	Mean	29.8	32.0	34.1	34.0	32.1	32.0	33.8	29.3
			STD	0.3	0.2	0.1	0.1	0.1	0.1	0.2	0.3
			CV	1.0	0.6	0.3	0.4	0.3	0.4	0.7	1.2
		400	Mean	29.1	32.0	34.3	34.1	32.2	32.2	34.1	29.1
			STD	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.3
			CV	0.9	0.3	0.3	0.3	0.2	0.2	0.3	0.9
		600	Mean	29.1	32.1	34.7	34.5	32.3	32.3	34.2	29.2
			STD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
			CV	0.3	0.2	0.2	0.4	0.3	0.2	0.2	0.3
18	1	200	Mean	29.0	31.6	33.7	33.4	31.2	31.3	33.2	28.7
			STD	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.5
			CV	0.2	0.5	0.5	0.4	0.3	0.3	0.3	1.7
		400	Mean	29.1	31.7	33.8	33.7	31.4	31.4	33.4	28.9
			STD	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.5
			CV	0.4	0.4	0.5	0.3	0.3	0.3	0.3	1.6
		600	Mean	29.3	31.8	34.0	33.7	31.6	31.6	33.6	29.0
			STD	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1
			CV	0.8	0.3	0.2	0.4	0.5	0.3	0.4	0.5
	3	200	Mean	29.6	31.9	34.0	33.8	31.8	31.8	33.6	29.2
			STD	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.2
			CV	0.9	0.2	0.3	0.3	0.2	0.2	0.4	0.8

	400	Mean	29.8	31.9	34.1	33.9	31.7	31.8	33.7	29.2
		STD	0.3	0.1	0.1	0.2	0.1	0.1	0.1	0.2
		CV	0.9	0.2	0.3	0.6	0.2	0.3	0.4	0.7
	600	Mean	29.8	31.9	34.1	34.0	31.9	31.9	33.6	29.4
		STD	0.3	0.1	0.1	0.1	0.1	0.1	0.2	0.3
		CV	0.9	0.2	0.3	0.4	0.2	0.3	0.7	1.2
24	1	200	Mean	29.4	31.8	32.4	31.9	31.3	31.2	31.7	28.4
			STD	0.3	0.6	0.4	0.5	0.5	0.6	0.7	0.3
			CV	1.0	1.8	1.1	1.5	1.6	1.9	2.1	1.0
		400	Mean	28.5	31.7	32.6	31.8	31.1	31.0	31.2	28.6
			STD	0.2	0.4	0.3	0.3	0.2	0.7	0.3	0.3
			CV	0.8	1.1	0.8	1.0	0.6	2.1	1.1	1.1
		600	Mean	29.0	31.2	33.4	33.1	31.0	31.0	32.7	28.6
			STD	0.2	0.1	0.3	0.1	0.1	0.1	0.1	0.2
			CV	0.5	0.1	1.1	0.4	0.1	0.1	0.3	0.8
	3	200	Mean	29.0	31.2	33.9	33.5	31.1	31.1	33.1	28.6
			STD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3
			CV	0.4	0.2	0.4	0.4	0.1	0.1	0.2	0.9
		400	Mean	29.0	31.3	33.7	33.1	31.1	31.1	33.1	28.5
			STD	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.3
			CV	0.2	0.4	0.6	0.1	0.2	0.1	0.4	0.9
		600	Mean	29.0	31.6	33.9	33.6	31.1	31.1	33.2	28.8
			STD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
			CV	0.2	0.5	0.3	0.3	0.1	0.1	0.4	0.3

Temp-1 = The inlet water jacket temperature
Temp-2 = The outlet water jacket temperature
Temp-3 = The temperature at the bottom of the fermenter
Temp-4 = The temperature at the top of the fermenter
Temp-5 = The mid height water jacket temperature on the right side
Temp-6 = The mid height water jacket temperature on the left side
Temp-7 = The temperature of the fermenter head space
Temp-8 = The laboratory ambient temperature
STD = Standard deviation
CV = Coefficient of variation
VVM = Volume of air per volume of reactor per minute
RPM = Revolution per minute

Table 3
The Effluent Cells, Lactose, and Oxygen Concentrations
at Various Hydraulic Retention Times, Air Flow Rates, and Mixing Speeds

Retention time, h	Airflow rate, VVM	Mixing speed, RPM	Cell Number			Lactose concentration			Oxygen concentration		
			Mean	STD	CV, %	Mean	STD	CV, %	Mean	STD	CV, %
			10^6 cells/mL			%			mg/L		
12	1	200	241.30	2.87	1.19	1.31	0.04	31.3	1.95	0.09	4.61
		400	368.30	7.18	1.95	1.15	0.01	3.13	2.16	0.07	3.33
		600	518.30	11.15	2.15	0.75	0.02	2.93	2.28	0.07	3.07
	3	200	400.0	12.79	3.20	0.82	0.16	1.95	2.24	0.10	4.46
		400	813.30	17.75	2.18	0.51	0.04	5.88	2.52	0.08	3.17
		600	1132.50	49.90	4.41	0.22	0.01	4.09	2.76	0.10	2.62
18	1	200	120.40	2.27	1.88	1.20	0.01	0.75	2.97	0.09	3.03
		400	139.30	3.94	2.83	1.00	0.01	1.95	3.08	0.07	2.27
		600	161.80	2.37	1.56	0.61	0.01	1.80	3.20	0.08	2.50
	3	200	149.70	2.83	1.89	0.70	0.01	1.86	3.10	0.08	2.58
		400	185.60	1.62	0.87	0.40	0.01	3.00	3.27	0.11	3.36
		600	285.60	1.16	0.41	0.10	0.01	6.00	3.43	0.09	2.62
24	1	200	42.80	1.75	4.32	1.12	0.02	1.70	3.15	0.06	1.90
		400	49.50	2.11	4.36	0.82	0.01	1.22	3.54	0.15	4.32
		600	64.80	2.08	3.31	0.45	0.01	3.33	3.75	0.09	2.40
	3	200	58.70	1.56	2.66	0.50	0.02	4.60	3.65	0.07	1.92
		400	85.80	3.74	4.34	0.11	0.01	4.54	3.82	0.09	2.35
		600	117.60	3.34	2.84	0.05	0.01	8.00	4.00	0.09	2.25

STD = Standard deviation.
CV = Coefficient of variation.
VVM = Volume of air per volume of reactor per minute.
RPM = Revolution per minute.

and oxygen concentration (1.90–4.61%) indicated that the fermenter was operating at the steady state condition.

The average temperature of the medium varied from 32.1 to 34.6°C (i.e., 33.35 ± 1.25°C). The optimum temperature for *K. fragilis* propagation is within the range 30–35°C (*2,11,12*). The yeast population size, the lactose concentration, and the dissolved oxygen concentration in the reactor were all effected by the hydraulic retention time, air flow rate, and mixing speed. The yeast population size varied from 58.7 to 1132.5 million cells/mL, the dissolved oxygen concentration varied from 1.95 to 4.00 mg/L, and the lactose consumption varied from 75.0 to 99.0%, depending on the hydraulic retention time, air flow rate, and mixing speed used.

Generally, decreasing the hydraulic retention time and/or increasing the air flow rate and/or the mixing speed increased the yeast cell number. A high cell number was observed at the 12 h hydraulic retention time. On the other hand, increasing the hydraulic retention time, and/or the air flow rate and/or the mixing speed increased the dissolved oxygen concentration and decreased the lactose concentration in the fermenter. Higher air flow rate and mixing speed resulted in higher oxygen transfer rate whereas longer retention times resulted in smaller microbial population and, thus, a higher dissolved oxygen concentration. Vananuvat and Kinsella (*13*) and Meiering et al. (*14*) observed higher lactose consumption and dramatic increases in yeast population when the agitation speed was increased to 700–800 RPM. In this study, the highest yeast population was observed at the 12 h retention time, 3VVM air flow rate, and 600 RPM mixing speed, which resulted in 95.6% lactose removal efficiency. Using a yeast cell density of 3.3×10^{-11} g/cell, given by Gancedo and Serrano (*15*), the yeast yield was found to be 0.78 g cell/g lactose removed from the system. This is in agreement with stoichiometric value of 0.79 g cell/g lactose calculated from Eq. (3).

Heat Balance

In order to determine the portions of lactose used for energy and growth, a heat balance was performed on the fermentation system, as shown in Fig. 4. The heat balance on the entire system includes:

1. Heat losses through the fermenter wall;
2. Heat losses through the fermenter lid;
3. Heat losses through the fermenter floor;
4. Heat losses with the exhaust gases;
5. Heat losses through the effluent;
6. Heat losses through the water jacket;
7. Heat generated by the metabolism of lactose; and
8. Heat generated by the mixing.

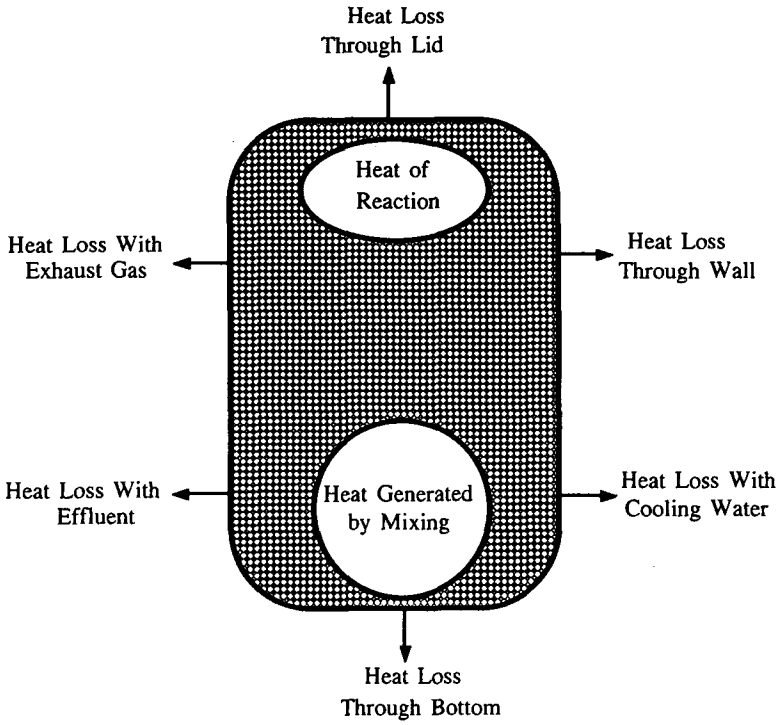

Fig. 4. Heat balance of the entire system.

$$q_y + q_m = q_b + q_w + q_t + q_a + q_e + q_c \quad (1)$$

Where:

q_y is the rate of heat generated by the lactose metabolism (kJ h^{-1})
q_m is the rate of heat generated by the mixing (kJ h^{-1})
q_b is the rate of heat lost through the fermenter floor (kJ h^{-1})
q_w is the rate of heat lost through the fermenter wall (kJ h^{-1})
q_t is the rate of heat lost through the fermenter lid (kJ h^{-1})
q_a is the rate of heat lost with exhaust gas (kJ h^{-1})
q_e is the rate of heat lost through the effluent (kJ h^{-1})
q_c is the rate of heat lost through the coolant(kJ h^{-1})

The rate of heat generated by mixing (q_m) is usually very small amount and can be neglected. With reference to Fig. 5, the values of q_b, q_w, q_t, q_a, q_c and q_e can be calculated from the following Eqs:

$$q_b = U_b A_b (T - T_a) \quad (2)$$

$$q_w = U_w A_w (T_c - T_a) \quad (3)$$

$$q_t = U_t A_t (T - T_a) \quad (4)$$

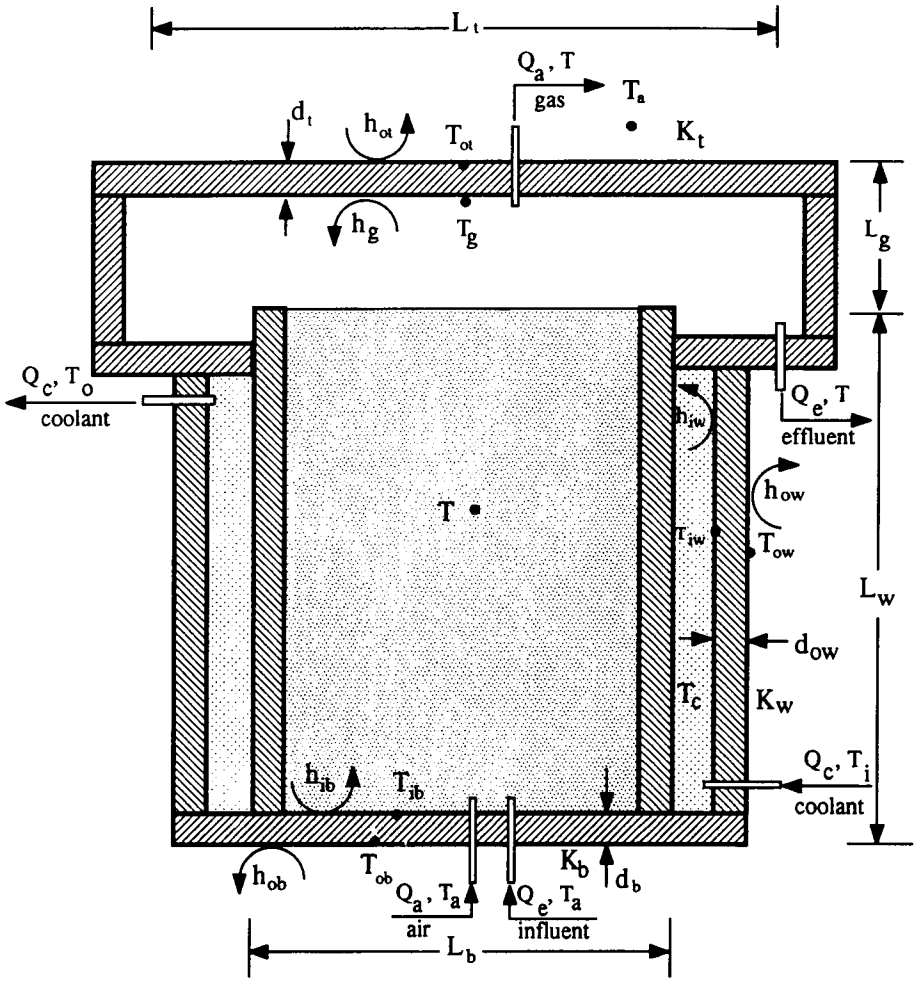

Fig. 5. Heat transfer of the entire system.

$$q_a = Q_a C_{pa}(T - T_a) \quad (5)$$

$$q_c = Q_c C_{pc}(T_o - T_i) \quad (6)$$

$$q_e = Q_e C_{pe}(T - T_a) \quad (7)$$

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 outer wall (m^2)
C_{pa} is the specific heat of the air (kJ kg^{-1} K^{-1})
C_{pc} is the specific heat of the coolant (kJ kg^{-1} K^{-1})

C_{pe} is the specific heat of the effluent (kJ kg^{-1} K^{-1})
Q_a is the mass flow rate of the air (kg h^{-1})
Q_c is the mass flow rate of the coolant (kg h^{-1})
Q_e is the mass flow rate of the effluent (kg h^{-1})
T is the temperature of the liquid medium (K)
T_a is the air ambient temperature (K)
T_c is the average temperature of the coolant (K)
T_i is the inlet temperature of the coolant (K)
T_o is the outlet temperature of the coolant (K)
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 of 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:

$$U_b = \frac{1}{\left(\frac{d_b}{K_b}\right) + \left(\frac{1}{h_{ob}}\right) + \left(\frac{1}{h_{ib}}\right)} \quad (8)$$

where

d_b is the thickness of the floor (m)
h_{ib} is the convective heat transfer coefficient between the medium and the fermenter floor (kJ m^{-2} h^{-1} K^{-1})
h_{ob} is the convective heat transfer coefficient between the fermenter floor and the ambient air (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 fermenter floor is by forced convection since the medium is stirred. The heat transfer from the fermenter floor to the air is by natural convection. Since the heat transfer coefficient due to forced convection (h_{ib}) is very large compared to that of the natural convection (h_{ob}), Eq (8) can be rewritten as follows:

$$U_b = \frac{1}{\left(\frac{d_b}{K_b}\right) + \left(\frac{1}{h_{ob}}\right)} \quad (9)$$

The convection heat transfer coefficient (h_{ob}) can be calculated as follows (Holman, 1990):

$$h_{ob} = 0.59 \left(\frac{T_{ob} - T_a}{L_b} \right)^{0.25} \quad (10)$$

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 heat leaving the fermenter through the inner wall is carried away by the water circulating in the water jacket. The conductive heat transfer across the outer wall of the water jacket can be calculated from Eq. 3. The heat transfer from the coolant water to the outside wall is by forced convection, whereas the heat transfer from the outside wall to the air is by natural convection. Since the heat transfer coefficient due to forced convection (h_{iw}) is very large compared to that of the natural convection (h_{ow}), the overall heat transfer coefficient of the outer wall (U_w) based on the outside area can be calculated as follows:

$$U_w = \frac{1}{\left(\frac{A_{ow} \ln (r_o / r_i)}{2\pi K_w L_w} \right) + \left(\frac{1}{h_{ow}} \right)} \quad (11)$$

Where:

A_{ow} is the surface area of outer side of the outer 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 coolant and the outer wall (KJ m^{-2} h^{-1} K^{-1})
h_{ow} is the convective heat transfer coefficient between the outer 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)

The convective heat transfer coefficients (h_{ow}) can be calculated as follows (*16*):

$$h_{ow} = 1.42 \left(\frac{T_{ow} - T_a}{L_w} \right)^{0.25} \quad (12)$$

where:

T_{ow} is the temperature of the outside surface of the fermenter wall (K)

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

$$U_t = \frac{1}{\left(\frac{d_t}{K_t}\right) + \left(\frac{1}{h_{ot}}\right) + \left(\frac{1}{h_g}\right)} \quad (13)$$

where:

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

The convective heat transfer coefficients (h_{ot}) and (h_g) can be calculated as follows (*16*):

$$h_{ot} = 1.32 \left(\frac{T_{ot} - T_a}{L_t}\right)^{0.25} \quad (14)$$

$$h_g = \frac{Nu \bullet K_a}{L_t} \quad (15)$$

Where:

K_a is the thermal conductivity of air (kJ m^{-2} h^{-1} K^{-1})
L_t is the characteristic length, diameter for disc (m)
T_{ot} is the temperature of the outside surface of the fermenter lid (K)

The Nesselt number (Nu) is calculated as follows (Holman, 1990):

$$Nu = 0.664 (Re)^{1/2} \bullet (Pr)^{1/3} \quad (16)$$

Where:

Re is Reynolds number (–)

A Fortran computer program was written to perform the heat balance on the system. The results are shown in Table 4. 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 values are presented in Fig. 6.

The portions of lactose used for energy and growth were affected by the hydraulic retention time, air flow rate, and mixing speed. Approximately 8–14% of the consumed lactose was used for energy, whereas

Table 4
The Calculated Heat Values for Continuous Culture Operation

Retention time, h	Air flow rate, VVM	Mixing speed, RPM	q_e, KJ/h	q_c, KJ/h	q_a, KJ/h	q_t, KJ/h	q_w, KJ/h	q_b, KJ/h	q_y, KJ/h
12	1	200	41.4	31.9	8.4	7.7	4.4	1.0	94.8
		400	46.3	33.3	9.4	9.2	4.2	1.2	103.6
		600	47.3	52.3	9.6	9.5	4.1	1.2	123.9
	3	200	41.4	34.7	25.1	7.7	4.3	1.0	114.2
		400	44.9	48.5	27.3	8.8	4.3	1.1	134.8
		600	47.3	51.6	28.4	9.5	4.2	1.2	142.1
18	1	200	27.2	39.7	8.2	7.5	3.8	1.0	87.5
		400	28.1	40.7	8.5	8.1	4.0	1.1	90.5
		600	28.2	39.1	8.6	8.3	4.2	1.1	89.4
	3	200	27.6	34.3	25.1	7.7	4.3	1.0	100.0
		400	27.9	33.0	25.5	8.1	4.6	1.0	100.1
		600	27.0	33.0	24.6	7.8	4.0	1.0	97.4
24	1	200	16.2	36.9	6.6	5.7	6.7	0.8	72.9
		400	15.7	64.7	6.3	5.6	2.4	0.7	95.4
		600	20.5	34.9	8.3	8.0	4.3	1.0	76.9
	3	200	22.3	35.0	27.1	8.4	4.4	1.1	98.4
		400	21.3	44.7	25.9	8.2	3.7	1.1	104.8
		600	21.7	40.9	26.4	8.5	4.0	1.1	102.7

q_y = heat generated by the metabolism of lactose.
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_c = heat lost with the coolant.
q_a = heat lost with exhaust gas.
q_e = heat lost with the effluent.
VVM = Volume of air per volume of reactor per minute.
RPM = Revolution per minute.

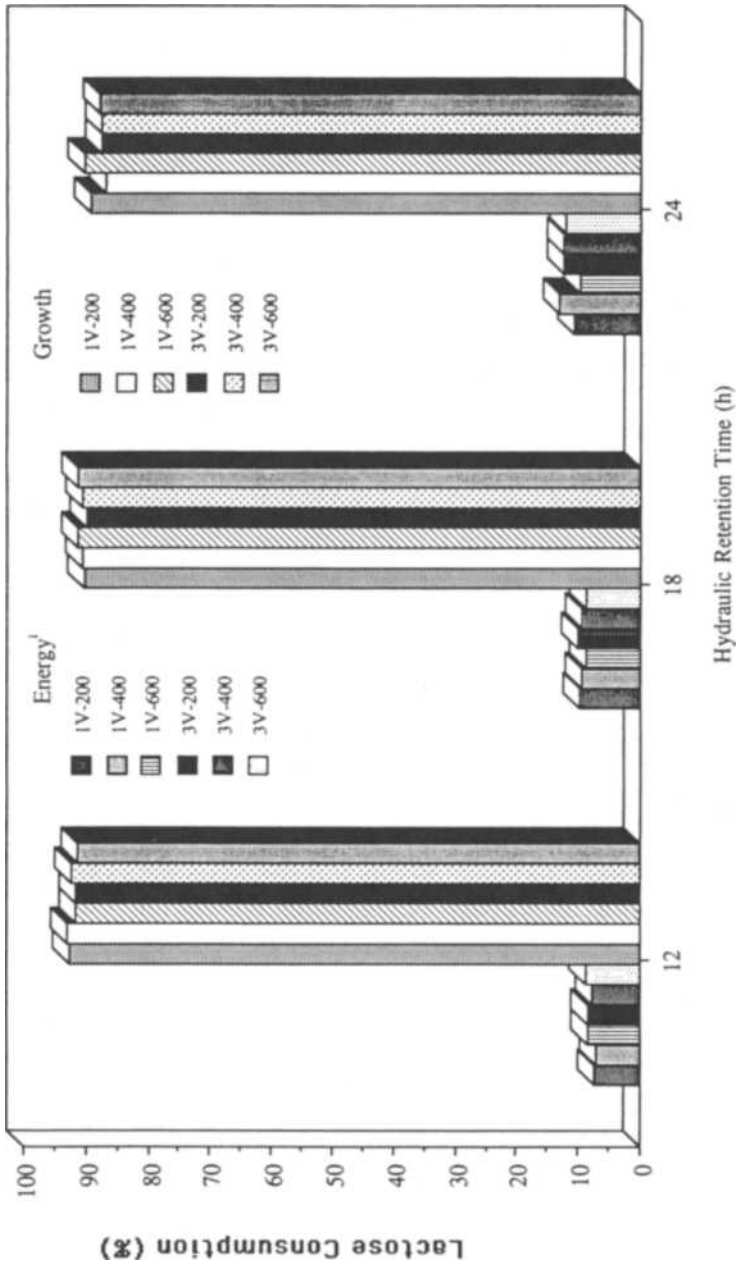

Fig. 6. Lactose consumption during the continuous culture operation.

86–92% was utilized for growth of *K. fragilis* (both individual cell growth and cell multiplication), depending on the hydraulic retention time, air flow rate, and mixing speed used. However, the hydraulic retention time had the most pronounced effect. For all air flow rates and mixing speeds, increasing the hydraulic retention time resulted in a substantial reduction in the yeast population size. At longer retention times (lower substrate loading rates), the yeast population appeared to be starving because of the lack of substrate and, thus, respiration was more predominant than growth. These results are in agreement with those obtained from the batch culture operation reported by Ben-Hassan et al. (*17*) as well as the stoichiometric value calculated from Eq. (3). Under optimum operating conditions, 89% of the lactose is used for growth, whereas 11% is used for energy. Of the total heat produced by the lactose metabolism, 16.46–44.69% was lost with effluent, 6.61–27.54% was lost with the exhaust gas, 30.39–67.82% was lost with cooling water, 0.74–1.23% was lost through the fermenter bottom, 5.87–10.40% was lost through fermenter lid, and 2.52–4.70% was lost through the fermenter wall depending on the hydraulic retention time, air flow rate, and mixing speed used. The results indicated that the heat transfer problem must be properly addressed in the design of large scale fermenters, otherwise the use of expensive cooling techniques may be required to keep the industrial fermentation process at its optimum temperature. This could be achieved by maximizing the ratio of wall area to the fermenter volume.

CONCLUSIONS

A 25 L aerobic fermenter has been designed and used for the production of SCP from cheese whey using the yeast *K. fragilis* under various levels of operating parameters (2 air flow rates, 3 mixing speeds, and 3 retention times). A heat balance was performed on the system to calculate the portions of cheese whey lactose used for energy and growth of *K. fragilis*. The yeast population size, the lactose concentration and the dissolved oxygen concentration in the reactor as well as the proportions of lactose used for energy and growth of *K. fragilis* were all affected by the hydraulic retention time, air flow rate, and mixing speed. A yeast population size of 58.7–11,325 million cells/mL and a lactose removal efficiency of 75.0–99.0% were achieved, depending on the hydraulic retention time, air flow rate, and mixing speed used. The results indicated that about 8.0–14.0% of the lactose consumed was utilized for energy and 86.0–92.0% was utilized for growth. The highest yeast population size was achieved at 12 h retention time, 3 VVM air flow rate, and 600 RPM mixing speed. A yield of 0.78 g cell/g lactose was achieved under these conditions.

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