

Heat Generated by Mechanical Agitation and Lactose Metabolism during Continuous Propagation of *Kluyveromyces fragilis* in Cheese Whey

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ABSTRACT

A procedure for measuring the rate of heat production by mixing in fermentation systems has been developed. The heat generated by mixing and lactose metabolism during the continuous production of single cell protein from cheese whey lactose was calculated using a heat balance equation. The technique quantified the heat produced in and lost from the fermentation system. Most of the heat generated by mixing in the cell-free system (98.93%) was lost with exhaust gas while a very small amount (1.07%) was lost through the fermenter lid, wall, and bottom. The heat generated by mixing was significant (19.69% of the total heat generated in the fermentation system with an active yeast population present) and, therefore, cannot be ignored in heat balance calculations. About 30.38% of the total heat generated in the reactor was lost through the coolant showing the need for jacketed fermenter for continuous operation. A yeast population size of 986 million cells.mL⁻¹ and a lactose removal efficiency of 95.6% were observed. About 88.5% of the lactose consumed was used for growth. A yield of 0.77 g(wb)cells.g⁻¹lactose was achieved. The heat released by unit biomass was 2.82 kJ.g⁻¹cells.

Keywords: Single cell protein, yeast, lactose, continuous, fermentation, growth, heat, temperature, mixing.

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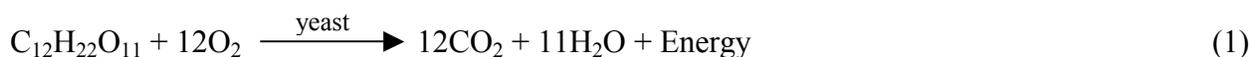
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INTRODUCTION

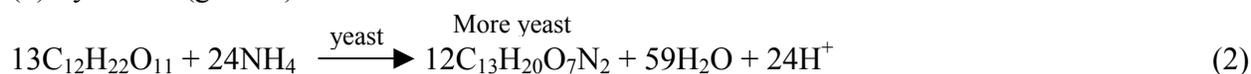
Mixing has found wide application in food, pharmaceutical, chemical, and waste treatment processes in which microorganisms are exploited to achieve complex biochemical conversions and to synthesize microbial mass (Ghaly et al., 1992). Mixing in fermentation processes is required (a) to maintain a homogeneous medium throughout the fermentation system (b) to provide a uniform temperature distribution in the medium and, thus, a better efficiency of heat removal (c) to provide intimate contact between the microorganisms and their substrates and (d) to ensure that adequate oxygen is obtained by all yeast cells (Ghaly et al., 1992; and Lyons, 1967).

Cheese whey is a liquid by-product (greenish yellow liquid) of cheese industry. The composition of whey varies according to the type of cheese produced as well as the applied process of manufacture. The composition of cheese whey is approximately 93% water, 5% lactose, 0.9% protein, 0.3% fat, 0.2% lactic acid and small amounts of vitamins. Production of single cell protein from cheese whey using the yeast *Kluyveromyces fragilis* requires both aeration and agitation (Bernstein et al., 1977). During aerobic fermentation of cheese whey, lactose is utilized by *K. fragilis* for the synthesis of microbial cells and production of energy. The process can be illustrated as follows:

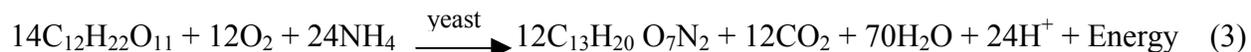
(a) Energy release (respiration)



(b) Synthesis (growth)



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



The production of SCP is an exothermic process in which heat is released causing a rise in the fermenter temperature, the rate of which depends on the activities of the yeast, the level of agitation, the heat losses from the fermenter, and whether or not a cooling unit is used. A value of 16.7 kJ.g^{-1} of carbohydrate substrate is generally accepted for heat of reaction of hydrocarbohydrates (Ghaly et al., 1992; Reisman et al., 1968; VonStocker and Birou, 1989; and Weast, 1988). However, most studies tend to neglect the heat produced by mixing, and the available methods for estimating the heat production on a dynamic basis in laboratory fermenters are either too expensive or too elaborate (Ghaly et al., 1992; and Walsh et al., 1980). The

objectives of this study were: (a) to perform heat balance on a reactor used for the production of SCP under continuous conditions, and (b) to determine the heat generated by mixing during the propagation of *K. fragilis*.

MATERIAL AND METHODS

Experimental Apparatus

The experimental apparatus (Figure 1) consisted of a fermenter, an air supply and a whey feeding and effluent removal system. A 25 L working volume, upright cylindrical fermenter (Figure 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: (a) since the cheese whey required for the entire experiment was collected, mixed and stored in freezer at -25° C, there was a logistical reason for designing a small capacity fermenter, and (b) 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 purposes. Lyons (1967) 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 mixing system consisted of (a) a stainless steel mixing shaft (10 mm diameter and 700 mm length) installed through the centre of the lid, (b) three, six-vaned flow disc impellers used to ensure adequate mixing in the vertical direction, (c) a heavy duty electric motor (G.K. Heller, Model No. 99P46-18, Floral Park, NY, USA) with a gear head reducer mounted on the lid and connected to a mixing speed controller (Cole-Parmer, Chicago, IL, USA) and (d) four standard baffles to reduce vortexing and to improve the top-to-bottom turnover.

Compressed air (PraxAir, Dartmouth, Nova Scotia, Canada) was supplied to the fermenter through a flow meter (Cole-Parmer, Chicago, IL, USA) with high resolution valve using tygon tubing of 10 mm diameter. The air was composed of 78.084% N₂, 20.996% O₂, 0.033% CO₂ and 0.937% other gases. A microfilter (Cole-Parmer, Chicago, IL, USA) 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, Montreal, Quebec, Canada). A condenser was used as a gas vent system. The condensation coil was made from a glass tube of 7 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 lower end of the outer cylinder and left through the water outlet located on the upper end. The moisture in the exhaust air was condensed and the water was returned to the fermenter through the condenser drain.

A whey feeding and effluent removal system was used with the continuous culture operation. It included a cheese whey feeding tank, a feeding pump and an effluent collection tank. The feeding tank was constructed of a PVC cylinder (6 mm thickness, 298 mm diameter

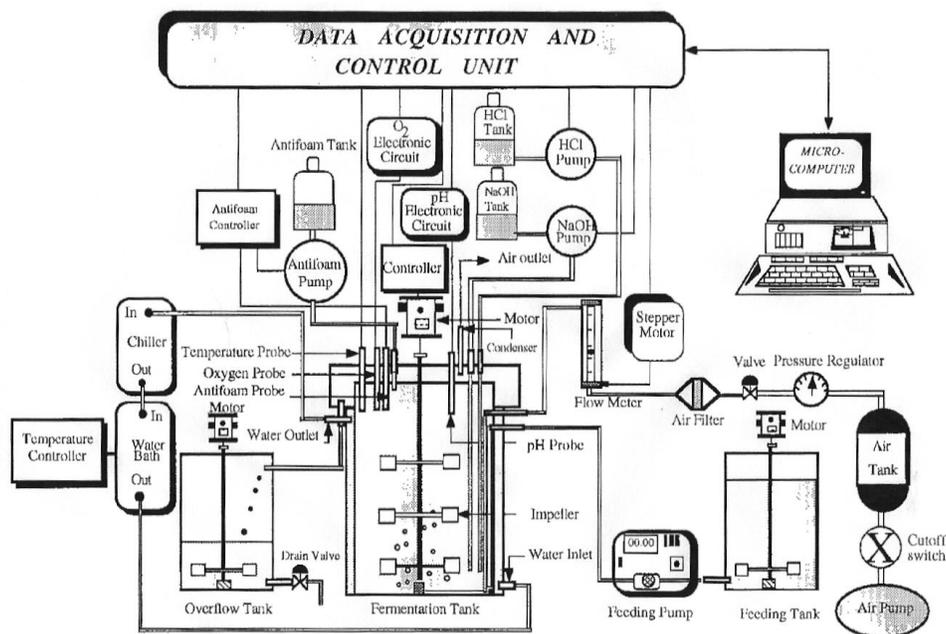


Figure 1. Schematic diagram of the experimental setup.

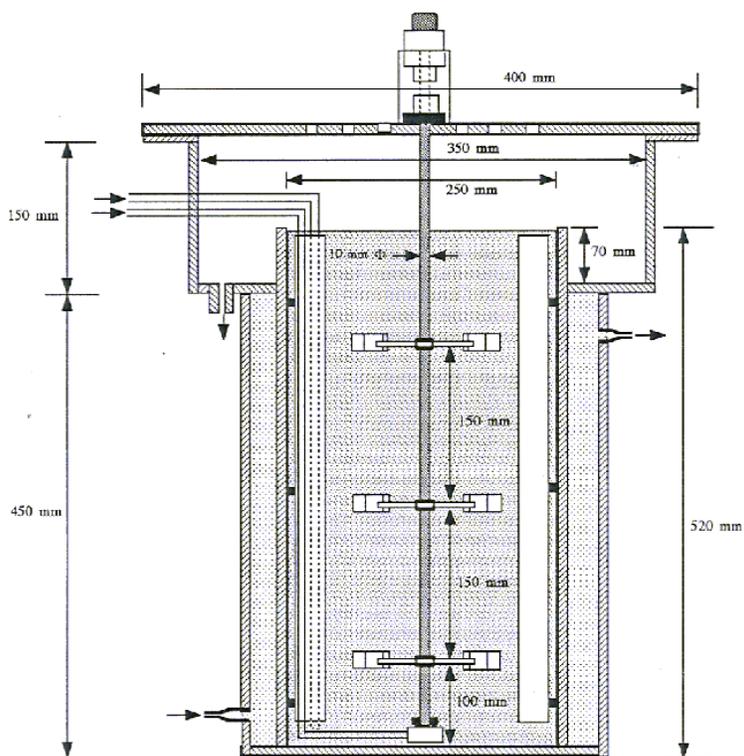


Figure 2. A cross-sectional elevation of the fermenter.

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and 560 mm height) and covered with a Plexiglas lid. An electric motor (Franklin Electric, Model No. 6105121401, Bluffton, IN, USA) with a speed-reducing gear arrangement was mounted on the lid to drive the shaft and a flat-bladed turbine impeller of 150 mm diameter. A pump with a variable speed motor (1-1000 rpm) and a precision optical tachometer (DIGI-STALTIC Digital Flow Controller, Cole Parmer, Chicago, IL, USA) was used to feed the whey into the reactor. An LED display gave readout for flow rate ($\text{mL}\cdot\text{min}^{-1}$), motor speed (rpm) and cumulative volume (mL). The cumulative volume resolution ranged from 0.01 to 1.00 mL while the motor speed resolution was 1 rpm. The effluent collection tank was constructed from PVC material. The thickness, diameter and height of the tank were 6, 298, 463 mm, respectively. A plastic tube of about 20 mm diameter connected the fermenter outlet to the lid of the overflow collection tank.

Whey Collection, Storage and Preparation

The 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 Biotechnology Laboratory at Dalhousie University, Halifax, Nova Scotia, where they were stored in a large freezer at -25°C until used. Some characteristics of the 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 (APHA, 1985).

Prior to placing the cheese whey into the fermenter, it was allowed to completely thaw at room temperature for 24 h. Raw cheese whey was first mixed and then pasteurized in several 4 L reagent bottles by heating the whey to 70°C for 45 min, rapidly cooling it to 1°C for 30 min, and letting it to stand at room temperature (20°C) for 24 h. The processes of heating and cooling were repeated three times to destroy any spores present in the whey. A plate count test was performed to insure the effectiveness of the pasteurization technique. The technique was developed by Ghaly and El-Taweel (1995) to avoid denaturing of protein during autoclave sterilization.

Inoculum Preparation

Freeze-dried pellets of *K. fragilis* (NRS 5790) culture were obtained from the Division of Biological Sciences, the National Research Council, Ottawa, Canada. A pellet of *K. fragilis* was dissolved in 5 mL of sterilized growth medium (DIFCO Laboratories, Detroit, MI, USA) 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 several Petri dishes. The Petri dishes were then placed in a controlled environment incubator at 35°C and left until visual growth appeared on the petri dishes (after about 72 h).

The pasteurized whey was transferred to several 250 mL sterilized Erlenmeyer flasks (150 mL per flask). The yeast culture was then transferred from the previously prepared stock

culture to the pasteurized whey in the sterilized Erlenmeyer flasks (the pure culture of *K. fragilis* in two Petri dishes were added to each flask containing 150 mL pasteurized whey). The flasks were capped with non-absorbent cotton plugs and mounted on a controlled environment-reciprocating shaker. The shaker, operated at a speed of 250 rpm for 48 h at a temperature of 35° C. Following the 48 h growth period, approximately 15000 mL of the yeast cultures were collected from the flasks and transferred to a large container and thoroughly mixed. The yeast culture was then stored in the refrigerator at 4° C until needed to inoculate the bioreactor.

Table 1. Some characteristics of the raw cheese whey used in the study

Characteristics	Measured Value	Unit
Total solids	63,835	mg.L ⁻¹
Fixed solids	9,100	mg.L ⁻¹
Volatile solids	54,738	mg.L ⁻¹
Percent volatile solids	85.74	%
Percent fixed solids	14.26	%
Suspended solids	22,150	mg.L ⁻¹
Fixed solids	185	mg.L ⁻¹
Volatile solids	21,965	mg.L ⁻¹
Percent volatile solids	99.16	%
Percent fixed solids	0.84	%
Total Kjeldahl nitrogen	1,690	mg.L ⁻¹
Ammonium nitrogen	270	mg.L ⁻¹
Organic nitrogen	1,420	mg.L ⁻¹
Percent organic nitrogen	84.02	%
Percent ammonium nitrogen	15.98	%
Total chemical oxygen demand	74,220	mg.L ⁻¹
Soluble chemical oxygen demand	59,640	mg.L ⁻¹
Insoluble chemical oxygen demand	14,580	mg.L ⁻¹
Percent soluble chemical oxygen demand	80.36	%
Percent insoluble chemical oxygen demand	19.64	%
Lactose	50,000	mg.L ⁻¹
pH	4.9	

Instrumentation and Measurements

During the continuous culture operation, the temperature of the fermentation medium was maintained at $33 \pm 1^\circ \text{C}$ by circulating a cooling water through the fermenter jacket using a temperature controlled water heating/cooling system. The temperatures were measured using temperature transducers (AD590) at 10 different locations (Figure 3). These were: (a) the inlet water temperature, (b) the outlet water temperature, (c) the temperature at the bottom of the fermenter, (d) the temperature at the top of the fermenter, (e) the temperature of the water at mid height on the right side of the jacket, (f) the temperature of the water at mid height on the left

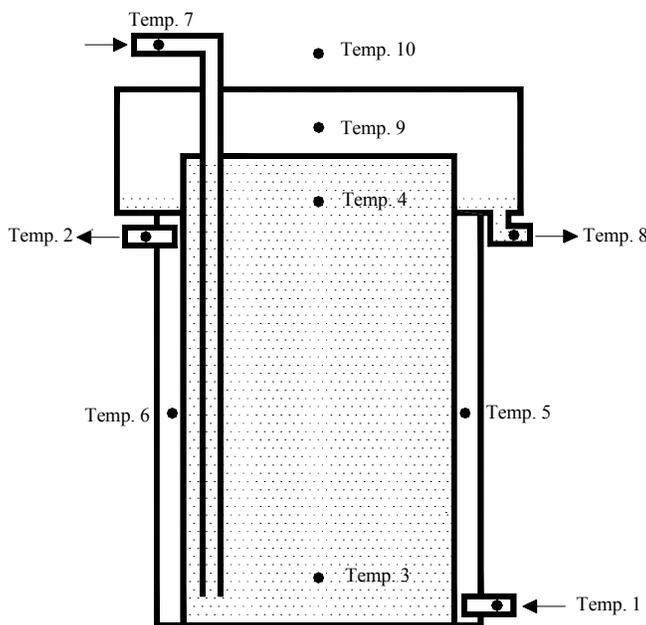


Figure 3. Positions of the temperature probes.

side of the jacket, (g) the inlet whey temperature, (h) the outlet whey temperature, (i) the temperature of the fermenter head space and (j) the laboratory ambient temperature. The temperature measurements were accurate to $\pm 0.1^\circ \text{C}$. The dissolved oxygen concentration was continuously monitored using an oxygen electrode probe (YSI Model 5739, Fisher Scientific, Montreal, Quebec, Canada) connected to a digital dissolved oxygen meter (YSI Model 8, Fisher Scientific, Montreal, Quebec, Canada). The pH was maintained at 4.5 ± 0.1 with the aid of a computer based pH measurement-and-control system, developed by Ben-Hassan et al. (1991). The moisture content of the exhaust gas was determined using a gas chromatography (HP Model 5890 series II, Palo Alto, California, USA).

Experimental Protocol

The fermenter and all accessories (mixing system, tubing and feeding tank) were chemically sterilized using 2% potassium meta-bisulfite solution, in order to eliminate any microbial contamination, and then washed with hot water several times before starting the experiment in order to remove any chemical traces.

For the yeast-free experiment, the fermenter was filled with pasteurized cheese whey, and the airflow and turbine drive were adjusted to 3 volume per volume per minute (VVM) and 400 rpm, respectively. The dissolved oxygen, temperature, and power input to the motor were monitored continuously. Samples were taken from the fermenter every 2 h and analyzed for microbial contamination and lactose concentration.

For the single cell protein experiment, the fermenter was filled with 17.5 L of cheese whey and then inoculated with 2.5 L of inoculum. The airflow (3 VVM), turbine drive (400 rpm), temperature controller ($33 \pm 1^\circ \text{C}$), pH controller (4.5 ± 1), power input to the motor, computer and data acquisition and control unit were started immediately and the dissolved oxygen, pH and temperature were continuously monitored. The remaining 5 L (to a full capacity) were made up with a continuous addition of whey at a hydraulic retention time of 12 h (a flow rate of 2.08 L.h^{-1}) until the fermenter reached the steady state condition (constant lactose and cell concentrations).

Samples were taken from the bioreactor every 12 h during the steady state conditions for a period of six days and analyzed for lactose and microbial cells. The lactose analysis was performed using a sugar analyzer (YSI Model 27, Fisher scientific Catalog No. 14-660). The plate count technique was used to measure the size of the yeast population and was carried out according to the procedure described in the Standard Methods for the Examination of Dairy Products (Messer et al., 1985). Gas samples of 0.5 mL each were taken from the gas stream using a pressure lock gas-tight syringe and analyzed for moisture content according to the procedure given by Hewlett Packard for Model HP 5890 Series II gas chromatograph. The samples were injected into the heated stainless steel packed column (Haye Sep Porous Polymer All Tech. Catalog No. C-5000, 2836, 762.0 mm L \times 3.2 mm o.d.).

HEAT BALANCE

A heat balance was performed on the fermentation system as shown in Figure 4. The heat balance on the entire fermentation system included (a) the heat generated by the metabolism of lactose, (b) the heat generated by mixing, (c) the heat loss through the fermenter floor, (d) the heat loss through the fermenter wall, (e) the heat loss through the fermenter lid, (f) the heat loss with the exhaust gas, (g) the heat loss through effluent (h) the heat loss through water jacket and (i) the heat required to raise the temperature of the liquid. The enthalpy of the base/acid added to control the pH and that contributed by the feed material flowing into the fermenter were neglected.

$$q_y + q_m = q_b + q_w + q_t + q_a + q_e + q_c + q_l \quad (4)$$

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})
 q_l is the rate of heat required to raise the temperature of the liquid (kJ.h^{-1})

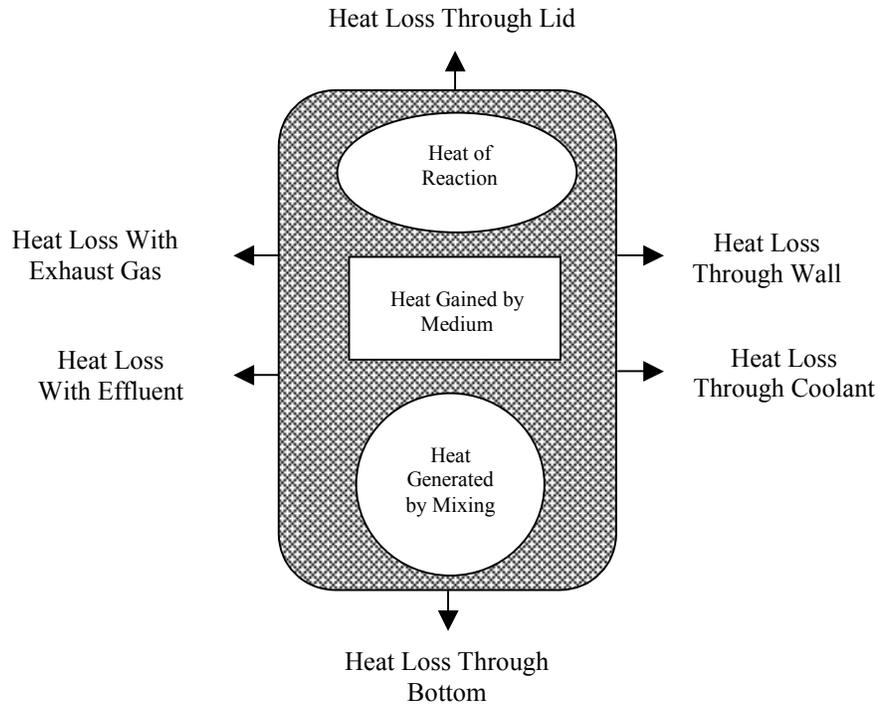


Figure 4. Sources of heat losses/gain for the entire fermentation system.

With reference to Figure 5, the values of q_b , q_w , q_t , q_a , q_c and q_e can be calculated from the following equations:

$$q_b = U_b A_b (T - T_a) \quad (5)$$

$$q_w = U_w A_w (T_c - T_a) \quad (6)$$

$$q_t = U_t A_t (T - T_a) \quad (7)$$

$$q_a = Q_a C_{pa} (T - T_a) \quad (8)$$

$$q_c = Q_c C_{pc} (T_o - T_i) \quad (9)$$

$$q_e = Q_e C_{pe} (T - T_a) \quad (10)$$

$$q_l = \frac{MC_{pl}(T - T_a)}{\Delta t} \quad (11)$$

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 ($kJ.kg^{-1}.K^{-1}$)
- C_{pl} is the specific heat of the liquid medium ($kJ.kg^{-1}.K^{-1}$)
- M is the mass of liquid medium (kg)
- 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 through the fermenter lid ($kJ.m^{-2}.h^{-1}.K^{-1}$)
- U_w is the overall heat transfer coefficient of the fermenter wall ($kJ.m^{-2}.h^{-1}.K^{-1}$)
- Δt is the time interval (h)

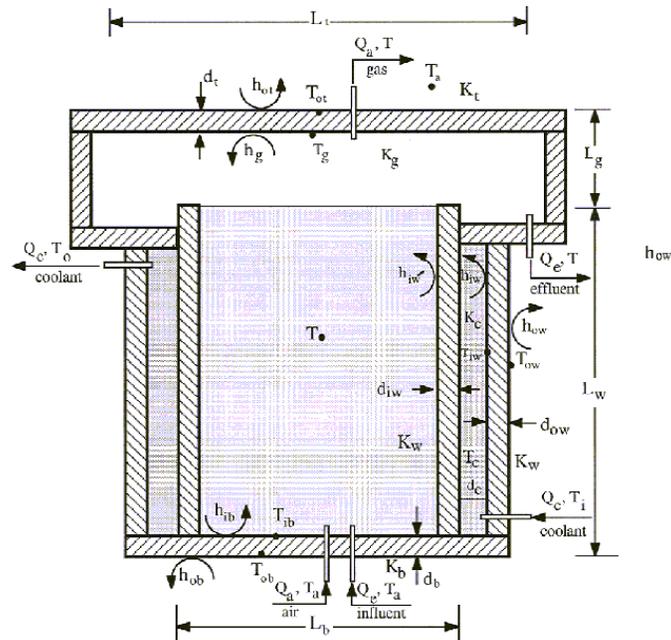


Figure 5. Diagram showing heat transfer during continuous operation.

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

$$U_b = \frac{1}{\left(\frac{d_b}{K_b}\right) + \left(\frac{1}{h_{ob}}\right) + \left(\frac{1}{h_{ib}}\right)} \quad (12)$$

Where:

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

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}), therefore equation 12 can be rewritten as follows:

$$U_b = \frac{1}{\left(\frac{d_b}{K_b}\right) + \left(\frac{1}{h_{ob}}\right)} \quad (13)$$

The convective heat transfer coefficients (h_{ob}) can be calculated as follows (Holman 1990):

$$h_{ob} = 0.59 \left(\frac{T_{ob} - T_a}{L_b} \right)^{0.25} \quad (14)$$

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:

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

Where:

- A_{ow} is the surface area of 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 convection heat transfer coefficient between the medium and the fermenter inside wall ($kJ.m^{-2}.h^{-1}.K^{-1}$)
 h_{ow} is the convective heat transfer coefficient between the fermenter outside 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 a vertical cylinder (m)

The heat transferred through the inner wall of the fermenter is carried away by the water circulating in the water jacket. The conductive transfer across the outer wall of the water jacket can be calculated from equation 6. The heat transfer from the coolant water to the outside wall of the fermenter is by forced convection while that 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:

$$U_w = \frac{1}{\left(\frac{A_{ow} \ln\left(\frac{r_o}{r_i}\right)}{2\pi K_w L_w} \right) + \left(\frac{1}{h_{ow}} \right)} \quad (16)$$

For an average film temperature of 300 K, a maximum temperature difference of 11° C and characteristic length of 325 mm, the calculated Rayleigh number is 3.55×10^7 . The flow is, therefore, laminar, and the convective heat transfer coefficients (h_{ow}) can be calculated as follows (Holman 1990):

$$h_{ow} = 1.42 \left(\frac{T_{ow} - T_a}{L_w} \right)^{0.25} \quad (17)$$

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:

$$U_t = \frac{1}{\left(\frac{d_t}{k_t}\right) + \left(\frac{1}{h_{ot}}\right) + \left(\frac{1}{h_g}\right)} \quad (18)$$

Where:

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

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

$$h_{ot} = 1.32 \left(\frac{T_{ot} - T_a}{L_t} \right)^{0.25} \quad (19)$$

$$h_g = \frac{N_u \cdot K_a}{L_t} \quad (20)$$

Where:

- K_a is the thermal conductivity of air ($\text{kJ.m}^{-1}.\text{h}^{-1}.\text{K}^{-1}$)
 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 Nusselt number (N_u) is calculated as follows (Holman, 1990):

$$Nu = (0.664)^2 (Re)^{1/2} (Pr)^{1/3} \quad (21)$$

Where:

- Re is the Reynolds number (-)
 Pr is the Prandtl number (-)

Yeast Propagation

Since the temperature of the media was maintained as constant by the cooling/heating system and therefore q_l can be deleted from equation 4. With reference to Figure 6-a, the heat balance equation will be as follows:

$$q_y + q_m = q_b + q_w + q_t + q_a + q_c + q_e \quad (22)$$

Yeast Free Operation:

In order to determine the heat generated by mixing during the continuous operation with yeast-free cheese whey, the q_y term is deleted from equation 4. The reactor was operated in the batch mode and the cooling system was not on operation, therefore q_c and q_e can be deleted from equation 4. At the steady state, the heat required to raise the temperature of the medium (q_l) is zero. With reference to Figure 6-b, the heat balance equation will be as follows:

$$q_m = q_b + q_w + q_t + q_a \quad (23)$$

Since there was no water circulation in the cooling system, the heat loss through the wall (q_w) can be calculated from the following equation:

$$q_w = U_w A_w (T - T_a) \quad (24)$$

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

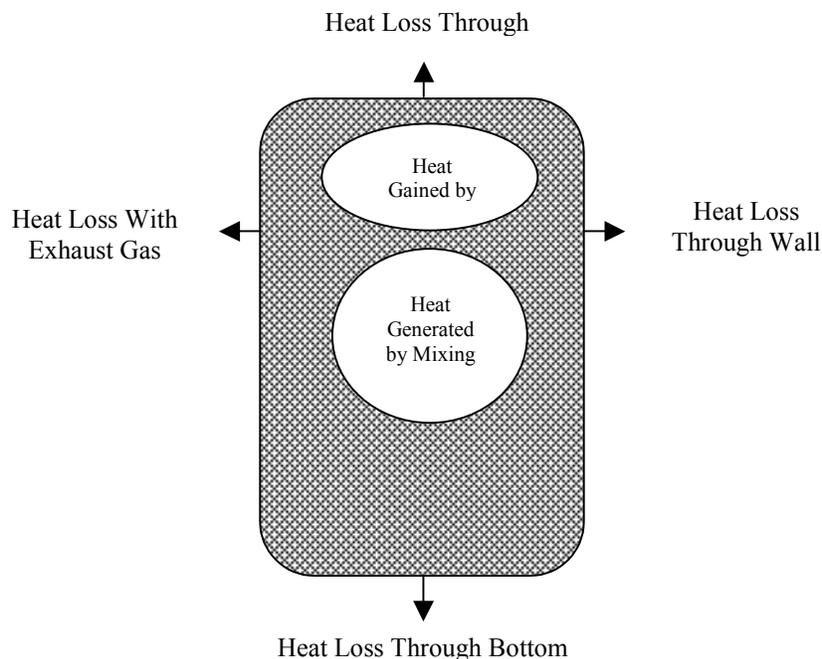
$$U_w = \frac{1}{\left(\frac{A'_{ow} \ln(r'_o / r'_i)}{2\pi K_w L_w} \right) + \left(\frac{A_{ow} \ln(r_o / r_i)}{2\pi K_c L_w} \right) + \left(\frac{A_{ow} \ln(r_o / r_i)}{2\pi K_w L_w} \right) + \left(\frac{A'_{ow}}{A'_{iw}} \right) \left(\frac{1}{h'_{iw}} \right) + \left(\frac{1}{h_{ow}} \right)} \quad (25)$$

Where:

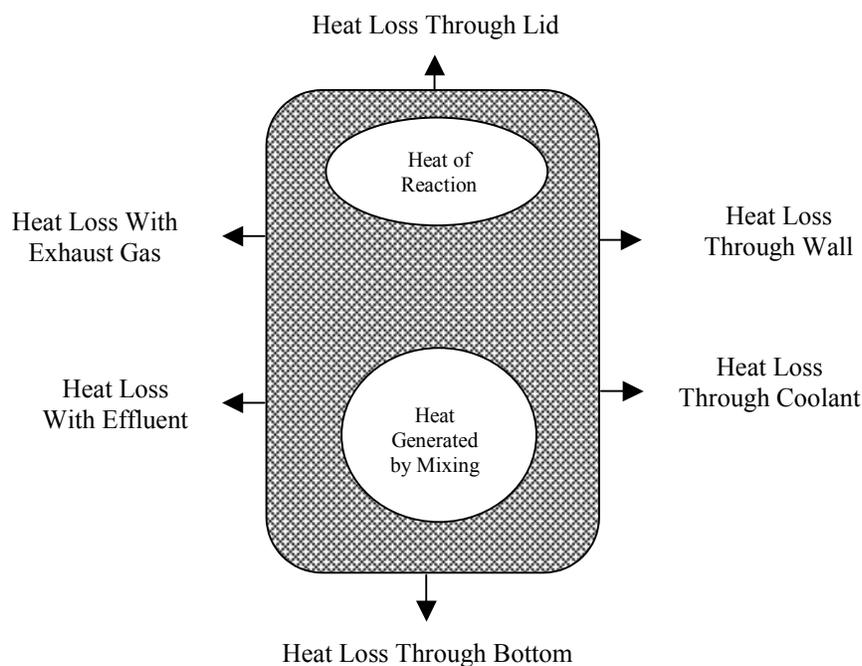
- A'_{iw} is the surface area of the inner side of the inner wall of the fermenter (m^2)
- A'_{ow} is the surface area of the outer side of the inner wall of the fermenter (m^2)
- r'_i is the inner radius of the inner wall of the fermenter (m)
- r'_o is the outer radius of the inner wall of the fermenter (m)
- K_c is the thermal conductivity of the coolant ($kJ.m^{-1}.h^{-1}.K^{-1}$)
- h_{iw} is the convective heat transfer coefficient between the medium and the inner surface of the fermenter inner wall ($kJ.m^{-2}.h^{-1}.K^{-1}$)

The heat transfer from the media to the inner wall of the fermenter is by forced convection while the heat transfer from the outer wall of the fermenter 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 transfer coefficient U_w can be calculated as follows:

$$U_w = \frac{1}{\left(\frac{A'_{ow} \ln(r'_o / r'_i)}{2\pi K_w L_w} \right) + \left(\frac{A_{ow} \ln(r_o / r_i)}{2\pi K_c L_w} \right) + \left(\frac{A_{ow} \ln(r_o / r_i)}{2\pi K_w L_w} \right) + \left(\frac{1}{h_{ow}} \right)} \quad (26)$$



(a) Batch yeast free medium with the non-use of coolant.



(b) Continuous yeast propagation with continuous flow of medium and coolant.

Figure 6. Heat balance.

A.E. Ghaly and N.S. Mahmoud. "Heat Generated by Mechanical Agitation and Lactose Metabolism during Continuous Propagation of *Kluyveromyces fragilis* in Cheese Whey". Agricultural Engineering International: the CIGR Journal of Scientific Research and Development. Manuscript FP 02 010. May, 2003.

EXPERIMENTAL RESULTS AND DISCUSSION

Yeast Free Operation

During the Cell-Free experiment, the lactose concentration was measured every 2 h while the dissolved oxygen concentration, the temperature of the medium and the ambient air temperature were measured continuously. The lactose and dissolved oxygen concentrations and room temperature remained unchanged over time at 5000 mg.L⁻¹ and 22° C, respectively. The initial medium temperature was 22° C, which increased gradually reaching 32.2° C after 16 h and then remained constant. The plate count test performed on the samples indicated that the medium was not contaminated with any type of microbes during the course of the experiment.

A Fortran computer program was written to perform the heat balance on the cell free system at the steady state using equation 23. The system reached steady state condition after approximately 16 h of operation. The heat losses were equivalent to the heat generated by mixing. The heat generated in the reactor by mixing was found to be 22.49 kJ.h⁻¹, most of which (98.95%) was lost with the exhaust gas whereas 0.04, 0.97 and 0.04% were lost through the lid, wall and bottom of the fermenter, respectively (Table 2).

Table 2. Calculated mixing heat in the cell-free system at the steady state condition.

Heat	Value	
	KJ.h ⁻¹	%
q _a	22.25	98.95
q _t	0.01	0.04
q _w	0.22	0.97
q _b	0.01	0.04
q _m	22.49	100.00

Yeast Propagation

The results of the temperature, pH, dissolved oxygen, cell number and lactose concentration obtained during the continuous propagation of yeast are presented in Tables 3 and 4. The lower coefficients of variation indicated that the fermenter was operating at the steady state condition.

The initial pH of the cheese whey was 4.9. 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. It has been recognized that keeping the pH at about 4.5 eliminates possible contamination by bacteria that grow at pH levels above 6 (Mahmoud and Kosilcowski, 1982; Wasserman, 1960). The samples taken from the bioreactor during the start up and steady state periods were plated on agar yeast peptone growth medium. The results showed that maintaining the pH at 4.5 eliminated possible contamination by other microorganisms. The colonies developed from the samples

exhibited typical elevated concave, smooth appearance and creamy color of *K. fragilis*. Staining specimen with crystal violet showed elongated clustered *K. fragilis* cells. The gram reaction showed yeast cells multiplying by budding.

Table 3. Measured temperatures of the bioreactor during continuous propagation of yeast at 12 h hydraulic retention time, 400 rpm mixing speed and 3vvm airflow rate.

Location	Mean (° C)	STD (° C)	CV (%)
Temp-1	29.0	0.3	0.9
Temp-2	32.2	0.1	0.3
Temp-3	34.1	0.1	0.3
Temp-4	34.0	0.1	0.3
Temp-5	32.0	0.1	0.2
Temp-6	32.1	0.1	0.2
Temp-7	33.8	0.1	0.3
Temp-8	29.3	0.3	0.9

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 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 = the standard deviation

CV = the coefficient of variation

Table 4. Measured pH dissolved oxygen, cell and lactose measurements during continuous propagation of yeast at 12 h hydraulic retention time, 400 rpm mixing speed and 3 vvm air flow rate.

Parameter	Mean	STD	CV
PH	4.5	0.20	2.0%
Dissolved oxygen concentration	2.78 mg.L ⁻¹	0.07 mg.L ⁻¹	2.6%
Cell number	986×10 ⁶ cells.mL ⁻¹	23×10 ⁶ cells.mL ⁻¹	2.2%
Lactose concentration	2200 mg.L ⁻¹	50 mg.L ⁻¹	4.4%

Influent lactose concentration = 50000 mg.L⁻¹

STD = the standard deviation

CV = the coefficient of variation

A.E. Ghaly and N.S. Mahmoud. "Heat Generated by Mechanical Agitation and Lactose Metabolism during Continuous Propagation of *Kluyveromyces fragilis* in Cheese Whey". Agricultural Engineering International: the CIGR Journal of Scientific Research and Development. Manuscript FP 02 010. May, 2003.

The average temperature of the medium was maintained at $34.1^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ with aid of a cooling system. The optimum temperature for *K. fragilis* propagation is within the range of $30 - 35^{\circ}\text{C}$ (Knight et al., 1972; Delaney et al., 1975; and Ghaly et al., 1992). Berstein et al. (1977) suggested that the temperature of the fermenter be kept below 35°C by running a low level of cooling water through a jacketed fermenter. This study proved the need for such system. The heat removed by the cooling water was 30.38% of the total heat generated in the fermenter.

The yeast population size was 986 million cells.mL⁻¹ and the residual lactose concentration was 2200 mg.L⁻¹ which resulted in lactose removal efficiency of 95.6%. Using a yeast cell density of 3.3×10^{11} gcell.L⁻¹ (Gancedo and Serrano, 1989; and Ben-Hassan et al., 1992), the yeast yield was found to be 0.77 gcell.g⁻¹ lactose removed from the system. This is very close to the stoichiometric value of 0.79 gcell.g⁻¹ lactose calculated from equations 1 and 2.

A Fortran computer program was written to perform the heat balance on the system using equation 22. The results are shown in Table 5. It was assumed that the rheological properties and hydrodynamic conditions of yeast free medium and medium containing cell mass will not significantly change and thus q_m will remain constant. The amount of lactose used for energy (yeast respiration) was calculated from q_y . A value of 16.7 kJ.g⁻¹ lactose utilized is generally accepted for heat of reaction for lactose oxidation (Ben Hassan et al., 1992; VonStockar and Birou, 1989; and Weast, 1988). The amount of lactose used for yeast growth was then calculated by subtracting the amount of lactose used for energy from the total lactose consumed by the yeast. The values of lactose used for cell growth and respiration (energy) were 88.5 and 11.5% of the total lactose utilized, respectively. According to equations 1, 2 and 3, the stoichiometric values for cellular growth and respiration (energy) are 93% and 7%, respectively. Stockor and Birou (1989) reported that the heat released per unit biomass range from 4 to 13 kJ.g⁻¹ cells depending on whether the fermentation is aerobic or anaerobic. In this study, the heat released was only 2.82 kJ.g⁻¹ cells showing that more lactose was utilized for the production of new cells. The total heat generated in the system during yeast propagation was 114.2 kJ.h⁻¹, of which 19.7% was generated by mixing.

CONCLUSIONS

A procedure for measuring the rate of heat production by mixing was developed using a 25L aerobic fermenter used for the propagation of the yeast *K. fragilis* in cheese whey. The technique presented accounts for all the heat produced in and lost from the fermentation system. The heat generated by mixing and lactose metabolism during the continuous production of yeast from cheese whey lactose was calculated using a heat balance equation. A heat balance was also performed on the system to calculate the portions of cheese whey lactose used for respiration and growth of *K. fragilis*.

Table 5. Calculated heat values during continuous propagation of yeast at 12 h hydraulic retention time, 400 rpm mixing speed at 3 vvm airflow rate.

Heat	Value	
	(kJ.h ⁻¹)	%
Losses		
q _b	- 1.00	0.88
q _w	- 4.30	3.77
q _t	- 7.70	6.74
q _a	- 25.10	21.98
q _c	- 34.70	30.38
q _e	- 41.40	36.25
Generated		
q _y	+ 91.71	80.04
q _m	+ 22.49	19.96
Total (Losses/Generated)	+114.20	100.00

Most of the heat generated by mixing in the cell free system (98.93%) was lost with the exhaust gas while a very small amount (1.07%) was lost through the fermenter lid, wall and bottom. The heat generated by mixing was significant (19.69% of the total heat generated in the fermentation system with an active yeast population present) and therefore, cannot be ignored in heat balance calculations. About 30.38% of the heat generated in the reactor was lost through the coolant showing the need for jacketed fermenter for continuous operation. The results, thus, showed the importance of incorporating the heat generated by mixing in heat balance calculations as well as the need for cooling system.

A yeast population size of 986 million cells.mL⁻¹ and a lactose utilization of 95.6% were observed. About 88.5% of the metabolized lactose was used for growth whereas 11.5% was used for energy. A yield of 0.77 g cell g⁻¹ lactose was achieved. The heat released due to respiration was 2.28 kJ.g⁻¹.cells.

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