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Kinetic modelling of continuous submerged fermentation of cheese whey for single cell protein production

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Abstract

A mathematical model describing the kinetics of continuous production of single cell protein from cheese whey using *Kluyveromyces fragilis* was developed from the basic principles of mass balance. The model takes into account the substrate utilization for growth and maintenance and the effect of substrate concentration and cell death rate on the net cell growth and substrate utilization during the fermentation process. A lactose concentration below 1.91 g/L limited growth of yeast cells whereas a lactose concentration above 75 g/L inhibited the growth of the yeast. The model was tested using experimental data obtained from a continuous system operated at various retention times (12, 18 and 24 h), mixing speeds (200, 400 and 600 rpm) and air flow rates (1 and 3 vvm). The model was capable of predicting the effluent cell and substrate concentrations with R^2 ranging from 0.95 to 0.99. The viable cell mass and lactose consumption ranged from 1.3 to 34.3 g/L and from 74.31% to 99.02%, respectively. A cell yield of 0.74 g cell/g lactose (close to the stoichiometric value of 0.79 g cell/g lactose) was achieved at the 12 h retention time—3 vvm air flow rate—600 rpm mixing speed combination. The total biomass output (viable and dead cells) at this combination was 37 g/L.

Keywords: Kinetics; Continuous fermentation; Cheese whey; Single cell protein; Microbial growth; Microbial decay

1. Introduction

Cheese whey is the liquid effluent generated during the cheese making process. The composition of the whey is about 92% water, 5% lactose, 1.9% protein and fats, 0.9% salt and a small amount of vitamins (Ghaly and Ben Hassan, 1995). The continuous flow aerobic process has been used successfully for the production of single cell protein from cheese whey using the yeast *Kluyveromyces fragilis* (Ghaly and Ben Hassan, 1995; Ghaly et al., 1992; Moresi et al., 1990; Mickle et al., 1974). Cheese whey fermentation for the production of SCP using the yeast *K. fragilis* can be described as a biochemical reaction of cells and lactose to produce microbial cells as the main product. During the fermentation process, some lactose is utilized for the production of energy required for cellular growth. A value of 16.7 kJ/g of lactose is generally accepted for heat of reaction (Reisman et al., 1968; Ghaly and Ben Hassan, 1994). The processes can be illustrated as follows:

(a) respiration

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

(b) growth

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

By combining Eqs. (1) and (2), a typical net reaction of the aerobic conversion of lactose to energy and new cells can be written as follows:

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Nomenclature

e	effluent
i	influent
K_d	is the endogenous microbial decay coefficient (h^{-1})
K_i	is the inhibition substrate concentration (g/L)
K_m	is the maximum substrate utilization coefficient (h^{-1})
K_s	is the saturation constant, equal to the substrate concentration at one-half the maximum specific growth rate (g/L)
O_o	is the oxygen flow rate (L/h)
Q	is the substrate flow rate (L/h)
q	is the substrate utilization rate, gram substrate utilized per gram cell per unit time (h^{-1})
S	is the initial concentration of the limiting substrate (g/L)
S_i	is the influent substrate concentration (g/L)
S_e	is the effluent substrate concentration (g/L)
V	is the reactor volume (L)
X	is the concentration of viable cells (g/L)
X_i	is the concentration of viable cells in the influent (g/L)
X_e	is the concentration of viable cells in the effluent (g/L)
$Y_{x/s}$	is the microbial yield coefficient, gram cells produced per gram substrate consumed
μ	is the specific cell growth rate (h^{-1})
μ_m	is the maximum specific growth rate (h^{-1})
dS/dt	is the rate of change in substrate concentration (g/L h)
dX/dt	is the growth rate (g/L h)

$$14C_{12}H_{22}O_{11} + 12O_2 + 24NH_4 \overset{\text{Yeast}}{\rightarrow} 12C_{13}H_{20}N_2O_7 + 70H_2O + 12CO_2 + 24H^+ + \text{Energy} \quad (3)$$

In theory, fermentation of 1 g of lactose (which requires 0.08 g O_2 and 0.09 g NH_4) results in 0.79 g of cells, 0.11 g of CO_2, 0.26 g of H_2O and 0.005 g of H^+ plus 82.5 J of energy.

The proper utilization of a continuous flow biological unit for single cell protein production depends upon thorough understanding of its kinetics as well as the effects of the operating parameters on microbial growth and substrate utilization. Mathematical models have been used to predict the influence of fermentation operating parameters on cell growth rate, cell concentration and substrate utilization rate. The use of models may lead to the development of better strategies for the optimization of the fermentation process and thus ensures its economic viability. Although there is a desire to concentrate lactose in cheese whey in order to maximize yeast growth rate and cell yield, the inhibitory lactose concentration in cheese whey on the *K. fragilis* is unknown. Furthermore, most models take into consideration substrate limitation and ignore substrate inhibition (Moresi et al., 1990; Ghaly and Ben Hassan, 1994). An optimum model must, therefore, take into account the effects of substrate limitation and inhibition as well as maintenance energy and cell death on the growth and metabolism of the microbes.

The main objectives of this study were: (a) to develop a mathematical model capable of predicting cell and substrate concentrations, specific growth rate and cell yield for a continuous aerobic submerged fermentation of cheese whey for single cell protein production using the yeast *K. fragilis*, taking into account substrate limitation and inhibition, cell death and maintenance energy (b) to evaluate the validity of the model using experimental data, and (c) to determine the inhibitory lactose concentration in cheese whey.

2. Microbial growth and kinetics

In the fermentation process, the components of individual microbial cells will first increase in size before cell division takes place. The time required for the cell to grow and divide into two cells is referred to as the generation time, which depends on the growth characteristics of the microorganism and the environmental conditions in which they live. However, the growth of microorganisms is normally measured in terms of the increase in the microbial population (i.e. the number of viable cells) rather than the increase in the size of a single organism. Further more, optimum growth depends on the optimum maintenance and transport of nutrients in the medium into the cell as well as environmental parameters. In a batch system, the growth of microorganisms usually undergoes four principal phases (the lag phase, the exponential growth phase, the stationary phase and the death phase). The exponential growth phase can be characterized by the following first order equation.

$$\frac{dX}{dt} = \mu X \quad (4)$$

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

Eq. (4) indicates that the rate of increase of cellular material is proportional to the quantity of viable cells present in the system and it can only be used to describe cell growth under optimum conditions. However, if one of the essential nutrients is present in a limited quantity, it will be depleted first and the growth will decline and then cease. Mulchandani and Luong (1989) reported that when the substrate concentration is below a given threshold limit, the specific growth rate is proportional to the increase in the substrate level and approaches a maximum value at the threshold limit. The relationship between the specific growth rate and the concentration of the limiting substrate can be described by following equation:

$$\mu = \frac{\mu_m S}{K_s + S} \tag{5}$$

where μ_m is the maximum specific growth rate (h^{-1}); S is the concentration of the limiting substrate (g/L); K_s is the saturation constant, equal to the substrate concentration (g/L) at one-half the maximum specific growth rate (g/L).

However, higher initial substrate concentration decreased the specific growth rate during ethanol fermentation (D'Amore and Stewart, 1987; Ghaly and El-Taweel, 1995) and lactic acid fermentation (Tango and Ghaly, 1999) as a result of inhibition caused by high osmotic pressure and low water activity. A model for specific growth rate must, therefore, take into account the inhibitory effect of high substrate concentration. Eq. (5) was, therefore, modified to account for the influence of substrate inhibition on specific growth rate.

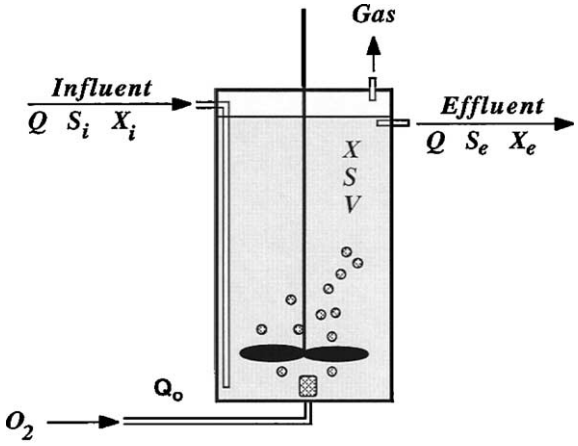
$$\mu = \frac{\mu_m S}{K_s + S} \cdot \frac{K_i}{K_i + S} \tag{6}$$

where K_i is the inhibition substrate concentration (g/L).

From Eqs. (4) and (6), the rate of change in microbial mass in a biological unit can be expressed as follows:

$$\frac{dX}{dt} = \frac{\mu_m S X}{K_s + S} \cdot \frac{K_i}{K_i + S} \tag{7}$$

A continuous fermentation system is shown schematically in Fig. 1. When a substrate is added at a constant rate to a continuous flow biological unit, the unit will eventually reach a steady state condition. At steady state, the following assumptions can be made for an industrial fermentation process: (a) the reactor is completely mixed; the composition of the effluent material is identical to that in the reactor ($S = S_e$; $X = X_e$), (b) there are no microbial cells entering the system ($X_i = 0$), (c) the microbial cell concentration in the reactor does not change with time; the quantity of microbial cells generated in the system is equal to that removed from the system during any given time increment ($dX/dt = 0$), and (d) the substrate concentration in the reactor does not change with time ($dS/dt = 0$). Based on these assumptions, the microbial mass balance, substrate mass balance and yeast yield can be computed. A microbial mass balance for a continuous submerged fermentation can be described as follows:



X = Concentration of viable cells (g/L)
S = Concentration of limiting substrate (g/L)
V = Reactor volume (L)
Q = Substrate flow rate (L/h)
Q_o = Oxygen flow rate (g/L)
i = influent
e = effluent

Fig. 1. Schematic of an aerobic continuous fermentation system.

$$\begin{bmatrix}\text{Cell}\\ \text{Change}\\ \text{Rate}\end{bmatrix} = \begin{bmatrix}\text{Cell}\\ \text{Input}\\ \text{Rate}\end{bmatrix} + \begin{bmatrix}\text{Cell}\\ \text{Growth}\\ \text{Rate}\end{bmatrix} - \begin{bmatrix}\text{Cell}\\ \text{Death}\\ \text{Rate}\end{bmatrix} - \begin{bmatrix}\text{Cell}\\ \text{Output}\\ \text{Rate}\end{bmatrix} \tag{8}$$

Mathematically, Eq. (8) can be written as follows:

$$V\frac{dX}{dt} = QX_i + \mu XV - K_d XV - QX_e \tag{9}$$

where V is the reactor volume (L), Q is the substrate flow rate (L/h), X_i is the concentration of viable cells in the influent (g/L), X_e is the concentration of viable cells in the effluent (g/L), k_d is the endogenous microbial decay coefficient (h^{-1}).

At a steady state condition ($dX/dt = 0$) and Eq. (9) can, therefore, be rewritten as follows:

$$(\mu - K_d)X = \frac{Q}{V}(X_e - X_i) \tag{10}$$

where $(\mu - K_d)$ is the net specific growth rate (h^{-1}).

Since no microbial cells enter the system ($X_i = 0$), the concentration of microbial cells in the system is the same

as that in the effluent ($X = X_e$) at the steady state condition and the retention time (R) of a system is equal to V/Q, then substituting for μ using Eq. (6), Eq. (10) can be rewritten as follows:

$$\frac{\mu_m S}{K_s + S} \cdot \frac{K_i}{K_i + S} - K_d = \frac{1}{R} = D \tag{11}$$

Eq. (11) indicates that the minimum retention time (R) required for the continuous culture system (or the maximum dilution rate, (D) is a function of the substrate concentration (S) and the system constants (μ_m, K_s, K_i and K_d).

A substrate balance for the continuous fermentation can be described as follows:

$$\begin{bmatrix}\text{Substrate}\\ \text{Change}\\ \text{Rate}\end{bmatrix} = \begin{bmatrix}\text{Substrate}\\ \text{Input}\\ \text{Rate}\end{bmatrix} - \begin{bmatrix}\text{Substrate}\\ \text{Output}\\ \text{Rate}\end{bmatrix} - \begin{bmatrix}\text{Substrate}\\ \text{Uptake}\\ \text{Rate}\end{bmatrix} \tag{12}$$

Mathematically, Eq. (12) can be written as follows:

$$V\frac{dS}{dt} = QS_i - QS_e - qXV \tag{13}$$

where dS/dt is the rate of change in substrate (g/L h), S_i is the influent substrate concentration (g/L), S_e is the effluent substrate concentration (g/L), q is the substrate utilization rate, gram substrate utilized per gram cell per unit time (h^{-1}).

At steady state condition ($dS/dt = 0$), $S = S_e$, and $R = V/Q$ and Eq. (13) can be written as follows:

$$X = \frac{S_i - S_e}{Rq} \tag{14}$$

Eq. (14) indicates that the cell concentration in the bioreactor is a function of the substrate removal ($S_i - S_e$), the relation time (R) and the substrate utilization rate (q). The specific growth rate (μ) is also related to the substrate utilization rate (q) as follows:

$$\mu = q \cdot Y_{x/s} \tag{15}$$

where $Y_{x/s}$ is the yeast yield coefficient, gram cells produced per gram substrate utilized (g/g).

The substrate is utilized by *K. fragilis* for cell growth and cell maintenance. Therefore, the term Xq in Eq. (14) can be replaced by the substrate uptake rate for growth ($R_{s/cg}$) plus the substrate uptake rate for cell maintenance ($R_{s/cm}$) modelled by Eqs. (16) and (17).

$$R_{s/cg} = \frac{dX}{dt} / Y_{x/s} \tag{16}$$

$$R_{s/cm} = m_s X \tag{17}$$

where m_s the maintenance energy coefficient, gram substrate per gram cell per hour (h^{-1}).

The maintenance energy coefficient term (m_s) has a significant effect on the fermentation process (Tango and Ghaly, 1999). The values of m_s can range from as little as 0.02 kg substrate per kg cell per hour to as high as 4.00 kg substrate per kg cell per hour (Bailey and Ollis, 1986). Substituting Eqs. (4), (6), (16) and (17) in Eq. (14) yields the following:

$$S_i - S_e = RX\left[\frac{1}{Y_{x/s}}\left(\frac{\mu_m S}{K_s + S} \cdot \frac{K_i}{K_i + S}\right) + m_s\right] \tag{18}$$

Eq. (18) indicates that the amount of substrate removed ($S_i - S_e$) is a function of the retention time (R), the microbial cell concentration (X), the substrate concentration (S) and the system constants (K_s, K_i, m_s and $Y_{x/s}$). Eq. (11) can be rearranged as follows:

$$\mu_m \cdot \frac{S}{K_s + S} \cdot \frac{K_i}{K_i + S} = \frac{1 + K_d R}{R} \tag{19}$$

Substituting Eq. (19) in Eq. (18) yields the following equation:

$$S_i - S_e = RX\left[\frac{1}{Y_{x/s}}\left(\frac{1 + K_d R}{R}\right) + m_s\right] \tag{20}$$

Rearranging Eq. (20) yields the following equation:

$$Y_{x/s} = \frac{X(1 + K_d R)}{(S_i - S_e) - (m_s RX)} \tag{21}$$

Eq. (21) indicates that the yeast yield is a function of the amount of substrate removed ($S_i - S_e$), the microbial cell concentration (X), the retention time (R) and the system parameters (K_d and m_s).

Since $1/R = \mu - K_d$ in Eq. (11), Eq. (21) can be rewritten as follows:

$$Y_{x/s} = \frac{\mu XR}{(S_i - S_e) - (m_s RX)} \tag{22}$$

3. Methods

3.1. Experimental apparatus

The experimental apparatus used in the continuous culture study (Fig. 2) consists of a 25 L working volume bioreactor, an air supply system, a whey feeding and effluent removal system and computer based data acquisition system. Details on the development of the bioreactor, measurement and control, air supply and feeding systems can be found in Ghaly et al. (1992) and Ben Hassan et al. (1991a,b).

3.2. Whey collection and preparation

The cheese whey was obtained from Farmer's Cooperative Dairy Plant in Truro, Nova Scotia in 60 L plastic containers and transported to the Cold Storage Facility

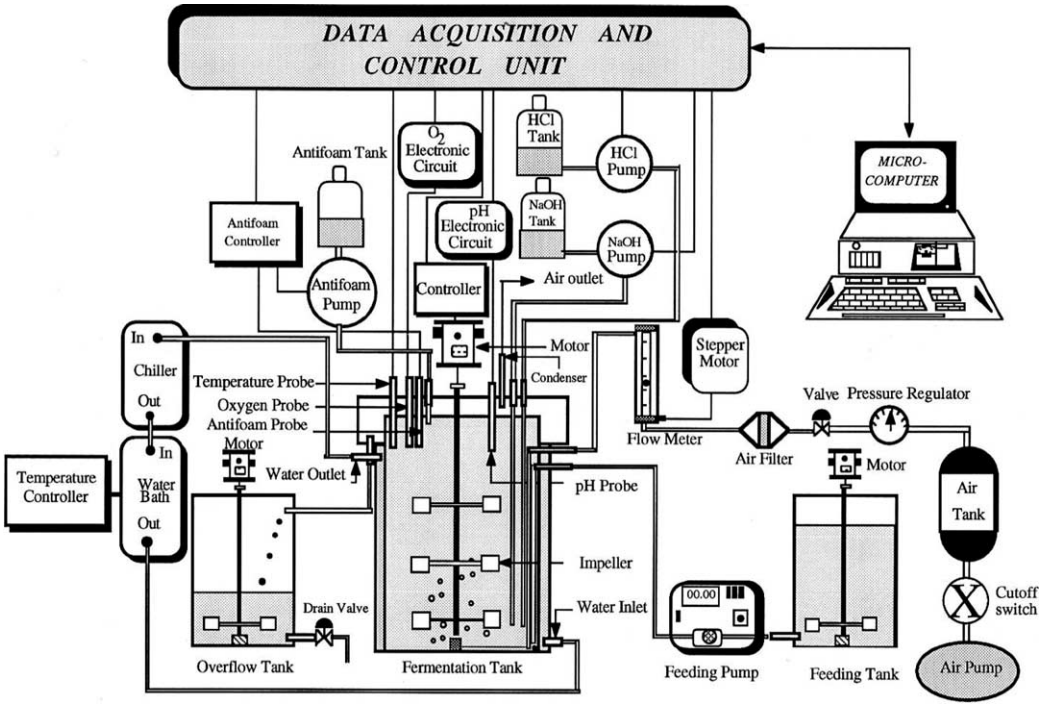


Fig. 2. Experimental apparatus.

of the Biotechnology Laboratory at Dalhousie University Halifax, Nova Scotia, Canada where they were stored in a large freezer at −25 °C until required. Prior to placing the cheese whey into the fermenter, it was allowed to completely thaw at room temperature for 24 h. Some characteristics of the cheese whey used in this study are presented in Table 1. The analyses were performed according to the procedures described in the Standard Methods for Examination of Water and Waste Water (APHA, 1998). The lactose concentration was determined using a sugar analyser (YSI Model 27, Yellow Spring Instrument, Yellow Spring, OH, USA).

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

Characteristics	Measured value	Unit
Total solids	67,070	mg/L
Total fixed solids	6650	mg/L
Total volatile solids	60,420	mg/L
Total dissolved solids	44,670	mg/L
Fixed dissolved solids	6090	mg/L
Volatile dissolved solids	38,580	mg/L
Total suspended solids	22,400	mg/L
Fixed suspended solids	650	mg/L
Volatile suspended solids	21,750	mg/L
Total chemical oxygen demand	74,750	mg/L
Soluble chemical oxygen demand	61,200	mg/L
Biological oxygen demand	49,500	mg/L
Total Kjeldahl nitrogen	1550	mg/L
Ammonium nitrogen	290	mg/L
Lactose	50,000	mg/L
Soluble phosphorus	470	mg/L
Potassium	1620	mg/L
Sulphur	150	mg/L
pH	4.9	–

3.3. Inoculum preparation

Freeze dried pellets of *K. fragilis* (NRS 5790) culture were obtained from the Division of Biological Sciences, National Research Council, Ottawa, Canada. A pellet of *K. fragilis* was dissolved in 5 mL sterile 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 dishes were then placed in a controlled controlled environment incubator at 35 °C and left until visual growth appeared on the petri dish (about 72 h). Fifteen litres of raw cheese whey were first pasteurized in several 4 L reagent bottles according to the procedure developed by Ghaly and El-Taweel (1995) and then transferred to several 250 mL sterilized Erlenmeyer flasks (150 mL per flask). The in-oculum was then transferred from the yeast stock to the pasteurized cheese whey in the sterilized Erlenmeyer flasks (two petri dishes of pure culture of *K. fragilis* were added to each flask containing 150 mL pasteurized cheese whey). The Erlenmeyer flasks were capped with non-absorbent cotton plugs and mounted on a controlled environment reciprocating shaker (Series 25, New Brunswick Scientific Co. Inc., Edison, New Jersey). The shaker was operated at a speed of 250 rpm for 48 h. Following the 48 h growth period. The yeast

cultures were collected from the flasks, transferred to a large container and then mixed thoroughly. The yeast culture was then divided into three equal parts of 5 L each and stored in the refrigerator at 4 °C until needed.

3.4. Experimental protocol

The fermenter and all accessories (mixing system, tubing and feeding tank) were chemically sterilized using 2% (w/v) potassium meta-bisulphite solution, and then washed with sterile hot water several times before starting the experiment in order to remove any traces of chemicals. The fermenter was filled with 17.5 L of cheese whey and then inoculated with 2.5 L of inoculum. The air flow (1 volume/volume/min), turbine drive (200 rpm), the temperature controller (33 °C), the computer and the data acquisition and control unit were started immediately. The remaining 5 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 every 6 h for six days during the steady state operation and analysed. The lactose analysis was performed using a sugar analyser (YSI Model 27, Yellow Spring Instrument, Yellow Spring, OH, USA), the viable cell number was calculated using dehydrogenase activity measurement described by Ghaly and Ben-Hassan (1993). The number of viable cells in the effluent was converted to cell mass using the conversion factor of 3.03×10^{-11} g/cell (Ghaly and Ben Hassan, 1995; Gancedo and Serrano, 1989). The mixing speed, hydraulic retention time and air flow rate were changed and the same procedure was used for samples collection and analysis during the steady state condition of all the hydraulic retention time—air flow rate—and mixing speed combinations.

4. Results and discussion

4.1. Operating parameters

Three retention times (12, 18 and 24 h), two air flow rates (1 and 2 vvm) and three mixing speeds (200, 400 and 600 rpm) were studied. For all experiments, the dissolved oxygen (DO) concentration was monitored every 2 h, while the pH and temperature were maintained at 4.5 ± 0.1 and 33 ± 1 °C, respectively. For each experimental run, the mean, standard deviation and coefficient of variation of the pH, temperature and DO measurements were calculated as shown in Table 2. The results indicate that the fermenter was operating at steady state conditions for all hydraulic retention times, airflow rates and mixing speeds as the coefficients of variation were very small (varying from 1.98% to 2.66% for pH, from 1.25% to 1.91% for temperature and from 1.92% to 4.61% for dissolved oxygen).

Both pH and temperature control units operated creditably. The initial pH of the cheese whey was 4.9. The pH of the medium ranged from 4.48 to 4.56 (4.52 ± 0.04), which is close to the optimum value of the 4.5 reported by Bernstein et al. (1977). The average temperature of the medium was 33.4 ± 1.3 °C (32.1–

Table 2
The effluent pH, temperature and dissolved oxygen at various hydraulic retention times, air flow rates and mixing speeds

Retention time (h)	Airflow rate (vvm)	Mixing speed (rpm)	pH			Temperature			Dissolved oxygen		
			Mean	STD	CV (%)	Mean (°C)	STD (°C)	CV (%)	Mean (mg/L)	STD (mg/L)	CV (%)
12	1	200	4.54	0.09	1.98	34.02	0.14	1.47	1.95	0.09	4.61
		400	4.52	0.11	2.43	34.02	0.13	1.39	2.16	0.07	3.33
		600	4.52	0.11	2.43	34.60	0.10	1.30	2.28	0.07	3.07
	3	200	4.54	0.10	2.20	34.06	0.12	1.35	2.24	0.10	4.46
		400	4.52	0.12	2.43	34.20	0.10	1.32	2.52	0.08	3.17
		600	4.56	0.10	2.19	34.60	0.10	1.30	2.76	0.10	2.62
18	1	200	4.51	0.11	2.43	33.61	0.14	1.43	2.97	0.09	3.03
		400	4.52	0.11	2.43	33.77	0.14	1.33	3.08	0.07	2.27
		600	4.50	0.12	2.66	33.97	0.11	1.31	3.20	0.08	2.50
	3	200	4.48	0.11	2.45	33.93	0.09	1.30	3.10	0.08	2.58
		400	4.53	0.12	2.65	34.02	0.16	1.47	3.27	0.11	3.36
		600	4.51	0.12	2.66	34.03	0.14	1.40	3.43	0.09	2.62
24	1	200	4.51	0.11	2.44	32.13	0.42	1.25	3.15	0.06	1.90
		400	4.51	0.06	1.99	32.22	0.29	1.91	3.54	0.15	4.32
		600	4.52	0.10	2.21	33.26	0.22	1.70	3.75	0.09	2.40
	3	200	4.54	0.10	2.20	33.67	0.13	1.40	3.65	0.07	1.92
		400	4.55	0.09	1.98	33.71	0.10	1.35	3.82	0.09	2.35
		600	4.52	0.12	2.65	33.77	0.10	1.30	4.00	0.09	2.25

The values are the average of 72 determinations. STD = standard deviation.

34.7 °C), which was, therefore, within the optimum temperature range reported in literature (Bernstein et al., 1977; Delaney et al., 1975). The dissolved oxygen concentrations varied from 1.95 mg/L to 4.00 mg/L depending on the hydraulic retention time, air flow rate and the mixing speed. Increasing the hydraulic retention time and/or the air flow rate and/or the mixing speed increased the dissolved oxygen concentration in the medium. Longer retention time resulted in smaller microbial populations (less oxygen uptake) and, thus, higher dissolved oxygen concentrations. Higher air flow rates and mixing speed resulted in higher oxygen transfer rate and thus, higher dissolved oxygen concentrations. Vananuvat and Kinsella (1975) and Meiering et al. (1978) observed higher lactose consumption and dramatic increases in yeast population when the agitation speed was increased up to 800 rpm. It should be noted that the dissolved oxygen concentration in this study was not a growth limiting factor.

4.2. Cell and lactose concentrations

For each experimental run, the mean, standard deviation and coefficient of variation of lactose concentration and active cell numbers were calculated (Table 3). The results also confirmed that the fermenter was operating at the steady state condition for all hydraulic retention time—air flow rate—mixing speed combinations as the coefficient of variation was relatively small (ranging from 0.75% to 5.88% for the lactose and 0.41–4.41% for the viable cell number).

The results indicated that at 12 h retention time, the yeast yield coefficient ranged between 0.339 and 0.743 g of yeast cell/g of substrate depending on the air flow rate and mixing speed used, while at 18 and 24 h retention times the values were much lower, ranging between 0.260 and 0.297 and 0.230 and 0.251 g yeast cell per gram substrate, respectively. For the purpose of single cell protein production, the 12 h retention time—3 vvm air flow rate—600 rpm mixing speed combination should be used. The value of the cell yield was 0.743 g cell/g substrate, which is very close to that of 0.78 g cell/g substrate determined from the stoichiometric Eqs. ((1)–(3). Bernstein and Everson (1973) reported that an optimum cell yield could be obtained if a shorter retention time (8 h) is used with a fermentation temperature of 30–35 °C, pH level of 4.5 and sufficient oxygen along with the essential nutrients. The values obtained in this study are higher than those reported in the literature of 0.58–0.67 kg of cells for each kilogram of whey lactose consumed (Bernstein and Everson, 1973 and Atkin et al., 1976). The high value of the growth yield coefficient obtained in this study was the result of proper reactor design and optimum levels of operation parameters (temperature, pH and DO).

It should, however, be noted that only viable cells were measured in this study. When single cell protein is produced, the total biomass (viable and non-viable cells) as well as non-cellular organic nitrogen would be of interest. Thus, the total biomass output would be much higher than the viable cell output at all retention times, mixing speeds and flow rates.

Table 3
The effluent lactose viable cell concentrations at various hydraulic retention times, air flow rates and mixing speeds

Retention time (h)	Airflow rate (vvm)	Mixing speed (rpm)	Lactose concentration			Cell number		
			Mean (g/L)	STD (g/L)	CV (%)	Mean (cell/mL × 10^6)	STD (cell/mL × 10^6)	CV (%)
12	1	200	13.1	0.41	3.13	241.3	2.87	1.19
		400	11.5	0.09	0.78	368.3	7.18	1.95
		600	7.5	0.22	2.93	518.3	11.15	2.15
	3	200	8.2	0.16	1.95	400.0	12.79	3.20
		400	5.1	0.30	5.88	813.3	17.75	2.18
		600	2.2	0.09	4.09	1132.5	49.90	4.41
18	1	200	12.0	0.09	0.75	120.4	2.27	1.88
		400	10.0	0.02	1.90	139.4	17.75	2.83
		600	6.1	0.11	1.80	161.8	49.90	1.46
	3	200	7.0	0.13	1.86	149.7	2.83	1.89
		400	4.0	0.12	3.00	185.6	1.62	0.87
		600	1.0	0.06	6.00	285.6	1.16	0.41
24	1	200	11.2	0.19	1.70	42.8	1.85	4.32
		400	8.2	0.10	1.22	49.5	2.11	4.26
		600	4.5	0.15	3.33	64.8	2.08	3.31
	3	200	5.0	0.23	4.60	58.7	1.56	2.66
		400	1.1	0.05	4.54	85.8	3.74	4.36
		600	0.5	0.04	5.00	117.6	3.34	2.84

The values are an average of 12 determinations.
Raw whey lactose concentration = 50 g/L. STD = standard deviation. CV = coefficient of variation.

5. Determination of model parameters

5.1. Determination of K_s, K_i and μ_m

A batch experiment was conducted using different initial lactose concentrations (ranging from 1 to 350 g/L) added to active cultures of *K. fragilis* yeast in separate flasks (500 mL). The flasks were then incubated in controlled environment shaker (Series 25, Incubator shaker, New Brunswick Scientific Co., Inc., New Jersey) at a temperature of 35 °C and shaking speed of 150 rpm until a complete growth curve was obtained for each culture. Three replications were made. The cell number was determined by measuring the dehydrogenase activity of the yeast cells at 2 h time intervals (Ghaly and Ben-Hassan, 1993). The effect of initial lactose concentration on the specific growth rate is shown in Fig. 3. The maximum specific growth rate (μ_m) and saturation constant (K_s) were determined according to the procedure described by Doran (1995). The Langmuir plot of (S/μ) versus the initial substrate concentration (S) should give a straight line as shown in Fig. 4. The intercept represents the value of (K_s/μ_m) while the slope of the line represents the value of ($1/\mu_m$). The numerical values of the maximum specific growth rate (μ_m), the saturation constant (K_s) and the substrate inhibition constant (K_i) for cheese whey fermentation using *K. fragilis* were 0.23 h^{-1}, 1.91 g/L and 75 g/L, respectively. The growth of the yeast is substrate limited at initial substrate concentration below 30 g/L, is a maximum ($\mu = \mu_m$) at initial substrate concentration between 30 and 75 g/L, and is substrate inhibited at substrate concentration above 75 g/L. The yeast growth is totally inhibited at substrate concentration of 350 g/L.

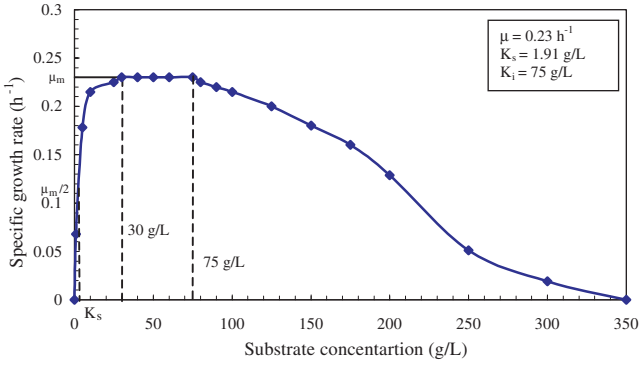


Fig. 3. Effect of initial lactose concentration on the specific growth rate.

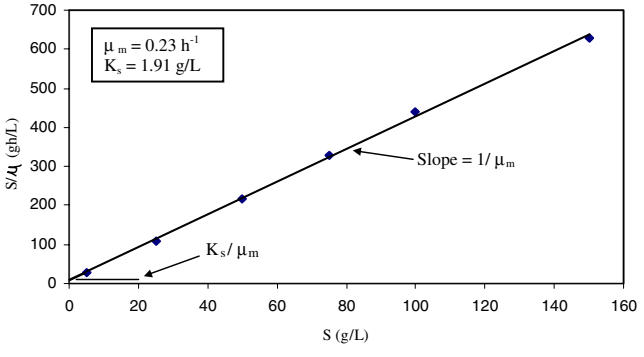


Fig. 4. Determination of the maximum specific growth rate (μ_m) and the saturation constant (K_s).

5.2. Determination of $Y_{x/s}$, K_d, q and m_s

The specific growth rate (μ), endogenous microbial decay coefficient (K_d), substrate utilization rate (q), the cell yield ($Y_{x/s}$) and the maintenance energy coefficient (m_s) were determined from the bioreactor data using Eqs. (6), (11), (14), (16) and (18). The values of these kinetic parameters are presented in Table 4.

6. Model validation

A FORTRAN program was developed to simulate the substrate (S) and the cell (X) concentration during the continuous fermentation process. The critical values of S and X were predicted by solving Eqs. (14) and (18). The yeast cell mass given by Gancedo and Cerrano (19) as 3.03×10^{-11} g/cell was used as a conversion factor to calculate the cell mass.

Table 4
Estimated model coefficients

Retention time (h)	Air flow rate (vvm)	Mixing speed (rpm)	μ (h^{-1})	K_d (h^{-1})	q (h^{-1})	$Y_{x/s}$ (g/g)	m_s (h^{-1})
12	1	200	0.150	0.067	0.442	0.339	0.619
		400	0.145	0.061	0.312	0.464	0.453
		600	0.125	0.042	0.241	0.518	0.406
	3	200	0.129	0.046	0.309	0.418	0.503
		400	0.108	0.025	0.159	0.680	0.308
		600	0.087	0.003	0.117	0.743	0.283
18	1	200	0.151	0.095	0.579	0.260	0.807
		400	0.148	0.093	0.527	0.282	0.744
		600	0.143	0.087	0.482	0.296	0.709
	3	200	0.141	0.085	0.527	0.267	0.786
		400	0.126	0.070	0.449	0.281	0.748
		600	0.091	0.036	0.307	0.297	0.706
24	1	200	0.151	0.110	0.658	0.230	0.914
		400	0.150	0.109	0.619	0.243	0.865
		600	0.144	0.102	0.604	0.238	0.882
	3	200	0.145	0.103	0.577	0.251	0.836
		400	0.130	0.088	0.561	0.232	0.907
		600	0.105	0.063	0.464	0.262	0.929

μ_{max} = 0.23 h^{-1}. K_s = 1.91 g/L. K_i = 75.00 g/L.

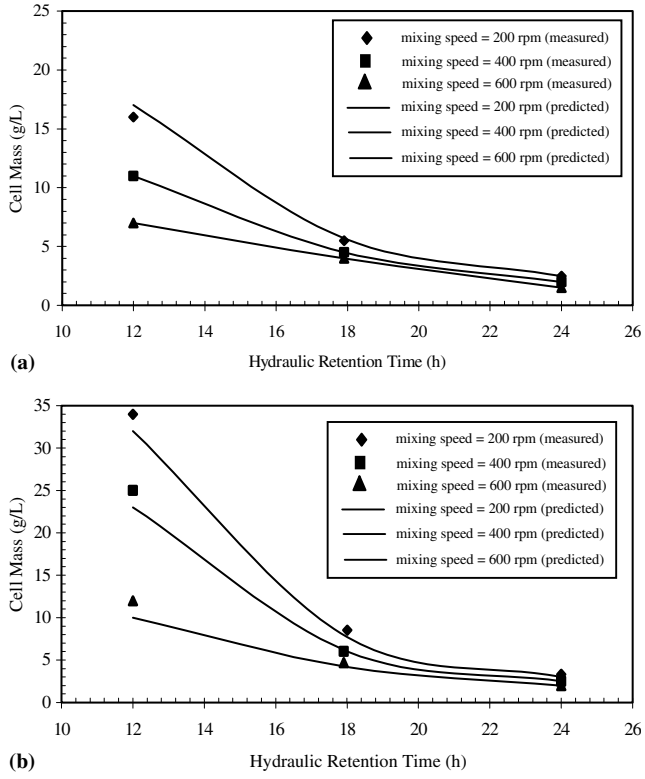


Fig. 5. Measured and predicted cell mass: (a) 1 vvm air flow rate, (b) 3 vvm air flow rate.

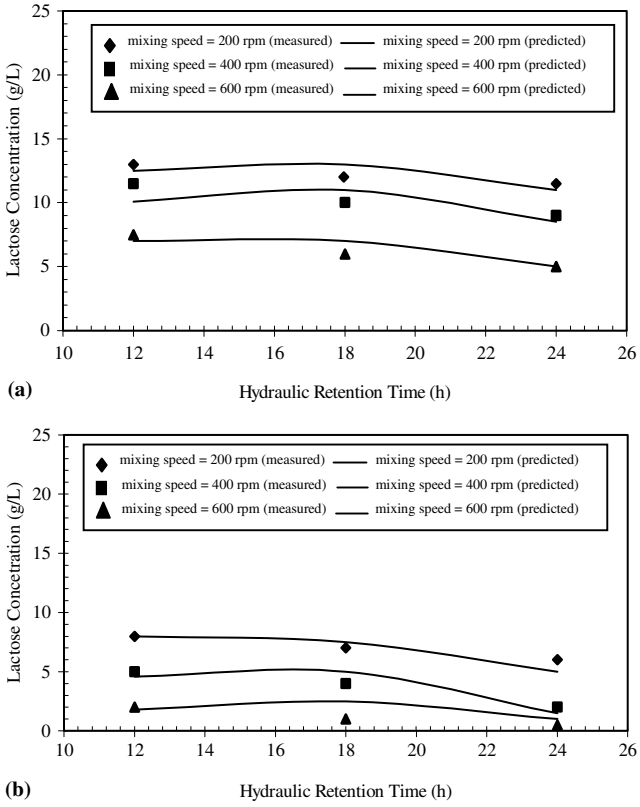


Fig. 6. Measured and predicted lactose concentration: (a) 1 vvm air flow rate, (b) 3 vvm air flow rate.

The observed and predicted values of the viable yeast cell concentration (X) and the substrate concentration (S) at different airflow rates, mixing speeds and hydraulic retention times are shown in Figs. 5 and 6. The experimental data are represented by points while the predicted values are represented by lines. A good agreement between the model predictions and the experimental data was obtained. A comparison of the predicted and observed cell and lactose concentrations are presented in Fig. 7. The correlation coefficient (R^2) for the cell and lactose concentration was 0.96 and 0.99, respectively. This confirms that the model is capable of predicting the experimental results with high accuracy.

In this study, the predicted viable cell yield at the optimum operating conditions (12 h retention time, 3 vvm air flow rate, 600 rpm mixing speed, temperature of 32 °C and pH of 4.5) was less (6.4%) than the actual cell yield. Atkin et al. (1976) predicted lower values (5.4%) of *K. fragilis* yield than the experimental values of a continuous culture operation. Although the model takes into consideration the effect of substrate concentration, it was only tested with raw cheese whey with lactose concentration of 50 g/L. Since the inhibitory lactose concentration was found to be 75 g/L, it is, therefore, recommended that the model be tested with cheese whey with a higher lactose concentration (75–200 g/L).

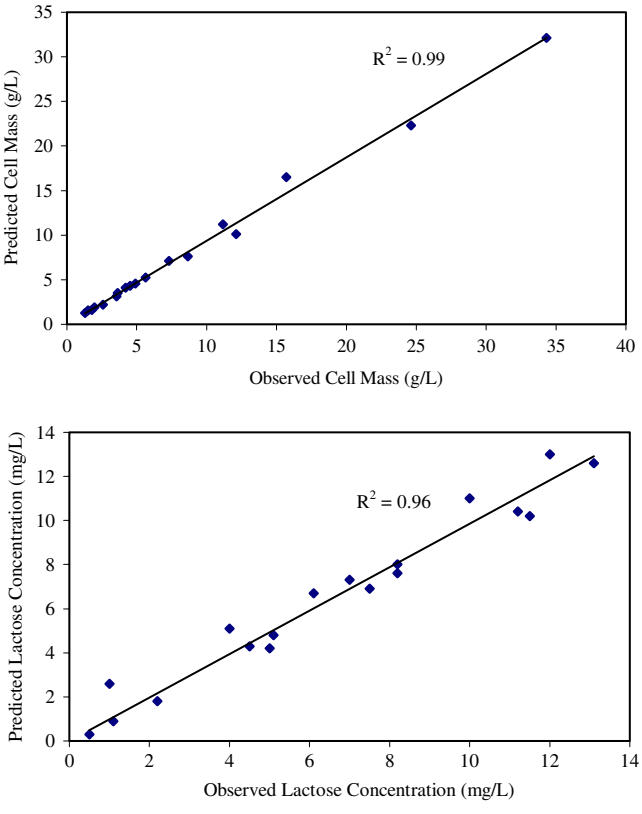


Fig. 7. Comparison of predicted and observed values of lactose concentration and cell mass.

7. Conclusions

A mathematical model describing the kinetics of a continuous production of single cell protein from cheese whey was developed from the basic principles of mass balance. The model takes into account the substrate utilization for cell growth and maintenance and the effect of substrate concentration and cell death rate on the net cell growth and substrate utilization during the fermentation process. A lactose concentration below 1.91 g/L was found to limit growth whereas a lactose concentration of 75 g/L was found to inhibit growth. The model was tested using experimental data obtained from a bioreactor operating at three hydraulic retention times (12, 18 and 24 h), three mixing speeds (200, 400 and 600 rpm) and two air flow rates (1 and 3 vvm). The predicted results of the substrate (S) concentration and yeast cell concentration (X) of the model compared very well with the experimental results with R^2 values ranging from 0.96 to 0.99.

A viable cell mass of 1.3–34.3 g/L and a lactose consumption rate of 74.31–99.02% was achieved depending on the retention time, air flow rate and mixing speed used. A cell yield of 0.74 g cells/g lactose (which is close to the stoichiometric value of 0.78 g cells/g lactose) was achieved at the 12 h retention time, 3 vvm air flow and 600 rpm mixing speed. For the purpose of single cell protein production a 12 h retention time with sufficient oxygen (good aeration and agitation) should be used. A total biomass output (viable and non-viable cells) at this condition was estimated at 37.0 g/L.

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