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Submerged yeast fermentation of acid cheese whey for protein production and pollution potential reduction

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Abstract

Bench-scale batch bioreactors were used to study the effectiveness of cheese whey fermentation for single-cell protein production using the yeast *Kluyveromyces fragilis* in reducing the pollution potential of whey as measured by solids, chemical oxygen demand (COD) and nitrogenous compounds concentrations. The four principal phases (lag, exponential, stationary and death) encountered in the history of a microbial culture grown under batch conditions were clearly recognized in the growth, temperature and dissolved oxygen curves. The lactose concentration and soluble COD displayed three distinct phases corresponding to the lag, exponential and stationary phases of the yeast growth. The minimum dissolved oxygen and maximum temperature observed in this study (at an air flow of 3 VVM, a mixing speed of 400 rpm and an ambient temperature) were 2.49 mg/L and 31.6°C, respectively. About 99% of lactose (90.6% of soluble COD) was utilized after 28 h. The total COD continued to decline due to cell death resulting in a reduction of 42.98%. The total nitrogen concentration remained unchanged while the organic nitrogen increased during the exponential phase and then declined during the death phase. The ash content remained unchanged while a substantial reduction (56%) of the volatile solids was observed. These results indicated that sufficient oxygen for yeast growth was present in the medium and no cooling system was needed for this type of fermenter under similar experimental conditions. Recovering the yeast biomass with ultrafiltration reduced the total COD by 98% of its initial value in the raw whey.

Keywords: Whey; Lactose; Yeast; SCP; Batch; Pollution; Solids; COD; Nitrogen

1. Introduction

Cheese whey is a liquid byproduct of the cheese-making process that contains most of the water soluble components, and water, present in milk (approximately 5% lactose, 0.9% nitrogenous compounds, 0.8% minerals and small amounts of vitamins). It has been estimated that annually as much as 24.0 and 1.7 million kg of whey are produced in the United States of America and Canada, respectively [1]. Only a little over one-half of the whey produced is utilized, much of which is in the form of a dried whey powder. About 17% of the whey produced is dumped in sewers whereas 26% is disposed of on the land. Because of its high biological oxygen demand (BOD) (40,000–60,000 ppm) whey disrupts the biological processes of sewage treatment plants [2–4]. Also, long-term land disposal of whey causes environmental pollution problems as reported by Ghaly et al. [5] and Fertizscha [6].

Whey exerts a considerable oxygen demand and its economic disposal/utilization becomes increasingly important for the dairy industry due to increased volume of whey being produced, increased centralization of processing sites and more stringent environmental laws related to effluent quality [7]. The dairy industry must, therefore, try to attain a position where handling the whey does not prevent the industry from meeting the

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market demand for its products. Since, lactose in cheese whey is the major contributor to BOD, using the whey as a substrate for the production of single-cell protein (SCP) may reduce its pollution potential while results in the production of a value added product. The term SCP refers to dried cells of microorganisms such as algae, actinomycetes, bacteria, yeast, molds, and higher fungi grown in large-scale culture systems for use as protein source in human food or animal feed [8]. The most important characteristic of these single-celled organisms is their high protein content, ranging from about 40% to 80% of their dry weight on a crude protein basis. Also, their protein tends to be of high quality, more closely resembling animal protein than plant protein, and is generally readily available nutritionally.

In searching for suitable microorganisms to be grown on cheese whey lactose as a substrate for SCP production, one can readily recognize that more research work has been carried out on yeast than on other microorganisms. The yeast *Kluyveromyces fragilis* has been used, by several researchers in several countries, for production of dried yeast and could be used as nutritional supplements for fortifying animal feed or human food from cheese whey [8–12]. It should be noted, however, that SCP contains a large percentage of nucleic acids that may be hard for some humans to digest.

The main aim of this study was to investigate the effectiveness of batch submerged fermentation of the yeast *K. fragilis* in acid cheese whey for the production of SCP and the reduction of the pollution potential of the whey as measured by the concentrations of chemical oxygen demand (COD), total solids and nitrogenous compounds.

2. Experimental apparatus

The fermentation system used in this study (Fig. 1) consisted of three fermenters, aeration system and temperature, pH and DO measuring equipment. The fermenter was constructed from plexiglas material and provided a liquid capacity of 4.8 L when the mixing device was submerged. Four holes were drilled and tapped through the lid: one hole for the oxygen probe, the second for the pH probe, the third for the temperature probe and the fourth for the exhaust gas outlet pipe. The fermenter was designed to be completely mixed, and hence, a steel mixing shaft of 7 mm diameter and 400 mm length was installed through the center of the lid. Two flat bladed turbine impellers of 75 mm diameter were used to mix the fermenter content. The first impeller was positioned at the end of the mixing shaft at about 30 mm from the fermenter floor, and the other impeller was positioned at 148 mm from the first impeller (or 178 mm from the fermenter floor). The mixing shaft was driven by a variable speed electric motor (Dayton Electric MFG Co. Model 4Z142, Chicago, IL). Four baffles were used in the fermenter to reduce vortexing and to improve the top-to-bottom

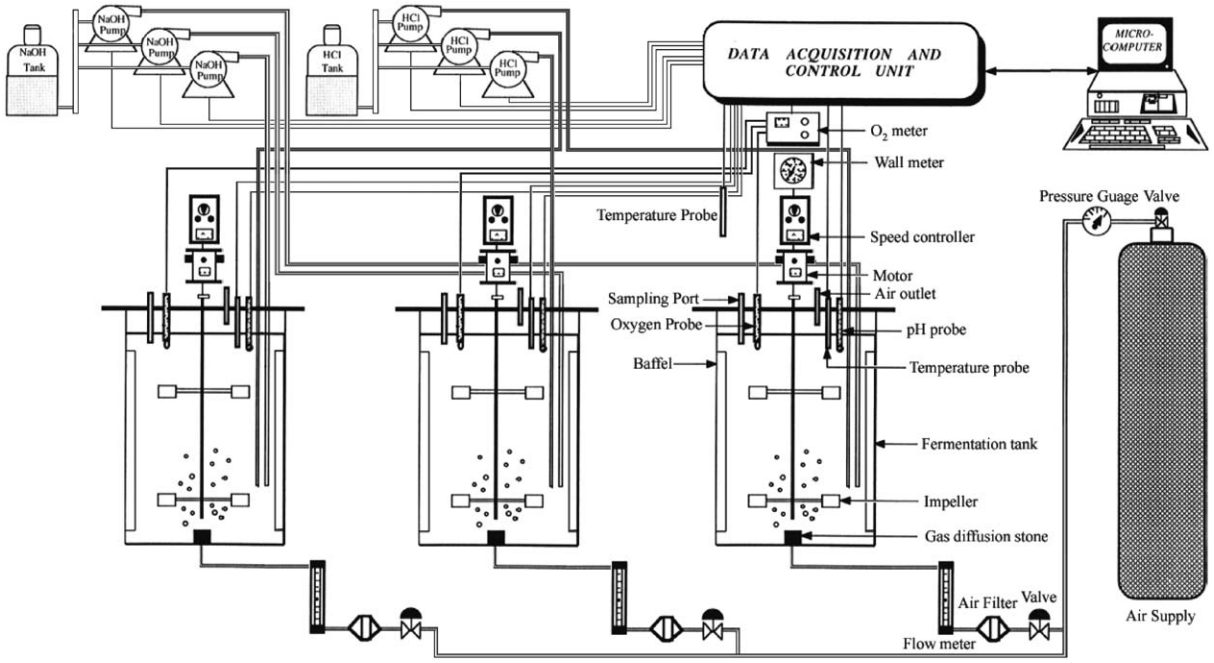

Fig. 1. The fermentation system.

turnover. The Standard design recommended for baffles, given in the Perry's Chemical Engineer's Handbook [13], was used.

Compressed air (Medigas Atlantic Limited Cat. No. T100172, Halifax, NS) was supplied to the fermenter through a flowmeter (Victor, CGA 590, Model 2581-AG, Boston, MA) with high-resolution valve using tygon tubing of 10 mm diameter. The air was composed of 78.084% N_2, 20.996% O_2, 0.033% CO_2 and 0.937% other gases. The moisture and impurities in the air were less than 10 and 2 ppm, respectively. A micro-filter (Cole-Parmer Cat. No. L-29701-00, Chicago, IL) was used to reduce the risk of microbial contamination. The air was introduced from the bottom of the fermenter through a gas diffusion stone (Fisher Scientific Cat. No. 11-139B, Yellow Spring, OH) of 26 mm height and 22 mm diameter.

The dissolved oxygen was monitored using an oxygen probe (YSI 5739, Fisher Scientific Cat. No. 13-299-43, Yellow Springs, OH) connected to a digital dissolved oxygen meter (YSI Model 58, Fisher Scientific Cat. No. 13-298-58, Yellow Springs, OH). A copper-constantan thermocouple inserted in the fermenter and connected to a data logger (Cole-Parmer Cat. No. L-08360-14, Chicago, IL) was used to measure the temperature. The pH was measured using a pH probe (Cole Parmer Cat. No. J-5990-40, Chicago, IL) connected to a pH control unit which included a data acquisition unit, signal conditioning circuit and two pumps.

2.1. The ultrafiltration system

A megaflow filtration apparatus (Model TM-100, New Brunswick Scientific Co., Inc., NJ) was used to recover the yeast biomass and organic nitrogen from the spent medium (Fig. 2). It is a high performance tangential flow filtration system designed for rapid molecular separation and concentration of cells and purification of wastewater. It achieves high recovery in the shorter time even with viscous fluids of high particulate concentration. The unit is operated with a heavy duty, large capacity peristaltic pump (Model 701 S/R, New Brunswick Scientific Co., Inc., NJ) which has a variable speed industrial motor (360 rpm with forward/off/reverse switch). The working capacity of the unit is the range of 8.4–2000 L/h. The flat sheet

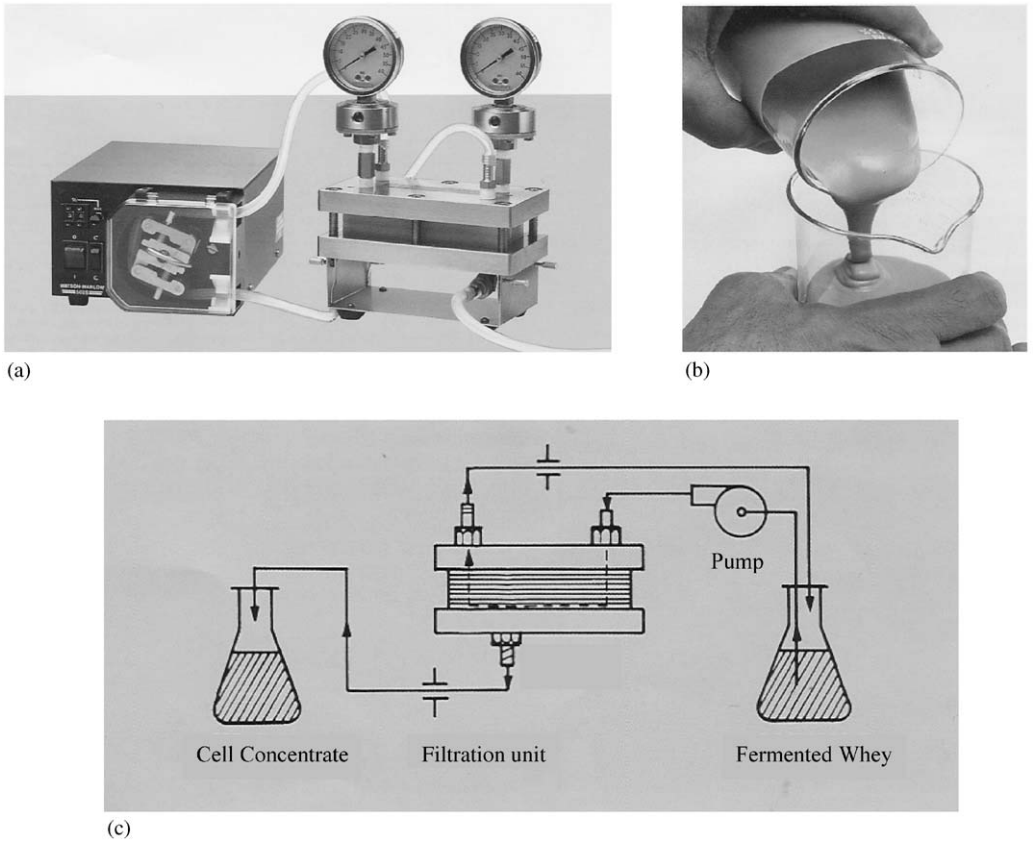

Fig. 2. Ultrafiltration system: (a) Model TM-100 filtration unit with Model 502S/R170 peristaltic pump; (b) concentrated yeast cell culture; (c) ultrafiltration unit in operation.

membrane is made of polypropylene and has pore size of 0.1 μm and an area of 64.5 cm^2. The dimensions (height × width × depth) of the filtration unit are 26 × 42 × 45 cm. The filtration unit is made of two stainless steel compression plates, a polypropylene channel plate, a polypropylene filtrate plate, two pressure gages, a filtration collection port and filtration plug. A silicon rubber tube of an inside diameter of 0.64 cm and a wall thickness of 0.25 cm was used to connect the mega flow filtration unit to the peristaltic pump and the medium container. The pump was operated at a flow rate of 1 L/h.

The inlet pressure varied from 103.4 to 137.88 kPa whereas the outlet pressure varied from 82.73 to 103.41 kPa. The temperature of the medium was 25°C.

3. Experimental procedure

3.1. Whey collection, storage and preparation

Acid cheese whey was obtained from the Farmer's Cooperative Dairy Plant in Truro, NS. 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 in Halifax, NS, 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. The solid, COD and nitrogen analyses were performed according to the procedures described in the Standard Methods for the Examination of Water and Wastewater [14]. The lactose concentration was determined using sugar analyzer (YSI Model 27, Yellow Sprigs, OH). Prior to placing the cheese whey into the fermenter, it was allowed to completely thaw at room temperature for 24 h. Fifteen liters of raw cheese whey were then pasteurized in several 4 L reagent bottles. The pasteurization technique included heating the whey to 60°C for 30 min, cooling it to 0°C for 30 min and letting it to stand at room temperature (21°C) for 24 h for any spore to germinate. The processes of heating, cooling and standing at room temperature was repeated three times to destroy any vegetative or spore cells present in the whey. The plate count test [15] was performed to insure the effectiveness of this pasteurization technique.

3.2. 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 sterilized growth medium containing 1% yeast extract, 2% peptone and 2% dextrose.

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

Characteristics	Measured value[a]	Unit
Total solids	66,830	mg/L
Fixed solids	10,100	mg/L
VS	56,730	mg/L
Percent VS	84.88	%
Percent fixed solids	15.12	%
Suspended solids	22,150	mg/L
Fixed solids	180	mg/L
VS	21,970	mg/L
Percent VS	99.19	%
Percent fixed solids	0.81	%
Total Kjeldahl nitrogen	1490	mg/L
Ammonium nitrogen	170	mg/L
Organic nitrogen	1320	mg/L
Percent organic nitrogen	88.59	%
Percent ammonium nitrogen	11.41	%
Total COD	74,220	mg/L
Soluble COD	59,640	mg/L
Insoluble COD	14,580	mg/L
Percent soluble COD	80.36	%
Percent insoluble COD	19.64	%
Lactose	50,000	mg/L
pH	4.9	—

[a] The measured values are the average of three replicates.

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 (50 Perti dishes were used). The Petri dishes were then placed in a controlled environment incubator at 35°C and left until visual growth appeared (after about 72 h). The yeast culture (visible colonies) was then scooped from the surface of the agar into two Perti dishes using sterilized loop and transferred to 200 mL pasteurized cheese whey in the sterilized Erlenmeyer flask (25 flasks were used). The Erlenmeyer flasks were then capped with non-absorbent cotton plugs and mounted on a controlled environment-reciprocating shaker (Series 25, Incubator Shaker, New Brunswick Scientific Co., Inc., Edison, NJ). The shaker was operated at a speed of 250 rpm for 48 h. Following the 48 h growth period, 5000 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 stored in the refrigerator at 4°C until needed.

3.3. Experimental protocol

The fermenter and all accessories were chemically sterilized using a 2% potassium meta-bisulfite solution

and washed with hot distilled–deionized water several times before starting the experiment in order to remove any chemical traces. The fermenter was filled with 4320 mL of pasteurized cheese whey. Then, 480 mL of the inoculum (10% by volume) were added as recommended by Ghaly et al. [16] and Ben-Hassan et al. [17]. The air flow (3 VVM) and turbine drive speed (400 rpm) recommended by Ben-Hassan et al. [17] were started immediately, and the dissolved oxygen, pH and temperature were monitored continuously. After the termination of the fermentation process (48 h), the ultrafiltration unit was used to recover the yeast biomass from the spent medium.

3.4. Sampling and analysis

Samples were drawn from the fermenters at 0 h and then every 2 h for the first 36 h and every 6 h for the next 12 h. The lactose concentration, the cell number, COD, solids and nitrogen analyses were performed on the samples collected from the fermentation system. Samples were also taken from the effluent of the ultrafiltration system for COD and solid analyses. Total Kjeldahl nitrogen and ammonium nitrogen analyses were performed on the samples whereas the organic nitrogen content was then calculated by subtracting the ammonium nitrogen values from those of the total Kjelhahl nitrogen. The plate count was carried out according to the procedures described by Messer et al. [15] in the Standard Methods for the Examination of Dairy Products. The COD, solids and nitrogen analyses were performed according to the procedures described in the Standard Methods for Examination of water and wastewater [14].

4. Results and discussion

4.1. Operating parameters

The measured values of pH, dissolved oxygen, temperature, cell number and lactose concentration are presented in Fig. 3. Each value is the average of three measurements performed on the samples taken from the three fermenters at any given sampling time. The results obtained from the three fermenters were comparable. The coefficient of variation varied from 1.2% (for pH) to 12.8% (for cell number).

4.2. pH

The initial pH value of the cheese whey used in this study was 4.9. Ghaly et al. [16] reported that maintaining the pH between 4.0 and 5.0 is very essential for the growth and survival of *K. fragilis*. It has also been recognized by Bernstein et al. [9] that keeping the pH at about 4.5 eliminates possible contamination by lethal bacteria that grow at pH levels above 6.0. In this study, the fermenter was operated under batch condition for approximately 48 h and the pH of the medium was maintained at 4.4 ± 0.2 by the addition of 1 N HCl solution.

4.3. Dissolved oxygen

The cheese whey was saturated with oxygen (6.6 mg/L), using compressed air, before the inoculum of *K. fragilis* was introduced. During the fermentation process, the dissolved oxygen displayed four distinct stages. The first stage corresponded to the lag phase of the yeast

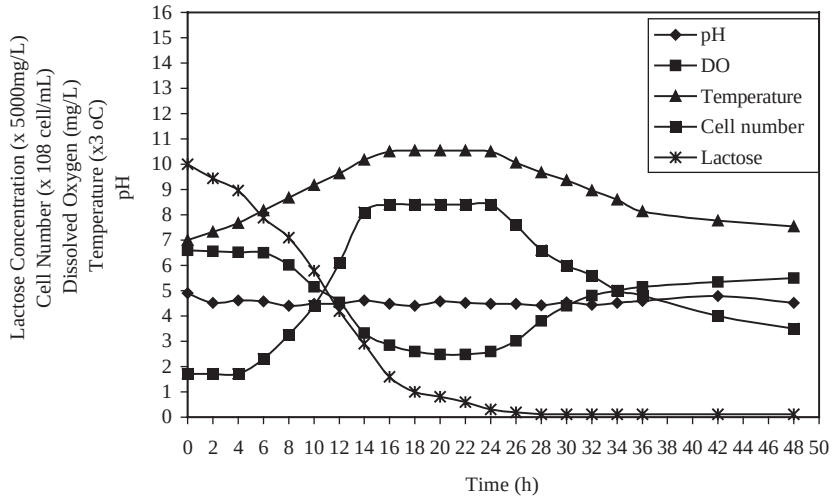

Fig. 3. The pH, temperature, dissolved oxygen concentration, cell number and lactose concentration measured during the batch culture operation.

growth curve, during which the dissolved oxygen decreased slowly (almost linearly) as the number of yeast cells remained constant and oxygen was mostly required for cell respiration and endogenous cell growth. The second stage corresponded to the exponential growth phase, during which the exponential growth of the cells was accompanied by a rapid decrease in the oxygen concentration. The third stage corresponded to the stationary growth phase of the yeast population, during which the number of cells remained constant at its maximum and the oxygen concentration reached a constant minimum value of 2.49 mg/L, as a temporary steady-state condition was achieved. During this steady-state period, the amount of oxygen added to the system by the aeration equipment was equivalent to that consumed by the yeast cell for respiration. The fourth stage corresponded to the death phase of the yeast, during which the number of cells decreased with time and as a result the oxygen concentration continuously increased. During this stage, the amount of oxygen used by the surviving yeast cells for respiration was less than that supplied by the aeration system.

The minimum dissolved oxygen values observed in this study appeared to be higher than that reported by several authors. Porges [18] and Ghaly et al. [19] reported 0.3 mg/L as being the critical value below which the oxygen uptake rate by micro-organisms is dependent on the oxygen concentration of the medium. Ghaly et al. [20] and Ghaly et al. [16] reported a minimum dissolved oxygen during batch fermentation of cheese whey under pH controlled and uncontrolled condition of 1.2 and 0.75 mg/L, respectively. In this study, the minimum oxygen concentration was 2.49 mg/L. Oxygen concentration in the medium was also found to correlate very well with the cell number ($R^2 = 0.97$) as shown in Fig. 4. Litchfield [21] and Burgess [22] reported a peak oxygen demand of *K. fragilis* in the range of 4.5–5.0 mM O_2/L/min. In this study, the air was supplied at 3 VVP given an oxygen input of 24.75 mmol O_2/L/min.

4.4. Temperature

The air ambient temperature in the laboratory remained constant during the course of the experiment at $21 \pm 0.2^{\circ}C$, whereas the temperature of the cheese whey was initially 21°C and then changed with time. The temperature curve displayed four distinct stages that corresponded to the lag, exponential growth, stationary, and death phases of yeast growth curve. The first stage corresponded to the lag phase, during which the temperature increased slowly by 1.0°C (from 21.0°C to 22.0°C) over a 2 h period as a result of lactose metabolism for maintenance. The second stage corresponded to the exponential growth phase, during which the temperature increased sharply by 9.7°C (from 22.0°C to 31.6°C) over the period of 16 h as a result of lactose metabolism for energy and growth. In the first two phases, the heat generated was higher than that lost from the fermenter. The third stage corresponded to the stationary phase, during which the substrate become limited and number of the yeast cells remained constant and as a result the temperature remained constant at 31.6°C for about 10 h as the heat generated by lactose metabolism was equivalent to that lost from the fermenter through the lid, bottom and wall and with the exhaust gas. The fourth stage corresponded to the death phase, during which the temperature decreased rapidly by 9.0°C (from 31.6°C to 22.6°C) due to the heat losses from the fermenter. The medium temperature was found to correlate with cell number ($R^2 = 0.94$) in fermenter as shown in Fig. 5.

The optimum temperature for *K. fragilis* propagation is in the range of 30–35°C [11,23,24]. Berstein et al. [9] suggested that the temperature of the fermenter medium must be maintained at 35°C by running a low level of

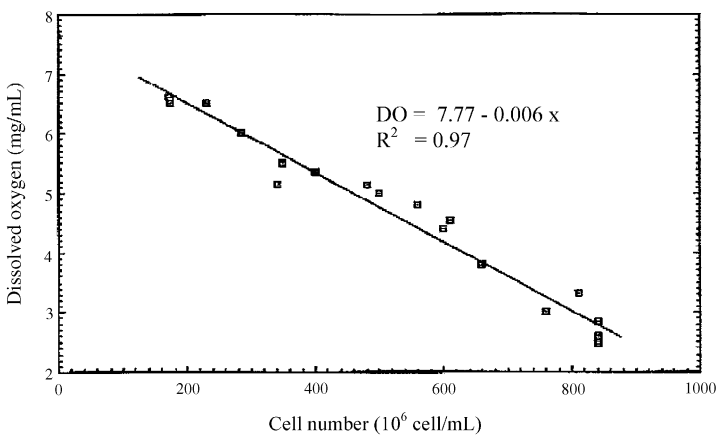

Fig. 4. Effect of cell number on dissolved oxygen.

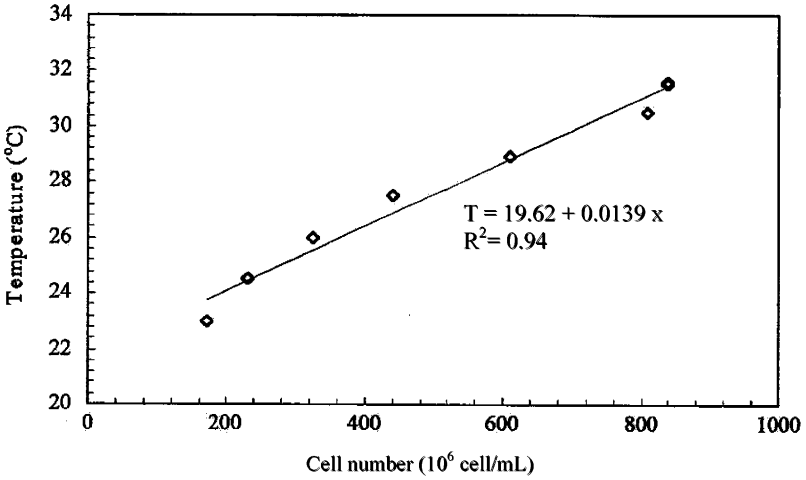

Fig. 5. Effect of cell number on temperature.

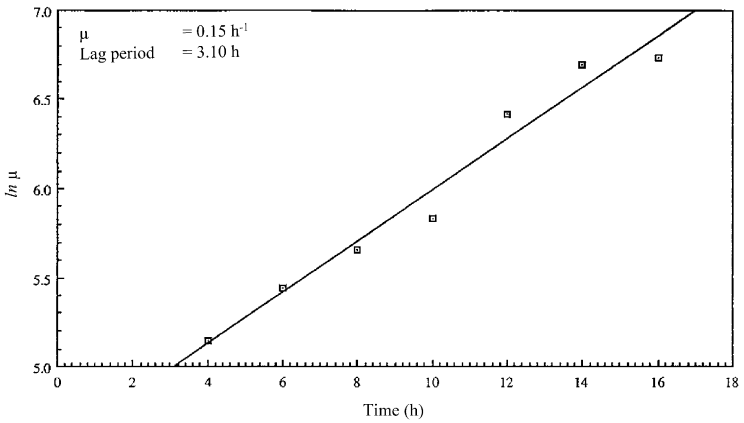

Fig. 6. Determination of the lag period and specific growth rate of *K. fragilis*.

water through jacketed fermenter. In this experiment, cooling was not found necessary as the temperature of the cheese whey in the fermenter reached a maximum value of 31.6°C. However, if an optimum fermentation is to be achieved, the fermenter should be insulated to minimize heat losses through the wall, top and the bottom of the fermenter and a heating system should be used during the initial growth phase to rise the temperature of the media near the optimum.

4.5. Yeast growth

The cheese whey samples taken from the reactor were plated on agar yeast–peptone–dextrose (YPD) growth medium. The colonies developed from the samples exhibited the typical elevated concave, smooth appearance and creamy color of *K. fragilis*. Staining specimens with crystal violet showed elongated clustered yeast cells. The Gram reaction showed that the yeast cell multiplied vegetatively by budding.

The four principal growth phases encountered in the history of a microbial culture grown under a batch operation can be clearly recognized in the growth curve. The length of the lag phase and specific growth rate were determined graphically as shown in Fig. 6. The duration of the lag phase was found to be approx. 3.1 h. Vananuvat and Kinsella [11] studied the production of yeast protein from lactose using *K. fargilis* and observed a lag phase of about 4.0–5.0 h. Ben-Hassan et al. [17] reported a lag phase of 4.0 h at similar conditions. The yeast grows exponentially between 3.1 and 16.0 h. The specific growth rate (μ) of *K. fargilis* population was found to be 0.15 h^{-1}. Alvarez and Ricano [25] reported that the specific growth rate of *K. fargilis* using batch culture fermentation for producing SCP was about 0.21 h^{-1} at a temperature of 37°C and a pH of

4.5. Ben-Hassan et al. [17] reported a specific growth rate of *K. fragilis* of $0.2\,h^{-1}$ at a temperature of 35°C and a pH of 4.5.

4.6. Lactose utilization

The initial value of lactose was 50,000 mg/L. Since the liquid volume was 4.8 L, the total amount of lactose in the fermenter at zero time was 240.0 g. The lactose concentration in the fermenter displayed three distinct stages that corresponded to the lag, exponential and stationary phases of the yeast growth curve. During the first stage, there was a slow reduction in the lactose concentration as lactose was utilized mainly for cell maintenance and cell endogenous growth. The second stage was a period of rapid lactose reduction, during which lactose was utilized by the yeast for both endogenous cell growth and the cell mass growth as well as cell respiration. In the third stage, the concentration of lactose was very low and, thus, an insignificant reduction of lactose was achieved during this period. This resulted in death of the yeast vegetative cells.

The results presented in Fig. 3 show that after 16 h of fermentation, the yeast cell number leveled off at a maximum value of 840×10^6 cells/mL. Since the environmental parameters (temperature, O_2 and pH) were at their optimum values, it can be concluded that a lactose concentration of 8000 mg/L was becoming insufficient to sustain the continuous growth of the yeast population. However, during the stationary growth phase (16–24 h), the number of dying cells was being replaced by an equivalent number of newborn cells. These new cells obtained the nutrient required for growth from the limited supply of lactose (8000–1500 mg/L) and the constituents of the dead cells after the rapture of their cellular membrane. Once the lactose concentration was below 1000 mg/L (during the death phase), the rate of cell death (or number of dead cells) was higher than the growth rate (or number of new cells) and the yeast population started to decline.

The lactose was reduced from 50,000 to 600 mg/L after 28 h. This resulted in a reduction of 99% of the initial lactose in the fermenter. Burgess [22] reported that the lactose consumption, under batch condition using a laboratory scale tower fermenter, was fairly low in the first 2 h (less than 10%), while the yeast was adapting to the new substrate. The lactose consumption then increased rapidly and the lactose concentration in the medium at the end of exponential growth phase was 1.75% and was almost totally depleted by the end of the experiment. Ben Hassan et al. [26] reported 99% lactose utilization after 20 h when operating a batch reactor at similar temperature and pH conditions. Ghaly et al. [20] reported 96% lactose removal efficiency after 12 h under similar conditions.

4.7. Pollution parameters

The measured COD, nitrogen, and solids concentrations in the medium are presented in Table 2. Each value is the average of three measurements performed on the samples taken from the three fermenters at any given sampling time. The results obtained from the three fermenters were comparable. The coefficient of variation varied from 3.9% (for ash) to 8.8% (for org-N).

4.8. COD

The aerobic decomposition of an organic matter such as lactose is a process which provides energy for growth and supplies nutrients for the synthesis of new microbial protoplasm. The process is illustrated in the following steps:

(a) energy release (respiration) reaction:

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

Table 2
The measured values of COD, ammonium nitrogen, and organic nitrogen concentrations

Time (h)	COD		Nitrogen		Solids	
	Total (mg/L)	Soluble (mg/L)	NH_4-N (mg/L)	Org-N (mg/L)	Total (mg/L)	Ash (mg/L)
0	74,220	59,640	170	1320	66,830	10,100
2	73,210	58,830	160	1330	63,020	10,090
4	73,120	56,290	130	1360	60,520	10,100
6	72,580	52,400	100	1390	57,680	10,100
8	72,050	44,330	80	1410	55,230	10,110
10	71,060	31,600	60	1430	53,010	10,100
12	69,560	24,450	40	1450	51,127	10,090
14	67,440	18,560	20	1470	49,500	10,080
16	65,280	13,240	10	1480	48,010	10,090
18	62,920	10,120	0	1490	46,830	10,110
20	60,300	7630	10	1480	45,620	10,100
22	58,210	5960	10	1480	44,330	10,100
24	54,730	5590	10	1480	42,940	10,100
26	53,920	5590	30	1460	41,480	10,110
28	52,160	5590	60	1430	39,990	10,100
30	50,010	5590	90	1400	38,510	10,100
32	48,470	5590	120	1370	37,270	10,110
34	46,350	5590	150	1340	36,350	10,110
36	44,430	5590	160	1330	35,710	10,110
42	43,440	5590	180	1310	35,230	10,100
48	42,320	5590	200	1290	35,100	10,090

The values are the average of three replicates each. The coefficients of variation (CV) were as follows: total COD = 6.7%; soluble COD = 6.9%; NH_4-N = 5.1%; Org-N = 8.8%; total solids = 7.6%; ash = 3.9%.

(b) Synthesis (growth) reaction:

$$13C_{12}H_{22}O_{11}+24NH_4^+ \xrightarrow{\text{yeast}} 12C_{13}H_{20}O_7N_2+59H_2O+24H^+. \quad (2)$$

A typical net reaction of the aerobic decomposition of organic matter is as follows:

$$14C_{12}H_{22}O_{11}+12O_2+24NH_4 \xrightarrow{\text{yeasts}} 12C_{13}H_{20}O_7N_2+12CO_2+70H_2O +24H^++\text{energy}. \quad (3)$$

The COD was used in this study as an indirect measurement of the soluble and insoluble organic matter in cheese whey. The difference between the initial and final values of the soluble COD is due to the consumption of the dissolved biodegradable organic matter (mostly lactose) by the yeast. A fraction of the soluble organic matter is oxidized by yeast to provide energy for growth with the production of CO_2 and H_2O according to Eq. (1). The other fraction is utilized by yeast for the synthesis of new microbial protoplasm according to Eq. (2).

In this study, the final reduction in soluble COD was 54050 mg/L (90.63% of the original value) which is higher than the reduction in the total COD of 31,900 mg/L (42.98% of the original value) as shown in Fig. 7. The reduction in the soluble COD is due to the oxidation of organic material for the production of energy (plus CO_2 and H_2O) required for growth and the conversion of soluble organic material to insoluble microbial cells (according to Eq. (3)), whereas the reduction in the total COD is due to the oxidation of organic matter to CO_2 and H_2O (according to Eq. (1)). Therefore, the difference between the total COD reduction and the soluble COD reduction represents the amount of soluble organic matter (soluble COD) used for the production of new yeast cells.

During the lag phase (first 3.1 h), all the reduction in the soluble COD was due to the oxidation of soluble organic material (mostly lactose) to CO_2 and H_2O while during the period of exponential growth (4–16 h), the amount of soluble organic material converted into microbial cells ranged from 67.16% to 88.75%. Further reductions in the total COD observed during the stationary growth and death phases were due mainly to the yeast endogenous respiration during the stationary phase and cell death during the death phase. Therefore, by the end of the experiment (48 h), approximately 40.98% of the cumulative reduction of the soluble organic material was converted to microbial cells and 59.02% was converted into CO_2 and H_2O. The yeast population could be further reduced with a longer fermentation time because most of the organic materials (yeast cells) will be converted to CO_2 and H_2O through the respiration process and final death of the cells.

Fig. 8 shows a breakdown of the reduction in the Soluble COD into microbial cells and energy (plus CO_2 and H_2O). Although there was no further reduction in the soluble COD after 24 h, the total COD continued to decline due to cell death. When the substrate (lactose) was limited (or absent) in the fermenter, the cells died and lyzed. Initially, their constituents were available as substrates for other living cells to grow which resulted in the stationary phase and finally when the death rate was faster than the growth rate, the cells death phase commenced.

Since the lactose in the cheese is about 75% of the total solids, and as such is the major contributor to the COD, the values of SCOD and TCOD concentrations measured in the media during the batch fermentation process were plotted against the lactose as shown in

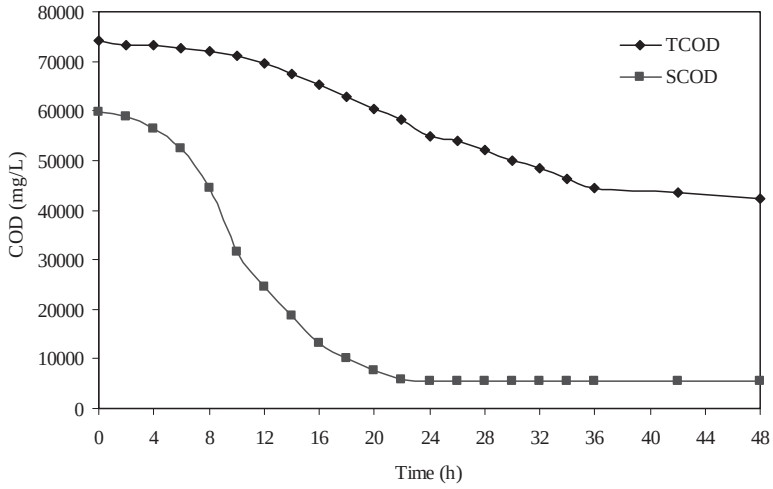

Fig. 7. The total and soluble COD measured during the batch culture.

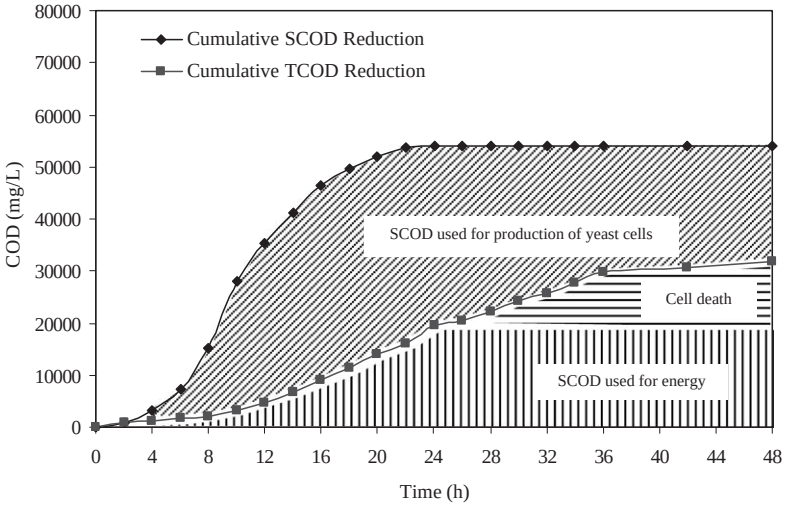

Fig. 8. Breakdown of soluble COD reduction into energy and yeast cells.

Fig. 9. Because lactose is soluble, it has contributed to most of the SCOD. Therefore, the relationship between the measured SCOD and lactose (L) during the fermentation process can be described by the following linear equation ($R^2 = 0.99$):

$$\text{SCOD} = 4.0594 + 1.1301\text{L}. \quad (4)$$

The reduction in TCOD was much slower than the reductions in lactose initially, as some of the lactose was converted to microbial cells which became a part of the TCOD, but become faster during death phase, Therefore, the relationship between the measured TCOD and lactose (L) during the fermentation process can be described by the following exponential equation ($R^2 = 0.96$):

$$\text{TCOD} = 50.824 + 6.099\ln \text{L}. \quad (5)$$

4.9. Nitrogen

The concentration of the ammonium nitrogen in raw cheese whey was very small (170 mg/L). No ammonium salts were added and the concentration of ammonium nitrogen in the raw whey was very small (11.41% of total nitrogen). The organic nitrogen in the raw cheese whey was approximately 1.9% (1320 mg/L) of the total solids which is within the reported values in the literature (1–2%). The organic nitrogen in cheese whey as measured in this study included amino acids, polypeptides and proteins. An increase in the amount of organic nitrogen is related to the synthesis of microbial cell; the opposite is an indication of microbial decline. Therefore, the measurement of organic nitrogen in a biological system would indicate how the system is functioning.

As shown in Fig. 10, the ammonium nitrogen decreased with time until it was completely utilized after 18 h (end of exponential growth phase). During the yeast exponential growth phase, some of the ammonium nitrogen as well as the organic nitrogen in the cheese whey were converted into cellular organic nitrogen and as a result the organic nitrogen increased from 1320 to 1490 mg/L. During the yeast death phase, the cells died, the cellular organic nitrogen was, thus, broken down and released to the media in the form of ammonium nitrogen. At the end of the experiment, the ammonium nitrogen concentration was 200 mg/L, which is higher than the initial concentration due to the conversion of cellular organic nitrogen to ammonium nitrogen. At the end of the fermentation process (after 48 h), the total nitrogen remained unchanged, although the organic nitrogen was reduced by 2.27% (30 mg/L) of its original value and the ammonium nitrogen was increased by 17.64% (30 mg/L) of the original value due to the conversion of nitrogen from its organic form to the inorganic form. Samples taken from the exhaust gas showed no loss of ammonia from the reactor. This was due to the low pH (4.4).

4.10. Solids

The concentration of the total solids in the raw whey was 66830 mg/L (56730 mg/L volatile solids (VS) and 10100 mg/L ash). The percentage of VS in raw whey was 84.88% whereas the percentage of ash was 15.12%. Most of the VS (50,000 mg/L or 88%) was lactose. The total solids decreased with time (due to decrease in VS) whereas the ash essentially remained unchanged as shown in Fig. 11. These results indicate that the fermentation process achieved a final reduction of

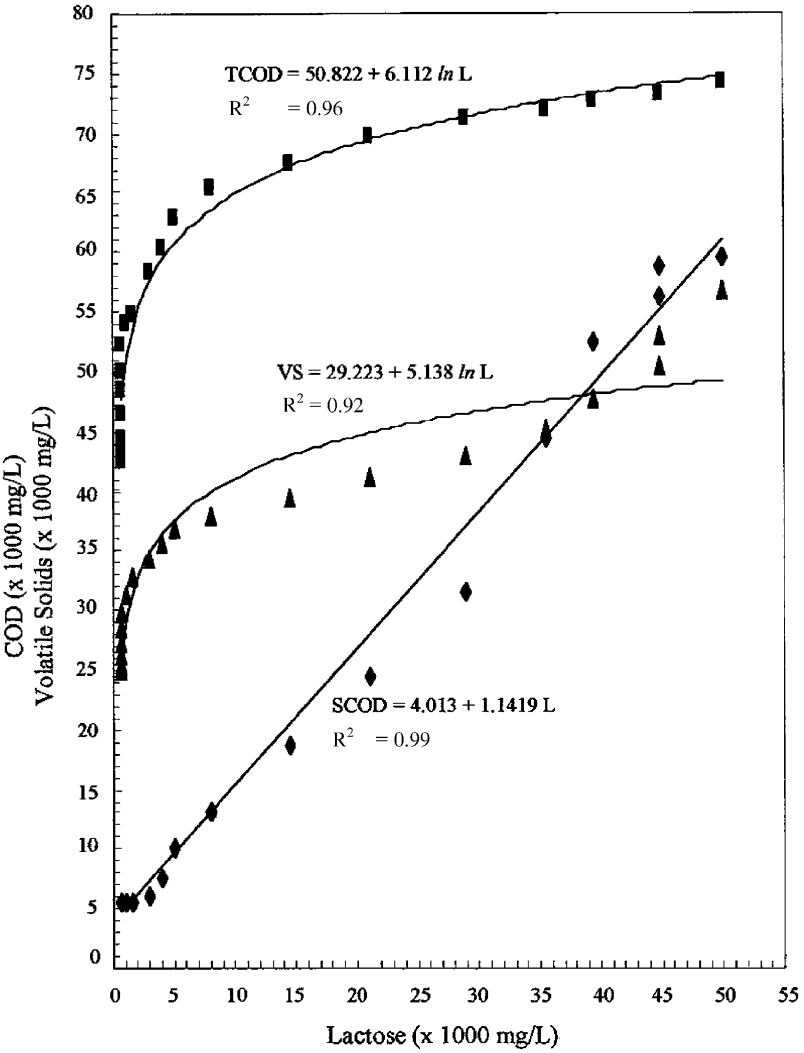

Fig. 9. The relationship between the lactose concentration and the concentrations of volatile solids, TCOD, and SCOD in the medium.

47.48% in total solids, and 55.91% in VS. The magnitude of reductions in the total and VS were essentially the same (31730 mg/L). The reduction in total solids corresponded to the reduction in the total COD which represented the conversion of lactose to energy, CO_2 and H_2O. The remaining solids in the medium represent the SCP (yeast biomass) and other useful products which could be recovered before final disposal of spent medium.

VS is a measure of the organic component of the solids in cheese whey. The lactose (L) in cheese whey is about 88% of the volatile solids. A plot of the volatile solids versus the lactose concentration is shown in Fig. 9. The reductions in lactose did not correspond to the reduction in volatile solids, as some of the lactose (non-cellular organic material) was converted to cells (cellular organic material). The relationship between VS and lactose (L) can, therefore, be described by the following exponential equation ($R^2 = 0.92$):

$$VS = 29.22 + 5.1318 \ln L. \quad (6)$$

4.11. Effluent quality

Although 99% of the lactose was utilized in this study by the yeast, only reductions of 42.98% and 47.48% in the TCOD and solids, respectively, were achieved and no change in the total nitrogen content was observed. The 90.63% reduction in the SCOD shows that the soluble organic material (lactose) was converted to insoluble material (yeast cells). Reduction in SCOD of about 85–95% of the initial values was reported for

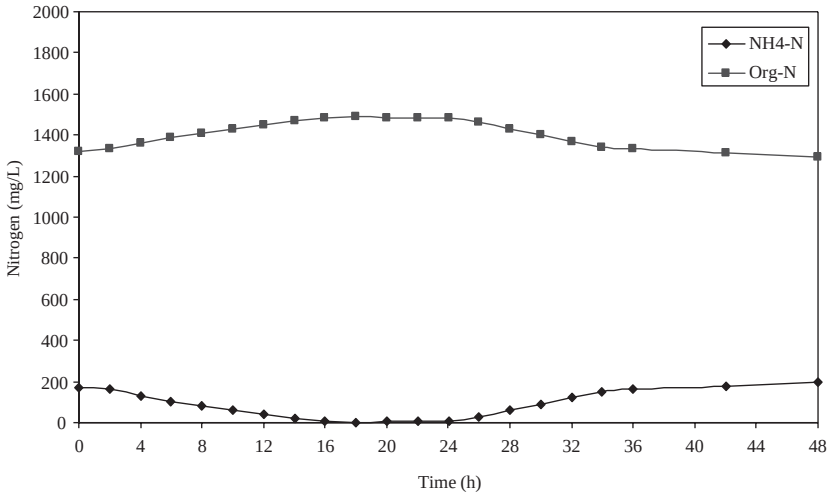

Fig. 10. The organic nitrogen and ammonium concentrations measured during the batch culture operation.

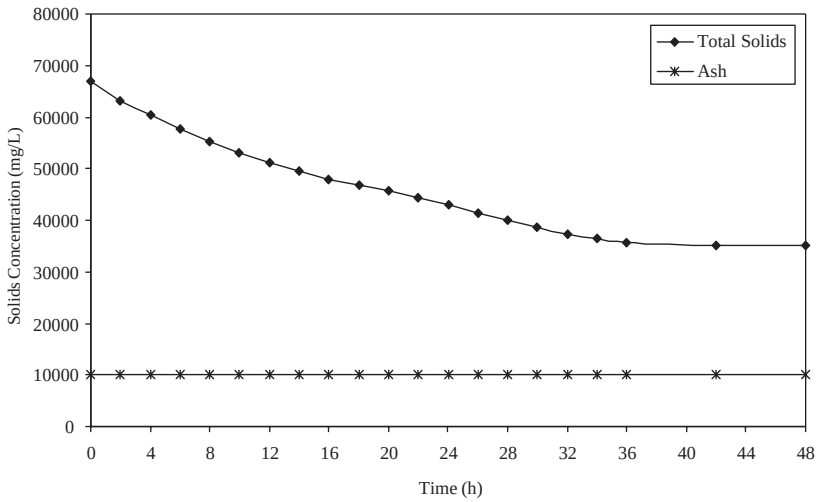

Fig. 11. The total solids and ash concentrations measured during the batch culture operation.

commercial plants [27]. Greater than 80% reduction in SCOD was observed in most laboratory and pilot operations ([11,12,22] Moresi et al., 1989; [28]). Therefore, a biomass recovery for use as animal feed supplements and in human food is necessary for further pollution potential reduction before the ultimate disposal of the spent medium. Ultrafiltration [29] and microfiltration [2,30] were used to separate the yeast from the fermentation media and resulted in up to 92% reduction of the initial COD of the whey. In this study, the ultrafiltration of the spent culture resulted in an effluent having a COD value of 1190 mg/L (TCOD and SCOD reductions of 98.4% and 98.0%, respectively). However, a waste stream having a COD value of 1190 mg/L can only be disposed into the stream. A final aerobic polishing of the effluent is likely to be required if the ultimate disposal point is a water body.

The potential of lactose bioconversion to produce useful products such as SCP while reducing the COD of the whey stream by at least 50% has been demonstrated. Further reduction (up to 98% of the original value) is achieved through the recovery of the biomass. The strategy for the industrial development of this fermentation process will depend on whether the objective is the production of cell mass or the reduction of COD. Table 3 shows the cell number, the concentration of lactose, TCOD and SCOD, COD removal efficiencies and the biomass yield at the end of the exponential growth phase (16 h), at the end of stationary phase (26 h), when constant minimum lactose and SCOD

Table 3
Lactose and COD removal efficiencies and cell yield

Parameter	End of phase			
	16 h	26 h	28 h	48 h
Cell number (10^6 cells/mL)	840	760	660	350
Lactose				
Concentration (mg/L)	8000	1000	600	600
Reduction (mg/L)	42,000	49,000	49,400	49,400
Removal efficiencies (%)	84	98	99	99
Yield (g cell/g lactose utilized)	0.66	0.50	0.44	0.23
TCOD				
Concentration (mg/L)	65,280	53,920	52,160	4232
Reduction (mg/L)	8940	20,300	22,060	31,900
Removal efficiencies (%)	12	27	30	43
Yield (g cell/g TCOD removed)	3.10	1.20	0.98	0.36
SCOD				
Concentration (mg/L)	13,240	5600	5590	5590
Reduction (mg/L)	46,400	54,040	54,050	54,050
Removal efficiencies (%)	78	91	91	91
Yield (g cell/g SCOD removed)	0.59	0.45	0.40	0.21

Note: 16 h = end of exponential growth phase; 26 h = end of stationary growth phase; 28 h = constant minimum concentrations of lactose and soluble COD; 48 h = end of experiment. Initial lactose = 50,000 mg/L; initial TCOD = 74,220 mg/L; initial SCOD = 59,640 mg/L.

concentration (28 h) were observed and at the end of the experiment (48 h). If the ultimate objective is the production of cell biomass, the fermentation should be terminated after 16 h (end of the exponential growth phase), when 88% of lactose will be utilized and a maximum cell number of 840×10^6 cells/mL (0.66 g cell/g lactose) will be achieved. However, this will result only in TCOD and SCOD removal efficiencies of 12% and 78%, respectively. Further fermentation of up to 28 h (beginning of the cell death phase) will reduce the microbial population by 21% and achieve 99% lactose utilization and addition reductions in the TCOD and SCOD of 18% and 13%, respectively. If the fermentation process continues to 48 h, a further reduction of 42% of the cell SCP is accompanied by 13% increase in the COD removal efficiency resulting in a total COD reduction of 43%. Nonetheless, the desire for biomass recovery, market potential and cost of ultrafiltration process versus the cost of extended fermentation will determine which strategy would be the most economical for a given operation.

5. Conclusions

Fermentation time of 18–24 h can be used for higher conversion rate of lactose into microbial cells. With longer fermentation time, the microbial population will be reduced and the cellular organic materials in the fermented whey will be converted to NH_4, CO_2 and H_2O. At constant ambient temperature of 21°C and air flow rate of 3 VVM, the dissolved oxygen and the temperature of the medium displayed four distinct stages corresponding to the lag, exponential, stationary and death phases with the lowest dissolved oxygen concentration (2.49 mg/L) and the highest temperature (31.6°C) being observed during the stationary phase. The lactose concentration in the fermenter displayed three distinct stages that corresponding to the lag, exponential and stationary growth phases of the yeast. Based on the results, a cooling system was not found necessary for this type of fermenter. However, for an optimum fermentation, a heating system may be required for the early stage of growth. A 99% lactose reduction was achieved after 28 h. The reduction in soluble COD was 90.63% whereas reduction in total COD was 42.98%. Only an estimated 41% of the reduction in the soluble COD was used for growth of new cells whereas the remaining 59% was converted to energy, CO_2 and H_2O. The total nitrogen concentration in the whey remained unchanged. No NH_3 was observed in the exhaust gas. Since the organic nitrogen in the cheese whey contains amino acids, polypeptides and proteins, all of which are the potential parts of cellular protoplasm, the measurement of organic nitrogen does not indicate the magnitude or the efficiency of converting the non-microbial organic nitrogen to microbial protoplasm. The ash content of cheese whey remained unchanged whereas a reduction of 55.91% in the volatile solids was observed. Recovery of the yeast biomass using ultrafiltration can reduce the COD by 98% making the effluent suitable for disposal in the sewer system. Further aerobic polishing may be required if the ultimate disposal is in water streams.

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