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# Pollution Potential Reduction of Cheese Whey Through Yeast Fermentation

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## ABSTRACT

Batch and continuous pilot-scale aerobic fermenters of 4.8 L operating volume were designed and constructed from plexiglass materials. The fermenters were used to study the kinetics of cheese whey fermentation using the yeast *K. fragillis* for pollution potential reduction and single cell protein production. Four retention times (6, 12, 18, and 24 h) were used in this study. The fermentation process was successful in reducing the total chemical oxygen deman (COD) by 42%, the soluble COD by 65%, the total solids by 53%, and the ammonium nitrogen by 90%. There were also gains in the suspended solids and the organic nitrogen of 60 and 17%, respectively. The reductions in the COD, total solids, and ammonium nitrogen, and the gains in the suspended solids and organic nitrogen were affected by the hydraulic retention time. More soluble material was converted to insoluble microbial cells at the 12-h hydraulic retention time, whereas greater pollution potential reduction was achieved at the 24-h hydraulic retention time for both batch and continuous operations.

**Index Entries:** Cheese whey; batch; continuous; aerobic; COD; solids; nitrogen; soluble; insoluble.

## INTRODUCTION

Cheese whey is a byproduct of the cheese industry that contains approximately 5% lactose, 1% nitrogenous compounds, 0.8% minerals, and

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small amounts of vitamins. It has been estimated that approximately 16 and 1.5 billion kilograms of whey are produced annually in the US and Canada, respectively (*1*). Only a little over one-half of the whey produced is utilized, most of which is in the form of dried whey powder, an industry that barely recovers its production cost because of poor markets and high energy expenditure. Other major uses include feeding liquid whey to hogs. Because of high trucking costs, liquid whey can only be used for hog feeding within an economic radius of 30–40 km. In many cases, however, the hog population is small in relation to the number of cheese plants and feeding liquid whey to hogs is becoming uneconomical (*2,3*).

Disposal of whey as a waste product has been undertaken in both the US and Canada. Of the 1.5 billion kg of liquid whey produced in Canada in 1985, 49% was used for whey powder, 8% was fed to hogs, 17% was dumped into sewers, and 26% was disposed of on land. In the US, of the 16 billion kg produced in the same year, 58% was utilized and 42% was disposed of as a waste product (*4*). The biochemical oxygen demand (BOD) of whey is between 40,000–60,000 ppm. Because of its high BOD value, whey may disrupt the biological process of sewage disposal plants (*1*). Long-term land disposal of whey may also cause environmental pollution problems, as reported by Ghaly and Singh (*1*) and Ghaly et al. (*4*).

Aerobic treatment of cheese whey can be used to reduce its pollution potential as well as to produce single cell protein (SCP). Since the major constituent of the whey is lactose, the selected organism must be able to readily metabolize lactose. Some species of yeast and mold have been known to grow easily on cheese whey. These include: *Kluyveromyces fragilis, Trichosporon cutaneum, Morchella crassipes, Morchella esculenta,* and *Morchella hortensis* (*3,5,6*).

Several researchers have used *K. fragilis* on cheese whey and on crude lactose to product SCP in batch fermenters (*3,7–10*). Limited work was also done by Vananuvat and Kinsella (*8*) on fermentation of crude lactose and by Singh and Ghaly (*3*) on fermentation of cheese whey in continuous cultures. Some of these studies suggested that during continuous fermentation the growth rate of yeast was considerably below the possible maximum predicted by batch fermentation. The minor nutritional deficiencies scarcely detectable in batch microbial culture become fully apparent during intensified biosynthesis of cells during continuous operation, as reported by Fancel and Burger (*11*). However, it has been observed by Vananuvat and Kinsella (*8*) that under optimum operating conditions, a 60% reduction in COD could be achieved.

## OBJECTIVES

The aim of this study was to design and operate batch and continuous pilot-scale aerobic fermenters to study the kinetics of cheese whey fermentation using *K. fragilis* for pollution potential reduction and single cell

protein production. The specific objectives were: to study the effect of the hydraulic retention time on the changes in the concentrations of the COD, total solids, suspended solids, organic nitrogen, and ammonium nitrogen; and to assess the conversion efficiency of soluble organic materials to insoluble microbial cells as measured by the changes in soluble COD, volatile suspended solids, and organic nitrogen.

## EXPERIMENTAL APPARATUS

The experimental set up is schematically presented in Fig. 1. Two fermenters were constructed of plexiglass material and used in this study; one for batch operation and one for continuous operation. The working volume of each fermenter was 4.8 L. The dimensions of the fermenter are shown in Fig. 2.

The fermenters were designed to be completely mixed and hence each was equiped with two turbine rotors and four baffles. The turbine rotor in each fermenter was driven by a variable speed electric motor (Dayton permanent magnet DC motor with Dayton SCR Control Model 42142). The motor shaft was attached to the turbine shaft through a chuck assembly.

Compressed air was supplied through a flowmeter (Cole Parmer FM082-03G) and then passed through a microfilter (Cole Parmer 3-2915-16). This special filter was used to sterilize the air. The filtered air was then introduced from the bottom of the reactor through a ceramic type diffuser (Fisher Gas Diffusing Stone 11-139 B). Separate feeding and overflow collection tanks were connected to the fermenters. The feeding was designed to be completely mixed and hence the feeding tank was equipped with turbine rotors and four baffles. A peristaltic pump (Harvard Model 1203) was used to provide a constant flowrate of whey from the feeding tank to the fermenter. Tugon tubing (9.5 mm *O.D.* × 6.4 mm *I.D.*) was used for the feed line.

## MEASURING INSTRUMENTS

Dissolved oxygen was monitored by a polarographic electrode (Beckman 39553 $O_2$ Sensor) connected to a dissolved oxygen meter (Beckman Field Lab Oxygen Analyzer Model 1008). The signal from the dissolved oxygen meter was recorded continuously on a data logger (Fluke Model 2240A). The pH was measured by a single electrode pH probe (Sargent-Welch S-30072-15) connected to a pH meter (Sargent-Welch Model LS-S-30005). The signal from the pH meter was also continuously recorded on the data logger. A copper-constantan thermocouple inserted in each fermenter and connected to the data logger was used to measure the whey temperature.

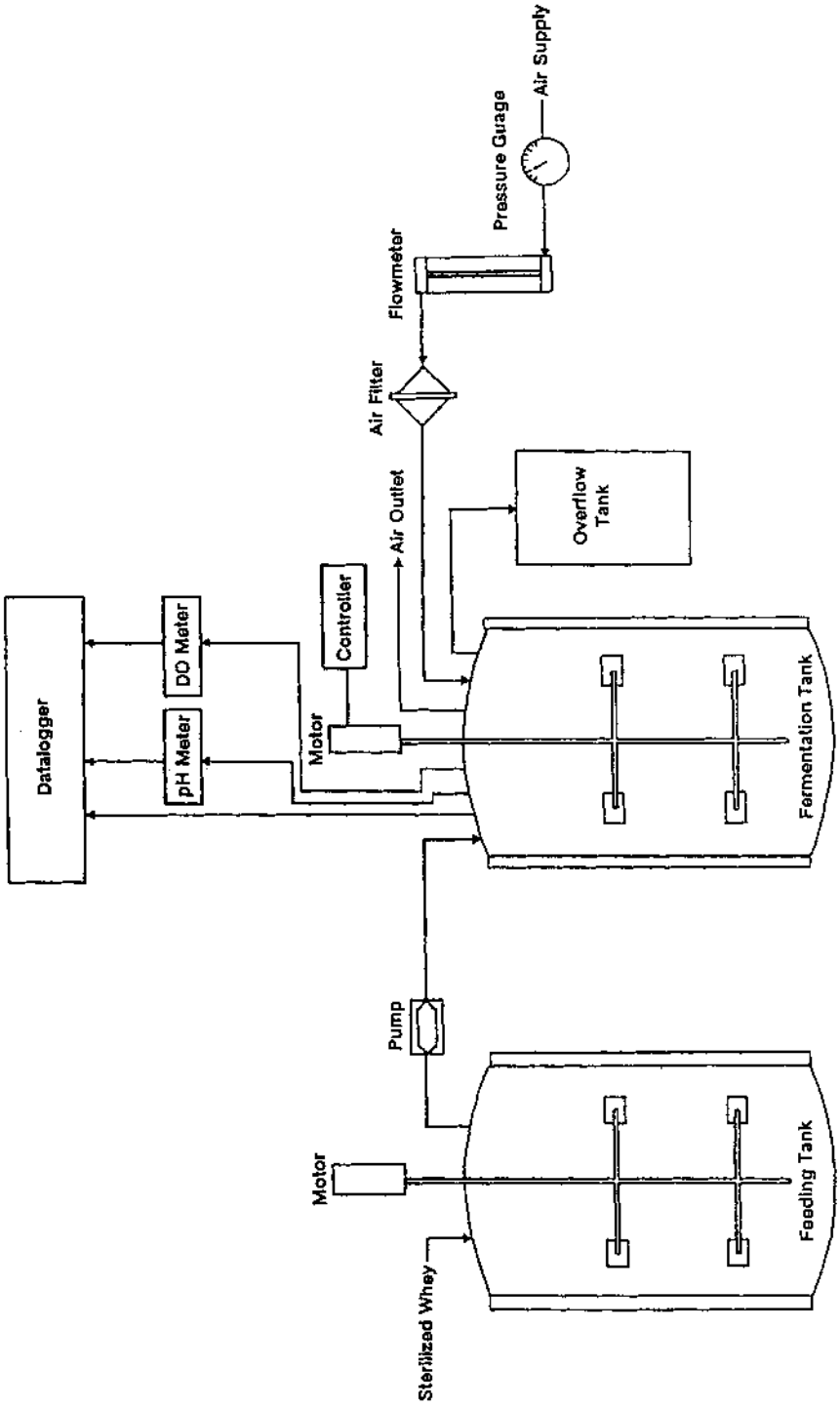


Fig. 1. The experimental setup.

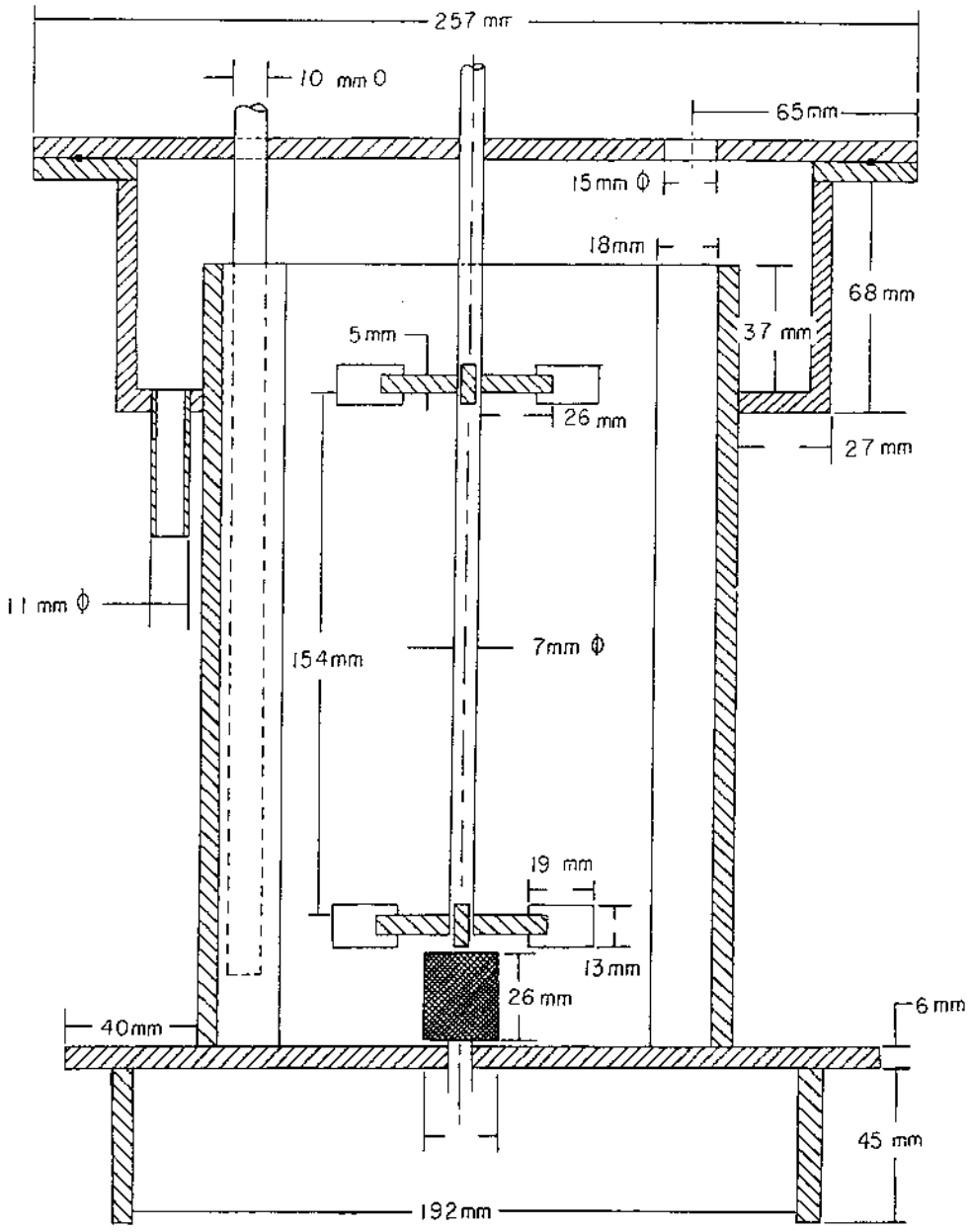


Fig. 2. Experimental apparatus.

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

| Characteristics | Measures value | Unit |
|---|---|---|
| Total solids | 56 800 | mg/L |
| Ash | 8 900 | mg/L |
| Volatile solids | 47 900 | mg/L |
| Percent volatile solids | 84.33 | % |
| Suspended solids | 21 820 | mg/L |
| Ash | 180 | mg/L |
| Volatile solids | 21 646 | mg/L |
| Percent volatile solids | 99.18 | % |
| Total Kjeldhal nitrogen | 1 500 | mg/L |
| Ammonium nitrogen | 270 | mg/L |
| Organic nitrogen | 1 230 | mg/L |
| Percent organic nitrogen | 82.00 | % |
| Nitrite and nitrate nitrogen | 30 | mg/L |
| Total chemical oxygen demand | 75 800 | mg/L |
| Soluble chemical oxygen demand | 58 000 | mg/L |
| Insoluble chemical oxygen demand | 17 800 | mg/L |
| Percent soluble chemical oxygen demand | 76.52 | % |
| pH | 5 | |

## EXPERIMENTAL PROCEDURE

### Cheese Whey Collection and Preparation

Cheddar cheese whey was acquired from Farmer's Dairy, Truro, Nova Scotia. The cheese whey was collected in 20-L screwtop carboys and transported to the Waste Management Laboratory at the Technical University of Nova Scotia in Halifax. The cheese whey was placed in 200-L tank and mixed thoroughly. It was then placed in 20-L plastic bags and stored in a freezer at −18°C until it was needed for feed preparation. Some characteristics of the cheese whey are presented in Table 1.

Prior to being fed into the fermenters, the cheese whey was removed from the freezer and allowed to thaw at room temperature for 24 h. It was then sterilized in an autoclave at 121°C and 105 KPa for 15 min. The sterilized cheese whey was placed in the feeding tank.

### Stock Culture and Inoculum Preparation

Freeze dried pellets of *K. fragilis* (Y-1109) was obtained from Northern Regional Research Center, Peoria, IL. The pellets were dissolved in 5 mL sterilized lactose broth solution (1.3% concentration). A loopful of this solution was streaked on tube slants of plate count agar. The tubes were

then placed in a controlled environment incubator at 20 ± 1°C. After visual growth appeared on these slants (after 72 h), the tubes were removed from the incubator and stored in a refrigerator at 4°C until required for inoculum preparation.

Several flasks, each with 200 mL lactose broth medium, were sterilized in an autoclave at 121°C and 105 KPa for 15 min. After the medium cooled down to room temperature, a loopful each of *K. fragilis* culture was taken from the culture slant in one of the tubes and transferred aseptically into each of the flasks containing lactose broth medium. The inoculated flasks were mounted on reciprocating shaker (Controlled Environment Incubator Model M52 NB) until a vigorous growth of *K. fragilis* was observed in the medium (after 24 h). The flasks were then stored in a refrigerator at 4°C until required for the inoculation of fermentation process.

### Fermenter Operation

The batch and continuous fermenters were chemically sterilized using 2% potassium meta-bisulfite solution, and then washed with hot water (90°C) several times before starting the experiment in order to remove any chemical traces. Each reactor was filled, up to two-thirds of its working volume, with sterilized cheese whey and was then inoculated by separate flasks of inoculum. The air flow and mixing turbine were then started. Dissolved oxygen, pH, and temperature were constantly monitored in each fermenter.

In the continuous fermenter, the culture was allowed to grow in batch mode until visual vigorous growth could be observed (after 24 h). Continuous feed then started and slowly increased until the desired hydraulic retention time was obtained. The system was allowed to operate for 5 hydraulic retention times before sampling started. It was judged from preliminary experiments that 5 hydraulic retention times would be sufficient for the system to reach the steady state condition.

## SAMPLING AND ANALYSIS

Sampling of the influent and effluent streams for continuous culture operation was done on a hydraulic retention time basis, regardless of the length of the hydraulic retention time being used. Sampling was not commenced until the reactor had been operated successfully for 5 hydraulic retention times in order to ensure that the steady state had been reached. Sampling was then continued until the end of the tenth hydraulic retention time so that 5 samples were collected during the steady state condition.

Sampling of materials in batch culture operation was done before the start of each experimental run (influent sample) and immediately after the termination of the experimental run (effluent sample). Five samples were also collected from the feed and spent materials of the batch operation.

The chemical oxygen demand, total solids, suspended solids, total Kjeldehal nitrogen, and ammonium nitrogen analyses were performed on the samples taken from the influent and effluent streams of batch and continuous culture operations. These were carried out according to the procedures described in the Standard Methods (*12*).

## RESULTS AND DISCUSSION

No dilution or concentration was made to the cheese whey used in the study. The effects of hydraulic retention time on the effluent quality (as measured by the chemical oxygen demand, the total and suspended solids, the organic nitrogen, and the ammonium nitrogen) were investigated under batch and continuous operations. Four hydraulic retention times (6, 12, 18, and 24 h) were studied.

The total and soluble chemical oxygen demand and the total and volatile solids data obtained during the continuous culture operation for all retention times were plotted in chronological order, as shown in Figs. 3 and 4. These results indicated that the steady state condition was achieved for all experimental runs of the continuous culture operation.

### Chemical Oxygen Demand

The values of total and soluble chemical oxygen demand (COD) of the effluent from batch and continuous operations are shown in Table 2. Each value is the average of 10 determinations. The reductions in the total and the soluble chemical oxygen demand for batch and continuous operations are also shown in Table 2. These results show that increasing the hydraulic retention time increased the reductions in both the total COD and the soluble COD. For example, increasing the retention time from 6 to 24 h increased the reductions in total COD from 2.90 to 42.88% under batch operation and from 3.83 to 42.08% under continuous operation, and increased the reduction in soluble COD from 3.97 to 65.17% under batch operation and from 6.03 to 64.41% under continuous operation. The reductions in the soluble COD are comparable to those reported by Vananuvat and Kinsella (*8*).

The chemical oxygen demand can be used as an indirect measurement of the change in microbial mass in cheese whey fermentation. According to Loehr (*13*), under aerobic conditions, microorganisms utilize soluble organic materials to provide energy for growth and supply nutrients for synthesis of new microbial protoplasm, as described by the following equations.

energy release (respiration) reaction

$$\text{organic matter} + O_2 \rightleftharpoons CO_2 + H_2O + \text{Energy} \qquad (1)$$

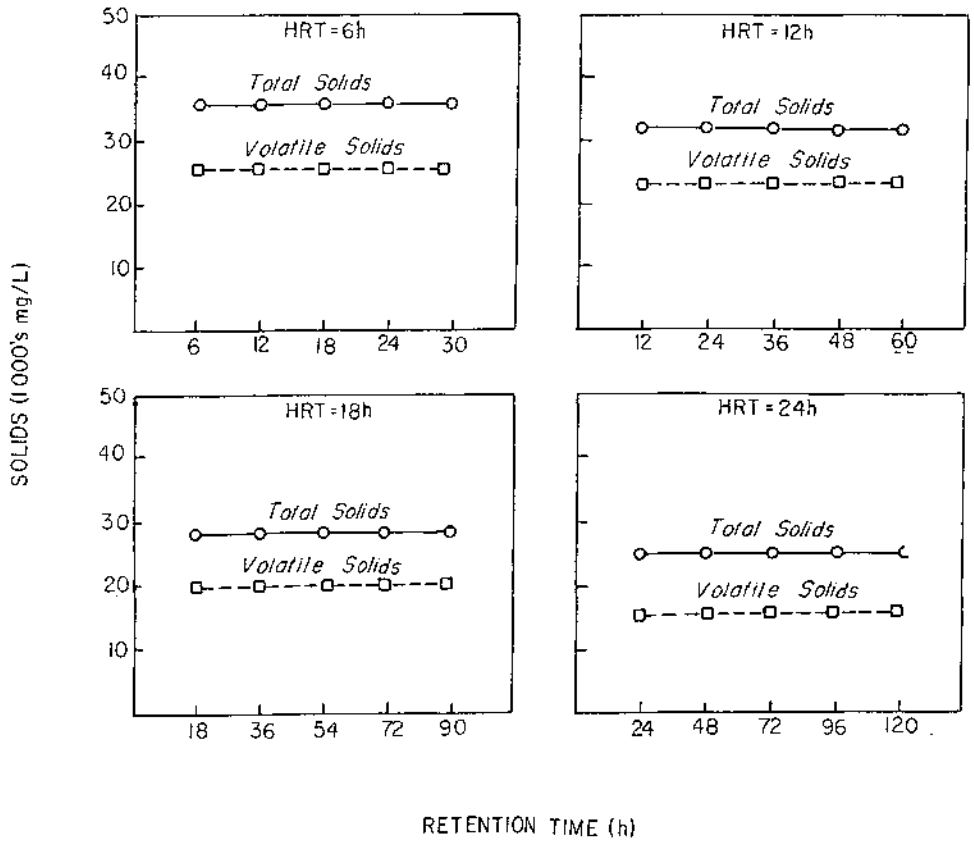


Fig. 3. The total and soluble chemical oxygen demand of the continuous culture effluent.

Synthesis (growth) reaction

$$\text{organic matter} + NH_4 \rightleftharpoons \text{microbial cells} + H_2O + H^+ \quad (2)$$

The difference between the initial and final values of the soluble COD is owing to the consumption of the biodegradable dissolved organic matter by microorganisms. This is called the total biological oxygen demand ($T_bOD$). The procedure was developed by Hiser and Busch (*14*), and has been used successfully to measure the $T_bOD$ in industrial waste water (*15*), in municipal sewage (*15*), and in animal manure (*16*). According to the $T_bOD$ test, a portion of the soluble organic matter is oxidized by microorganisms to provide energy for growth with the production of $CO_2$ and $H_2O$ according to Eq. (1). The other portion of the soluble organic matter is utilized by microorganisms for the synthesis of new microbial protoplasm according to Eq. (2). A typical net reaction of the aerobic decomposition of organic matter is as follows.

$$\text{organic matter} + O_2 + \text{aerobes} \rightleftharpoons \text{more aerobes} + CO_2 + H_2O \quad (3)$$

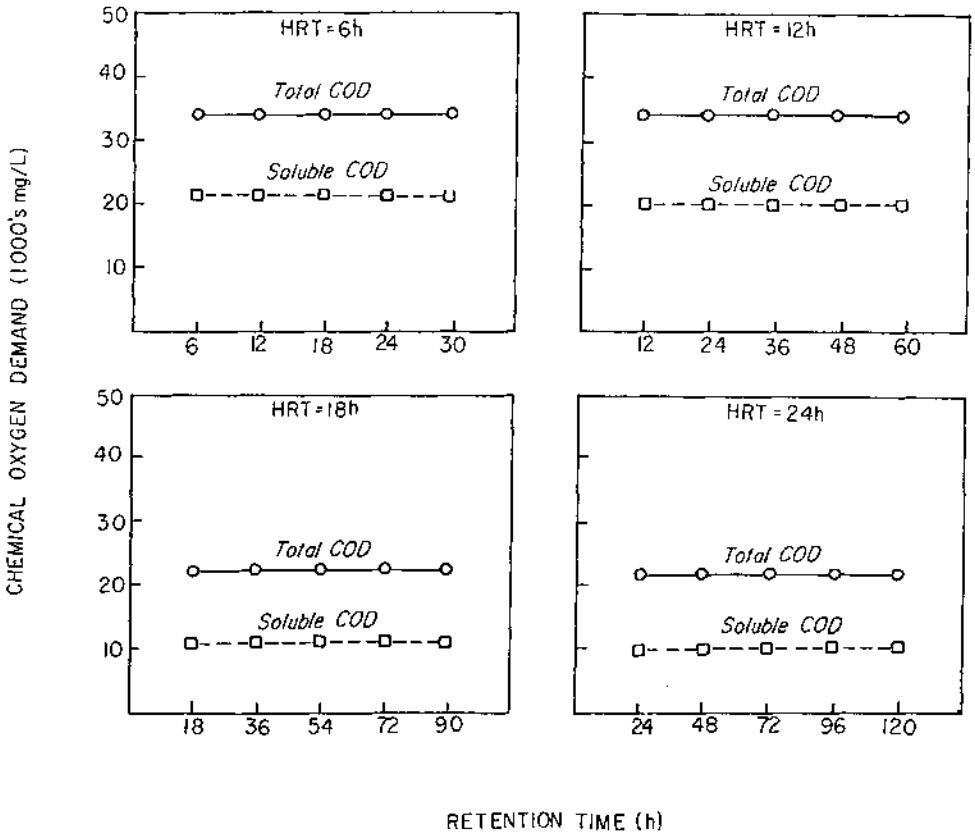


Fig. 4. The total and volatile solids of the continuous culture effluent.

Table 2
The Effluent Chemical Oxygen Demand Concentrations at Various Hydraulic Retention Times[a]

| Operation | Hydraulic retention time, h | Total COD, mg/L | Total COD reduction mg/L | Total COD reduction % | Soluble COD, mg/L | Soluble COD reduction mg/L | Soluble COD reduction % |
|---|---|---|---|---|---|---|---|
| Batch | 6 | 73 600 | 2 200 | 2.90 | 55 700 | 2 300 | 3.97 |
| | 12 | 67 000 | 8 800 | 11.61 | 38 600 | 19 400 | 33.45 |
| | 18 | 49 400 | 26 400 | 34.82 | 25 200 | 32 800 | 56.55 |
| | 24 | 43 300 | 32 500 | 42.88 | 20 200 | 37 800 | 65.17 |
| Continuous | 6 | 72 900 | 2 900 | 3.83 | 54 500 | 3 500 | 6.03 |
| | 12 | 67 700 | 8 100 | 10.69 | 40 100 | 17 900 | 30.86 |
| | 18 | 54 000 | 21 800 | 28.76 | 30 900 | 27 100 | 46.72 |
| | 24 | 43 900 | 31 900 | 42.08 | 21 800 | 36 200 | 64.41 |

[a]The values are the average of 10 determinations; Raw whey total COD = 75 800 mg/L; Raw whey soluble COD = 58 000 mg/L.

Table 3
Portions of the Soluble COD Converted to Carbon Dioxide and Microbial Cells at Various Hydraulic Retention Times

| Operation | Hydraulic retention time, h | Total reduction in soluble COD, mg/L | Converted to carbon dioxide[a] | | Converted to microbial cells[b] | |
|---|---|---|---|---|---|---|
| | | | mg/L | % | mg/L | % |
| Batch | 6 | 2 300 | 2 200 | 95.65 | 100 | 4.35 |
| | 12 | 19 400 | 8 000 | 45.24 | 11 400 | 58.76 |
| | 18 | 32 800 | 26 400 | 80.49 | 6 400 | 19.51 |
| | 24 | 37 800 | 32 500 | 85.98 | 5 300 | 14.02 |
| Continuous | 6 | 3 500 | 2 900 | 82.86 | 600 | 17.14 |
| | 12 | 17 900 | 8 100 | 45.25 | 9 800 | 54.75 |
| | 18 | 27 100 | 21 800 | 80.44 | 5 300 | 19.55 |
| | 24 | 36 200 | 31 900 | 88.12 | 4 300 | 11.88 |

[a]Microbial respiration
[b]Microbial synthesis.

It is clear from the COD results that the reductions in the soluble COD were higher than the reductions in the total COD at all hydraulic retention times for both batch and continuous operations. This is owing to the conversion of soluble organic material to insoluble microbial cells. The difference between the initial and final values of the total COD is owing to the oxidation of organic matter to $CO_2$ and $H_2O$, whereas the difference between the reduction in the total COD and soluble COD is owing to the conversion of organic matter to microbial cells.

The portion of soluble COD converted to microbial cells and that converted to $CO_2$ and $H_2O$ are shown in Table 3 and Fig. 5. The percentage of of soluble material converted to microbial cells was affected by the hydraulic retention time. Higher conversion percentages were obtained at the 12-h retention time for both batch and continuous operation. At this hydraulic retention time, approximately 11 400 mg/L (58.76%) and 9800 mg/L (54.75%) of the soluble material were converted to microbial cells for batch and continuous operations, respectively. The results thus suggested that a hydraulic retention time of less than 12 h should not be used. On the other hand, microbial population may also be reduced with longer retention times because most of the organic materials will be converted to $CO_2$ and $H_2O$ through the respiration process.

## Total Solids

The various solids components of the raw cheese whey are presented in Fig. 6. The concentration of the total, fixed, and volatile solids of the effluent from batch and continuous operations are presented in Table 4. Each value is the average of 10 determinations. The volatile solids was

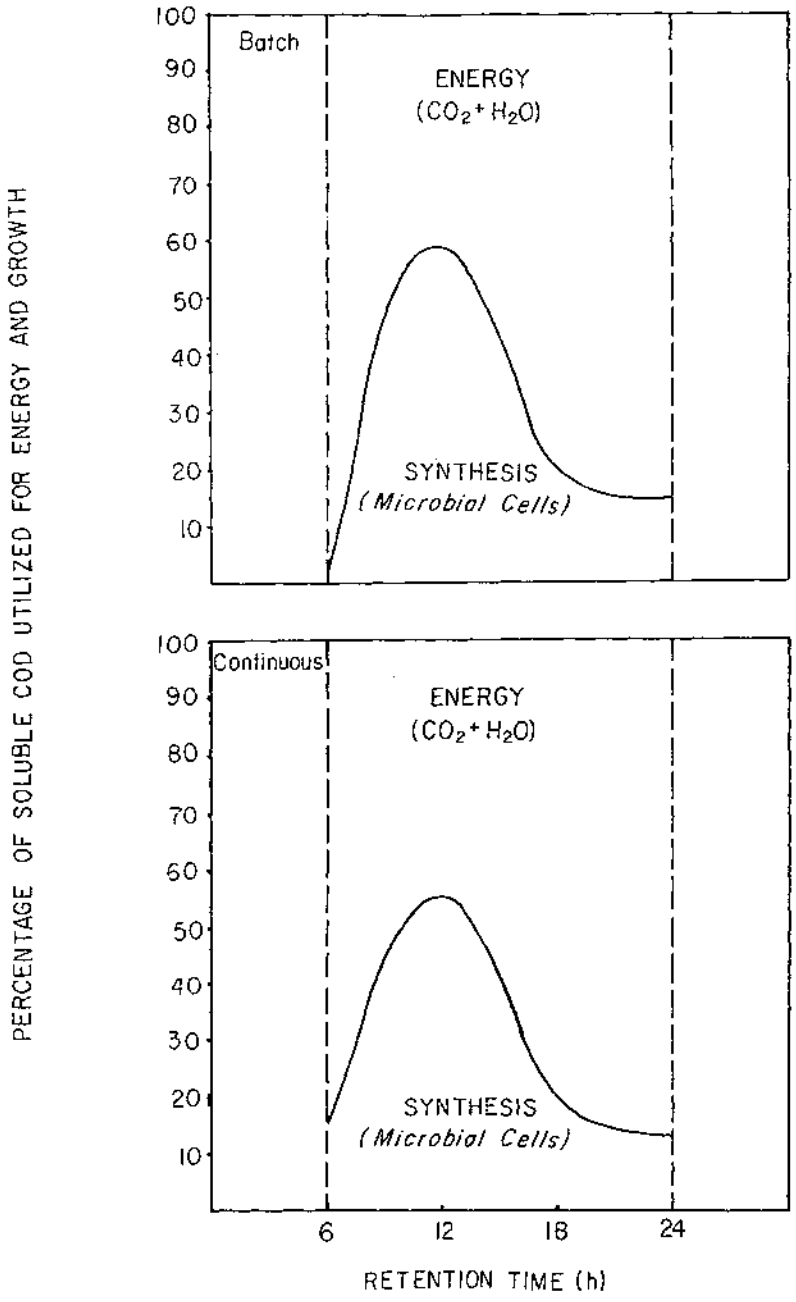


Fig. 5. Percentage of soluble chemical oxygen demand utilized for energy and microbial synthesis.

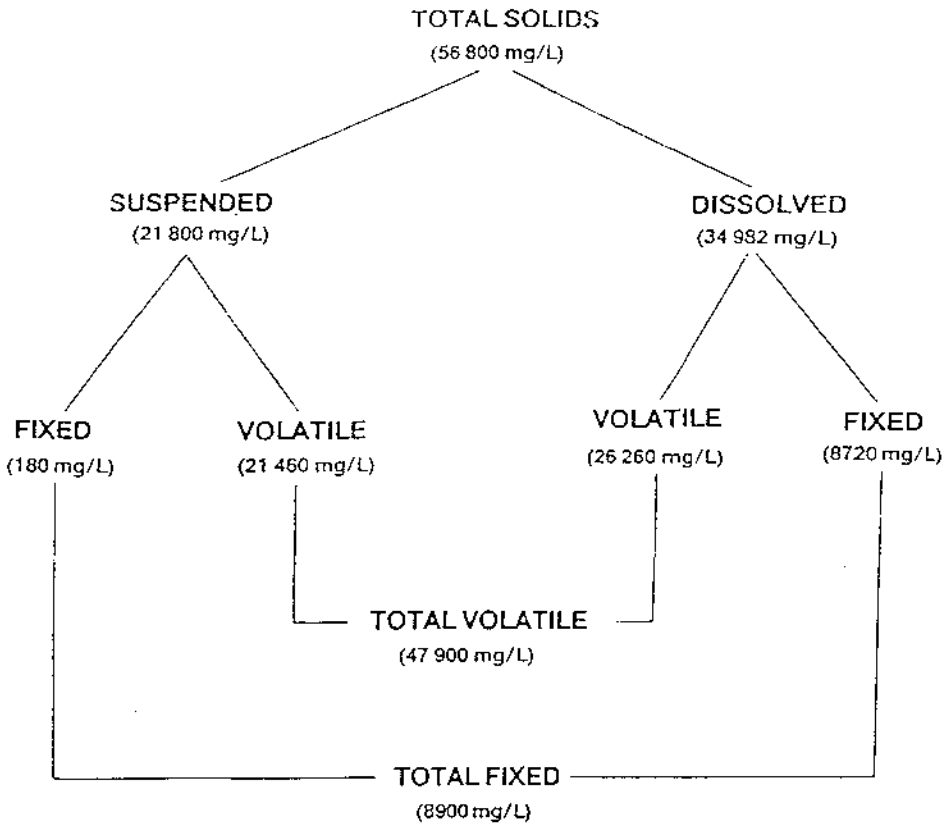


Fig. 6. The various components of solids in the cheese whey.

Table 4
The Effluent Total Solids Concentrations at Various Hydraulic Retention Times[a]

| Operation | Hydraulic retention time, h | Total, mg/L | Fixed, mg/L | Volatile | |
|---|---|---|---|---|---|
| | | | | mg/L | %[b] |
| Batch | 6 | 40 400 | 8 800 | 31 600 | 78.22 |
| | 12 | 35 600 | 8 900 | 26 700 | 75.00 |
| | 18 | 27 800 | 9 000 | 18 800 | 67.63 |
| | 24 | 25 200 | 8 800 | 16 400 | 65.08 |
| Continuous | 6 | 35 800 | 8 900 | 26 900 | 75.14 |
| | 12 | 32 000 | 9 000 | 23 000 | 71.88 |
| | 18 | 29 500 | 8 900 | 20 600 | 69.83 |
| | 24 | 26 800 | 8 800 | 18 000 | 67.16 |

[a]The values are the average of 10 determinations.
[b]Percent of the raw whey total solids; Raw whey total solids = 56 800 mg/L; Raw whey ash = 8 900 mg/L; Raw whey volatile solids = 47 900 mg/L; Raw whey percent volatile solids = 84.33%.

Table 5
The Reductions in the Total and Volatile Solids at Various Hydraulic Retention Times

| Operation | Hydraulic retention time h | Total solids reduction[a] mg/L | % | Volatile solids reduction[b] mg/L | % |
|---|---|---|---|---|---|
| Batch | 6 | 16 400 | 28.87 | 16 300 | 34.03 |
| | 12 | 21 200 | 37.32 | 21 200 | 44.26 |
| | 18 | 29 000 | 52.08 | 29 300 | 61.17 |
| | 24 | 31 600 | 55.63 | 31 500 | 65.76 |
| Continuous | 6 | 21 000 | 36.97 | 21 000 | 43.84 |
| | 12 | 24 800 | 43.66 | 24 900 | 51.98 |
| | 18 | 27 300 | 48.06 | 27 300 | 56.99 |
| | 24 | 30 000 | 52.82 | 29 900 | 62.42 |

[a]Raw whey total solids=56 800 mg/L.
[b]Raw whey volatile solids=47 900 mg/L.

84.33% of the total solids in raw whey. However, the ratio of the volatile solids to the total solids decreased during the fermentation process reaching 65.08% for batch operation and 67.16% for continuous operation at the 24-h hydraulic retention time.

The fixed solids (ash) in the raw whey were approximately 8900 mg/L (15.67% of the total solids), which essentially remained the same for both the batch and continuous operations at all hydraulic retention times. On the other hand, the concentrations of the total solids (and thus the volatile solids) decreased during the fermentation process. The reductions were affected by the hydraulic retention time, the longer the hydraulic retention time the higher were the reductions in the total volatile solids for batch and continuous operations. Since there were no changes in the concentration of the fixed solids, the magnitudes of the reductions in the total and the volatile solids were essentially the same as shown in Table 5.

The percentage reductions in the volatile solids at various hydraulic retention times for both batch and continuous operations are presented in Fig. 7. The results show that at the 24-h hydraulic retention time, the fermentation process achieved volatile solids reductions of 65.76 and 62.42% for batch and continuous operations, respectively. These correspond to total solids reductions of 55.63 and 52.82% for batch and continuous operations, respectively.

## Suspended Solids

The concentration of the mixed liquor suspended solids (MLSS) and the volatile portion of the suspended solids (MLVSS) have traditionally been used to assess the effectiveness of aerobic treatment systems. In the

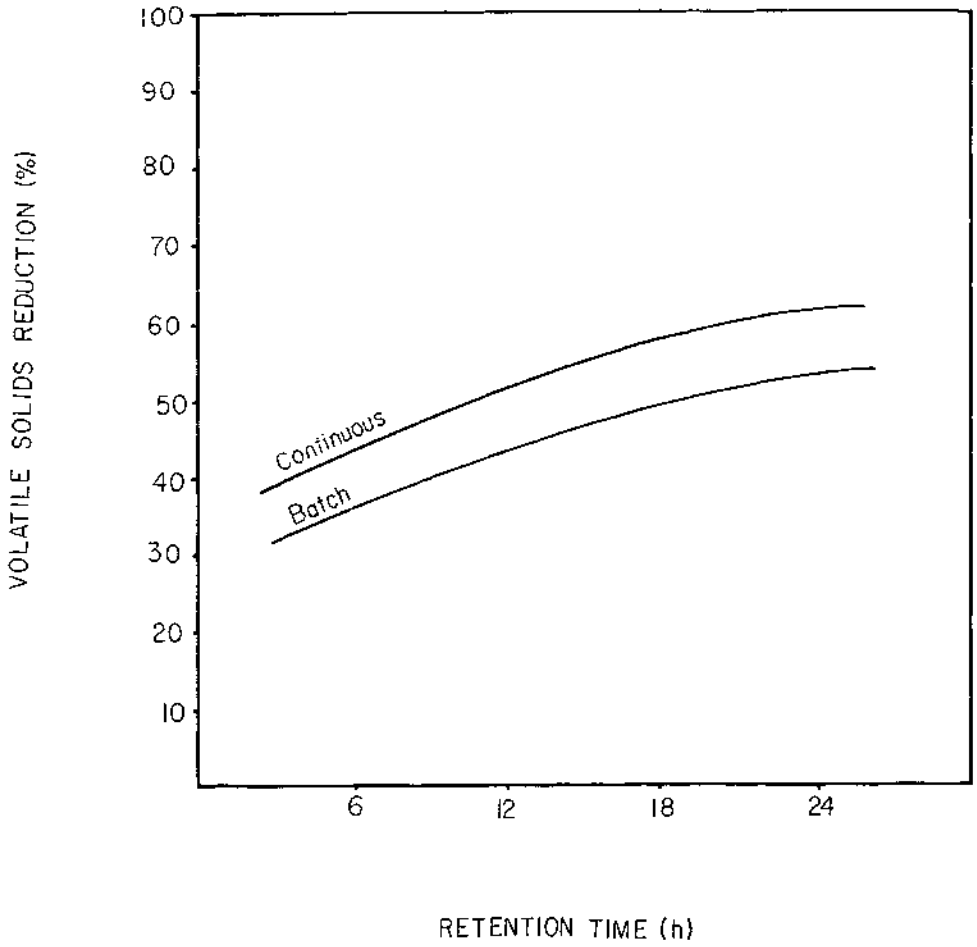


Fig. 7. The volatile solids reductions at various hydraulic retention times.

present study, suspended solids analysis was performed on both the influent and effluent streams to assess the conversion of soluble material to insoluble microbial mass.

The suspended solid data are presented in Table 6. Each value is the average of 10 determinations. The concentration of the suspended solids in the raw cheese whey was 21 820 mg/L (approx. 38.41% of the total solids). The fixed and volatile portions of the suspended solids were 180 mg/L and 21 640, respectively. The volatile portion of the suspended solids in the total suspended solids of the raw cheese whey was 99.18%.

There was a considerable gain in the suspended solids and their volatile and fixed portions for all hydraulic retention times, as shown in Table 7. The gains in the total suspended solids varied from 4.17 to 59.99% and from 17.00 to 55.00% for batch and continuous operations, respectively. Corresponding gains in the fixed solids portion (from 5.56 to 55.56% for batch operation and from 11.11 to 50.00% for continuous operation) and

Table 6
The Effluent Organic and Ammonium Nitrogen Concentrations at Various Hydraulic Retention Times[a]

| Operation | Hydraulic retention time, h | Organic nitrogen, mg/L | Gain in organic nitrogen | | Ammonium nitrogen mg/L | Reduction in ammonium nitrogen | |
|---|---|---|---|---|---|---|---|
| | | | mg/L | % | | mg/L | % |
| Batch | 6 | 1 360 | 130 | 10.60 | 90 | 180 | 66.66 |
| | 12 | 1 410 | 180 | 14.63 | 70 | 200 | 74.07 |
| | 18 | 1 400 | 170 | 13.82 | 10 | 260 | 96.30 |
| | 24 | 1 390 | 160 | 13.01 | 10 | 260 | 96.30 |
| Continuous | 6 | 1 350 | 120 | 9.76 | 60 | 210 | 77.78 |
| | 12 | 1 440 | 210 | 17.07 | 50 | 220 | 81.48 |
| | 18 | 1 430 | 200 | 16.26 | 20 | 250 | 92.59 |
| | 24 | 1 390 | 160 | 13.01 | 10 | 260 | 96.30 |

[a]The values are the average of 10 determinations; Raw whey organic nitrogen = 1230 mg/L; Raw whey ammonium nitrogen = 270 mg/L.

Table 7
The Gain in Suspended Solids at Various Hydraulic Retention Times

| Operation | Hydraulic retention time, h | Total[a] | | Fixed[b] | | Volatile[c] | |
|---|---|---|---|---|---|---|---|
| | | mg/L | % | mg/L | % | mg/L | % |
| Batch | 6 | 910 | 4.17 | 10 | 5.56 | 900 | 4.16 |
| | 12 | 13 090 | 59.99 | 100 | 55.56 | 12 990 | 60.03 |
| | 18 | 4 360 | 19.98 | 30 | 16.67 | 4.330 | 20.01 |
| | 24 | 3 270 | 14.99 | 20 | 11.11 | 3 250 | 15.02 |
| Continuous | 6 | 3 710 | 17.00 | 20 | 11.11 | 3 690 | 17.05 |
| | 12 | 12 000 | 55.00 | 90 | 50.00 | 11 910 | 55.04 |
| | 18 | 4 360 | 19.98 | 30 | 16.67 | 4 330 | 20.01 |
| | 24 | 2 610 | 11.96 | 10 | 5.56 | 2 600 | 12.01 |

[a]Raw whey total suspended solids = 21 820 mg/L.
[b]Raw whey fixed suspended solids = 180 mg/L.
[c]Raw whey volatile suspended solids = 21 640 mg/L.

the volatile solids portion (from 4.16 to 60.03% for batch operation and from 17.05 to 55.04% for continuous operation) were observed.

Higher gains in the suspended solids (volatile and fixed) were also observed at the 12-h hydraulic retention time for both the batch and continuous operations. This would indicate that the 6-h hydraulic retention time was not sufficient (short) for the optimal growth of the *K. fragilis,*

and the hydraulic retention times of 18 and 24 h were long. During the longer hydraulic retention times, the yeast population may have been decreased because of the limited food supply. According to Loehr (*13*), organisms can use previously accumulated internal food supplies for their metabolism in the absence of external food sources in a process called endogenous metabolism. Under nutrient limiting conditions, microorganisms die and lyse, releasing their nutrient for utilization by surviving cells, as described by the following equation.

$$\text{Microorganisms} \rightleftharpoons \text{endproducts} + \text{fewer microorganisms} \quad (4)$$

It is evident from the results that considerable amounts of solids (organic and inorganic) were converted from the soluble (dissolved) phase to the insoluble (suspended) phase. The gain in the suspended volatile solids is an indication of the conversion of the organic portion from the dissolved phase to a suspended microbial mass. Also, the gain in the ash portion of the suspended solids was a result of the transformation of mineral from the soluble form to the insoluble microbial mass. The trend of the suspended solids results is in agreement with those of the soluble COD.

## Nitrogeneous Compounds

Samples obtained from both the influent and effluent streams of the reactors were analyzed for total Kjeldahl nitrogen (TKN) and ammonium nitrogen. The total Kjeldahl nitrogen as measured in this study included both the ammonium and organic nitrogen but did not include nitrite and nitrate nitrogen (*12*). The organic nitrogen was, thus, calculated by subtracting the ammonium nitrogen values from those of the total Kjeldahl nitrogen.Nitrite and nitrate nitrogen analyses were performed on the influent samples only. No attempt was made to perform these analyses on the effluent samples since this was beyond the scope of this study.

The organic nitrogen and ammonium nitrogen values obtained for batch and continuous operations are presented in Table 8. Each value is the average of 10 determinations. These analyses indicated that the concentration of the organic nitrogen in the raw whey was approximately 1230 mg/L (82% of the TKN).

The organic nitrogen in cheese whey as measured in this study included amino acids, polypeptides, and proteins (*12*), all of which are products of biological processes and are essential parts of the cellular protoplasm. An increase in the amount of organic nitrogen in the effluent of a biological process is, therefore, related to the assimilation of soluble organic and inorganic nitrogen and the synthesis of microbial cells; the opposite is an indication of microbial decline and the mineralization of organic nitrogen. Since the two processes of microbial growth and death occur simultaneously in any biological system (*13*), the measurement of organic nitrogen would indicate how the system is functioning.

Table 8
The Effluent Suspended Solids Concentrations at Various Hydraulic Retention Times[a]

| Operation | Hydraulic retention time, h | Total, mg/L | Fixed, mg/L | Volatile, mg/L |
|---|---|---|---|---|
| Batch | 6 | 22 730 | 190 | 22 540 |
| | 12 | 34 910 | 280 | 34 630 |
| | 18 | 26 180 | 210 | 25 970 |
| | 24 | 25 090 | 200 | 24 890 |
| Continuous | 6 | 25 530 | 200 | 25 330 |
| | 12 | 33 820 | 270 | 33 550 |
| | 18 | 26 180 | 210 | 25 970 |
| | 24 | 24 430 | 190 | 24 240 |

[a]The values are the average of 10 determinations; Raw whey total suspended solids = 21 820 mg/L; Raw whey fixed suspended solids = 180 mg/L; Raw whey volatile suspended solids = 21 640 mg/L; Raw whey percent volatile suspended solids = 99.18%.

The organic nitrogen results obtained from the study showed substantial gain in organic nitrogen. This was affected by the hydraulic retention time for both the batch and continuous operations. The gain in organic nitrogen varied from 10.60 to 14.63% for batch operation and from 9.76 to 17.07% for continuous operation. It appears from the results that the 12-h hydraulic retention was favourable for the growth of the yeast under both batch and continuous operations as higher gain in organic nitrogen (14.063 and 17.07% for batch and continuous operations, respectively) was achieved. The gain in organic nitrogen corresponded to a reduction in ammonium nitrogen. However, the reduction in ammonium nitrogen was slightly higher than the gain in organic nitrogen at all hydraulic retention times for batch and continuous operations.

The ammonium nitrogen as measured in this study included all ammonium salts and free ammonia, if present in the samples (*12*). The concentration of the ammonium nitrogen in the raw cheese whey was relatively small (270 mg/L or 18% of the TKN). Total ammonium reductions of 180–260 mg/L (66.66–96.30%) for batch operation and 210–260 mg/L (77.78–96.30%) for continuous operation were achieved. In an aerobic treatment unit (with satisfactory environmental conditions), ammonium is converted to microbial cells by the assimilation process. Ammonium is also converted to the oxidized forms of nitrites and nitrate in a process called nitrification. A third pathway for ammonium reduction is a nonbiological process called "ammonia volatilization." In this process, ammonium is decomposed to ammonia that is released to the atmosphere at a rate dependent on the concentration of ammonia, pH, and the degree of

Table 9
The pH, Dissolved Oxygen, and Temperature at Various Hydraulic Retention Times[a]

| Operation | Hydraulic retention time, h | pH | Dissolved oxygen, mg/L | Temperature °C |
|---|---|---|---|---|
| Batch | 6 | 4.8 | 3.5 | 35 |
| | 12 | 4.5 | 2.5 | 36 |
| | 18 | 4.2 | 1.4 | 37 |
| | 24 | 3.9 | 1.6 | 38 |
| Continuous | 6 | 4.9 | 2.9 | 37 |
| | 12 | 4.6 | 1.9 | 38 |
| | 18 | 4.6 | 2.1 | 38 |
| | 24 | 4.6 | 2.4 | 37 |

[a]Initial pH = 5.00; Initial DO = 4.8 mg/L; Initial temperature = 21°C.

agitation. Most of the ammonium nitrogen in the cheese whey (57–95%) was assimilated by the yeast and converted to microbial protein. The higher conversion values were observed at the 12-h hydraulic retention time, whereas the lower conversion values were observed at the 6-h hydraulic retention time. These results are in agreement with the COD and suspended solids results.

## pH

The initial pH value of the cheese whey used in this study was 5.0. The whey pH decreased with time during the fermentation process, reaching 3.9–4.8 for batch operation and 4.6–4.9 for continuous operation, as shown in Table 9. The pH values for batch culture are the minimum values observed at the end of each experimental run (spent medium), whereas the pH values of the continuous operation are those obtained during the steady state of each experimental run. The observed pH values of 3.9–4.9 are within the optimum pH range of 3.8–5.7 reported in the literature for the growth of *K. fragilis* (*3,7,9*). Bernstein et al. (*9*) and Reddy and Erdman (*17*) concluded that a pH of 4.5 is the optimal level for the growth of *K. fragilis* and recommended continuous pH monitoring and control of the fermentation process. However, the results obtained from this study showed that sustaining a constant pH level of 4.6 at the optimum hydraulic retention time of 12 h for both batch and continuous operations was not a problem. A pH level of 4.6 will secure efficient yeast propagation and is safer in that the lethal bacteria cannot live at this low pH level, thus making contamination less of a problem.

## Dissolved Oxygen

The average dissolved oxygen (DO) measurements for batch and continuous operations are presented in Table 9. The DO varied from 1.6 to 3.5 mg/L for batch operation and from 2.1 to 2.9 mg/L for continuous operation, depending on the hydraulic retention time. The DO values of the continuous fermenter are those measured during the steady state operation, whereas the batch values are the minimum values measured at peak growth. From monitoring the batch fermenter, it appeared that *K. fragilis* required large quantities of oxygen during the first 2–4 h of growth, since the DO reached peak low during this period for all hydraulic retention times. For the remainder of the experiment DO rose again to the values reported in Table 9. Similar results were obtained by Wasserman et al. (*7*), who observed a peak oxygen demand of 5 milimoles per liter of medium per minute, which was twice the oxygen demand during the major portion of the yeast growth curve.

Oxygen transfer is achieved more effectively at higher agitation speeds and with larger diameter impellers in Newtonian systems (*20,21*). In this study, an agitation speed of 500 rpm and an impeller to tank diameter ratio of 0.48 were used with an air flowrate of one volume of air for each volume of medium per minute (IVVM). Reisman et al. (*20*), Meiering et al. (*10*), and Wasserman et al. (*7*) recommended an impeller to tank diameter ratio of 0.31–0.38, 0.36, and 0.38, respectively. Vananuvat and Kinsella (*8*) and Meiering et al. (*10*) reported that the speed of agitation affects yield, lactose consumption, and specific growth rate, and observed dramatic increases in yeast yield when the agitation speed was increased to 700–800 rpm. At these higher agitation speeds, lower air flowrate of one volume of air for each volume of medium per minute can be used (IVVM) (*19*). Wasserman et al. (*7*) reported an air flowrate of 4 VVM at 300 rpm as optimum. In the present study, the impeller to tank diameter ratio was higher than those reported in the literature (*7,10,20*) in order to compensate for the medium agitation speed of 500 rpm used. Reisman et al. (*20*) reported that higher agitation speeds of 700–800 rpm did not use unattainable amount of power and would not, therefore, pose a threat to the economic feasibility of the fermentation process.

## Temperature

The average temperature measurements for batch and continuous operations are presented in Table 9. The temperature varied from 35 to 38°C, depending on the hydraulic retention used. The temperature of the whey in batch fermenter rose quickly in the first 2–3 h, and reached the reported value after 5–6 h. The temperature values of the continuous fermenter are those measured during the steady state conditions. The optimum temperature for *K. fragilis* propagation is within the range of 30–36°C (*7–9,18,19*).

The fermentation process in which carbohydrates are used as the fermenting substrates and *K. fragilis* as the growth culture is an exothermic process in which heat is produced. A value of 4 kcal/g of substrated is generally accepted for heat of combustion of carbohydrates (7). Although, no cooling system was used with the fermenters in this study and the laboratory temperature was above the average ($25 \pm 3$°C) during the experiment, the temperature of the fermenting fluid did not rise above 38°C because of the proper design of the fermenters.

However, the problem of the excess temperature is often overlooked when scaling up a fermentation system from the laboratory pilot scale to the industrial full scale. On the one hand, the volume of a cylindrical fermenter is proportional to the radius cubed, whereas the available surface area for heat transfer increases only by the radius squared in a jacketed type of fermenter. On the other hand, tall, narrow tanks, in spite of having the advantages of better heat transfer, longer bubble residence time, and higher oxygen transfer owing to increase in pressure, have the disadvantages of being unstable and require a substantial amount of power for aeration. Nonetheless, if the heat transfer problem is not closely monitored in the design of the large-scale fermenter, the use of expensive refrigeration techniques may be required to keep the industrial process at its optimum temperature. With a proper design of large-scale fermenters, cooling can be accomplished by running a low level of water through jacketed fermenters, as reported by Bernstein et al. (9).

## CONCLUSIONS

1. Five hydraulic retention times of operation proved to be sufficient period for the continuous culture system to reach steady state condition.
2. The reductions in the soluble COD were higher than the reductions in the total COD because of the conversion of soluble organic material to microbial protoplasm. Both the total and soluble COD reductions were affected by the hydraulic retention time, the longer the hydraulic retention time the greater were the reductions.
3. The ash content of the total solids in cheese whey remained unchanged under batch and continuous operations. However, substantial reductions in the volatile solids were observed under both batch and continuous operations. The reductions in volatile solids were, however, related to the hydraulic retention time, the higher the hydraulic retention time the greater were the reductions.
4. The increases in the suspended solids (both fixed and volatile) are owing to the conversion of soluble material to insoluble

microbial mass. Higher conversion rates were observed at the 12-h hydraulic retention time for both batch and continuous operations.

5. The organic nitrogen concentration in the effluent increased, whereas the ammonium nitrogen concentration decreased under both batch and continuous operations. The gain in the organic nitrogen is an indication of converting the nonmicrobial inorganic nitrogen to microbial protoplasm. Higher conversion rates were observed at the 12-h hydraulic retention time for batch and continuous operations.
6. A hydraulic retention time of less than 12 h should not be used in the fermentation of cheese whey using *K. fragilis*. For a higher conversion rate of soluble organic material to microbial cells a hydraulic retention time of 12 h is recommended. A hydraulic retention time of 24 h would be appropriate for pollution potential reductions, i.e., reductions in COD and total solids.

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