Effect of pH Control on the Growth and Survival of *Kluveromyces fragilis* in Cheese Whey Under Aerobic Condition

A. E. GHALY,[*,1] R. M. BEN-HASSAN,[1] AND M. H. MANSOUR[2]

[1]*Agricultural Engineering Department, Technical University of Nova Scotia, PO Box 1000, Halifax, Nova Scotia, Canada B3H 2X4; and* [2]*Engineering Department, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H6*

ABSTRACT

Many biological and chemical systems involve acid-base equilibria and thus depend critically on the pH of the solution. Small fluctuations of pH must be corrected by the addition of an acid or base to maintain the pH at the required optimum level during the fermentation process. The effect of pH control on the growth of *Kluveromyces fragilis*, used for the production of single-cell protein from cheese whey, was investigated. Maintaining the pH at 4.5 was found essential for the survival of the yeast. Without pH control, the pH of the medium continued to rise, resulting in the death and/or sporulation of the yeast cells. Diauxic growth was observed, caused by the growth of contaminant bacteria when the pH was not controlled.

Index Entries: Yeast; bacteria; pH; temperature; dissolved oxygen; lactose; diauxic growth; spores.

INTRODUCTION

In industrial fermentation processes, biological activities tend to substantially alter the pH of the system from the desirable optimum

---

values. Denitrification, organic nitrogen breakdown, and sulfate reduction are examples of biological reactions that can cause increases in pH. A decrease in pH may be caused by sulfate oxidation, nitrification, and organic carbon oxidation (1). However, many biological and chemical systems involve acid-base equilibria and therefore depend critically on the pH of the solution. Albert (2) stated that the viability and growth of organisms in these systems depends to a large extent on the pH of the cell fluid and the media in which the cells grow. Ghaly and Singh (1) reported that the yield and efficiency of single-cell protein production were considerably influenced by the pH value of the growth medium because the stability and function of the cell membrane and protein are pH-dependent.

Fiechter, Kapplei, and Meussdoerffer (3) stated that in order to maintain a constant intracellular pH value, a gradient of hydrogen ion concentration across the membrane has to be maintained. Small pH fluctuations therefore should be corrected by the addition of an acid or base to maintain the pH at the optimum level during fermentation. Since the response of pH to the addition of an acid or base is nonlinear and difficult to control by conventional metering devices, a computer-based pH measurement-and-control system was developed by Ben-Hassan, Ghaly, and Mansour (4) for such fermentation process. In this study, the effect of pH control on the growth and survival of the yeast *K. fragilis*, used for the production of single-cell protein from cheese whey under batch condition, was investigated.

The specific objectives of this study were to investigate the effect of pH on the maximum specific growth rate of *K. fragilis* and the possible growth of contaminant microorganisms originally present in the cheese whey, and to determine the lactose consumption, oxygen concentration, and temperature of the fermentation system with and without pH control.

EXPERIMENTAL APPARATUS

The experimental apparatus used in this study is shown in Fig. 1. The fermenter was constructed of a plexiglass cylinder of 5 mm thickness. The dimensions of the fermenter are shown in Fig. 2. The bottom of the fermenter was constructed from a 6 mm thick and 195 mm diameter plexiglass circular plate, cut with a 4 mm groove around the circumference, and glued in place with plexiglass cement. Baffles were used in the fermenter to reduce vortexing and improve the top-to-bottom turnover. Standard design recommended for baffles, given by *Perry's Chemical Engineer's Handbook* (5), was used. Four vertical baffles made from plexiglass material (15 mm wide×5 mm thick) were positioned every 90°C on the inside of the fermenter wall. A 6 mm thick and 195 mm diameter lid was
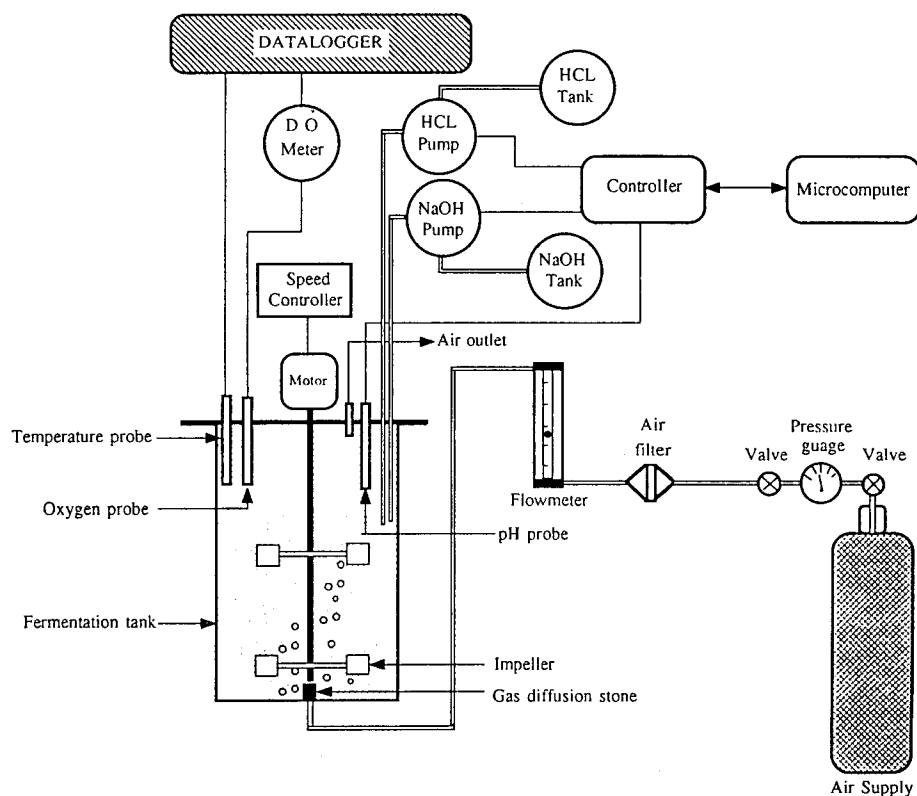
Fig. 1. The experimental apparatus.

held in place by six bolts. A gas-tight seal was provided by a 2 mm diameter neoprene ring gasket coated with vaseline. Four holes were drilled and tapped through the lid to take: a 20 mm diameter tube for cheese whey loading, a 40 mm diameter oxygen probe, a 25 mm diameter pH probe, a 10 mm temperature probe, and a 15 mm diameter air outlet.

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 ~ 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).

Compressed air (Medigas Atlantic Ltd., Cat. No. T100172) was supplied to the fermenter through a flowmeter with a high-resolution valve using tygon tubing of 10 mm diameter. The air was made 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 microfilter
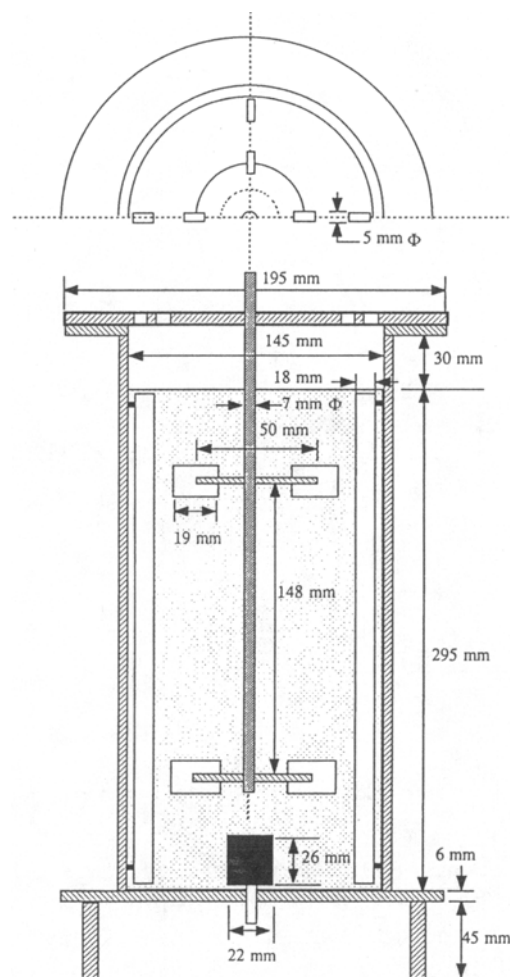
Fig.   2.   The vertical cross-section of the fermenter.

(Cole-Parmer, Cat. No. L-29701-00) was used to reduce the risk of cross contamination. The air was introduced from the bottom of the fermenter through a gas diffusion stone (Fisher Scientific, Cat. No. 11-139B) 26 mm height and 22 mm diameter.

Dissolved oxygen was monitored by an oxygen electrode probe (YSI 5739, Fisher Scientific, Cat. No. 13-299-43) connected to a digital dissolved oxygen meter (YSI Model 58, Fisher Scientific, Cat. No. 13-298-58). A copper-constantan thermocouple inserted in the fermenter and connected to a data logger (Cole-Parmer, Cat. No. L-08360-14) was used to measure the temperature. The pH was measured using a pH probe (Cole-Parmer, Cat. No. J-5990-40) connected to a pH control system. The pH control system included a microcomputer (TRS-80 Model 100), a data acquisition unit, a signal conditioning circuit, and two pumps. More elaborate dis-

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

Characteristics	Measured values	Unit
Total solids	63835	mg/L
Fixed solids	9100	mg/L
Volatile solids	54738	mg/L
Percent volatile solids	85.74	%
Percent fixed solids	14.26	%
Suspended solids	22150	mg/L
Fixed solids	185	mg/L
Volatile solids	21965	mg/L
Percent volatile solids	99.16	%
Percent fixed solids	0.84	%
Total Kjeldhal nitrogen	1690	mg/L
Ammonium nitrogen	270	mg/L
Organic nitrogen	1420	mg/L
Percent organic nitrogen	84.02	%
Percent ammonium nitrogen	15.98	%
Total chemical oxygen demand	74220	mg/L
Soluble chemical oxygen demand	59640	mg/L
Insoluble chemical oxygen demand	14580	mg/L
Percent soluble chemical oxygen demand	80.36	%
Percent insoluble chemical oxygen demand	19.64	%
Lactose	5.00	%
pH	4.90	—

cussion of the pH control system was done by Ben-Hassan, Ghaly, and Mansour (4).

EXPERIMENTAL PROCEDURE

Whey Collection, Storage, and Preparation

The cheese whey was obtained from the Farmer's Cooperative Dairy Plant (Truro, Nova Scotia). The cheese whey was pumped from the plant cheese whey storage tank into 60 L plastic containers. The containers subsequently were sealed and transported to the Cold Storage Facility of the Biotechnology Laboratory at the Technical University of Nova Scotia. They were stored in a large freezer at −25°C until required. Prior to placing the cheese whey into the feeding tank, it was allowed to completely thaw at room temperature for 48 h. Some characteristics of the cheese whey used in this study are presented in Table 1. These analyses were performed according to the procedures described in *The Standard Methods for Examination of Water and Wastewater (6)*.

Stock Culture and 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 sterilized 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 dish was then placed in a controlled environment incubator at 35 °C and left until visual growth appeared on the Petri dish (about 72 h). The Petri dish was removed from the controlled environment incubator and stored in a refrigerator at 4 °C until required for inocululm preparation.

Raw cheese whey was first pasteurized in several 250 mL Erlenmeyer flasks. The pasteurization process involved heating the whey to 70 °C for 45 min and then cooling it to 1 °C for 30 min. The processes of heating and cooling were repeated three times. The yeast was transferred from the Petri dish to the pasteurized cheese whey in the 250 mL sterilized Erlenmeyer flasks. Two Petri dishes of pure culture of *K. fragilis* were added to each flask containing 100 mL pasteurized cheese whey in order to start with a large seed size and a high cell count. The Erlenmeyer flasks were capped with nonabsorbent cotton plugs and mounted on a controlled environment reciprocating shaker. The shaker was operated at a speed of 250 rpm for 48 h. Following the 48-h growth stage, 950 mL of the yeast cultures were transferred to the fermenter.

Fermenter Operation

The fermenter was chemically sterilized using a 2% potassium metabisulfite 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 pasteurized cheese whey and immediately inoculated using separate flasks of inoculum (950 mL). The air flow (2 vum) and turbine drives (350 rpm) were started immediately, and dissolved oxygen, pH, and temperature were monitored continuously. The ambient temperature remained at 21 ± 1 °C during the experiment.

Samples Collection and Analyses

At zero hour, samples were drawn from the fermenter immediately. Then, samples were drawn every 2 h. For each sample, the percentages of lactose, plate count, and staining tests were conducted. The lactose analysis was performed using a sugar analyzer (YSI Model 27, Fisher Scientific, Cat. No. 14-660). The plant count and staining tests were carried out according to the procedures described by Messer, Behney, and Leudecke (7) in *Standard Methods for the Examination of Dairy Products* and by Pelczar and Chan (8), respectively.

Table 2
pH, Dissolved Oxygen, Temperature, Lactose Concentration, and Cell Number

Time, h	pH		Oxygen concentration, mg/L		Temperature, °C		Lactose concentration, %		Cell number (10⁶ cell/mL)	
	WPHC	NPHC	WPHC	NPHC	WPHC	NPHC	WPHC	NPHC	WPHC	NPHX
0:00	4.38	4.30	5.50	5.50	22.0	24.0	5.00	5.00	171.0	171.0
2:00	4.38	4.42	5.40	5.45	22.5	24.5	4.90	4.95	171.0	171.0
4.00:	4.22	4.60	5.10	5.25	23.0	25.0	4.60	4.77	172.0	171.0
6:00	4.17	5.20	4.40	4.50	25.2	27.0	3.82	3.61	298.0	228.0
8:00	4.24	6.70	2.60	2.92	28.0	29.0	2.70	2.55	520.0	412.0
10:00	4.36	6.73	1.60	2.85	31.7	30.0	1.43	2.41	670.0	412.0
12:00	4.48	6.80	1.20	2.80	33.0	31.0	0.12	2.33	840.0	462.0
14:00	4.45	6.90	1.20	1.50	33.0	32.0	0.10	0.10	840.0	1005.0
16:00	4.50	6.90	1.20	1.00	33.0	32.0	0.08	0.05	840.0	1310.0
18:00	4.48	7.20	2.20	0.85	31.5	32.0	0.06	0.04	640.0	1330.0
20:00	4.53	7.50	2.80	0.75	28.5	32.0	0.05	0.04	580.0	1330.0
22:00	4.55	7.60	3.10	0.75	25.5	32.0	0.05	0.04	550.0	1330.0
24:00	4.55	7.70	3.20	0.75	22.5	32.0	0.05	0.04	520.0	1330.0

WpHC = with pH control;
NpHC = no pH control.

RESULTS

The measured pH, dissolved oxygen, temperature, lactose concentration, and cell number data are presented in Table 2.

pH

The initial pH value of the cheese whey used in this study was 4.30 (Fig. 3). In one experiment, the pH was maintained in the range of 4.17–4.55 by the addition of HCl (1N) during the course of the experiment. In the other experiment, when the fermentation system was operated without pH control, the pH continued to rise, reaching 8.37 at the end of the experiment.

Microbial Growth

Samples of cheese whey were taken from the reactor at time intervals and plated on agar yeast peptone dextrose (YPD) growth medium. Stain test also was performed. The colonies developed from the samples taken from the reactor while maintaining the pH at 4.5 exhibited the typical elevated concave, smooth appearance and creamy color of *K. fragilis*. Staining specimens with crystal violet showed elongated clustered yeast cells (Fig. 4). The gram reaction showed that the yeast cells multiplied vegetatively by budding.
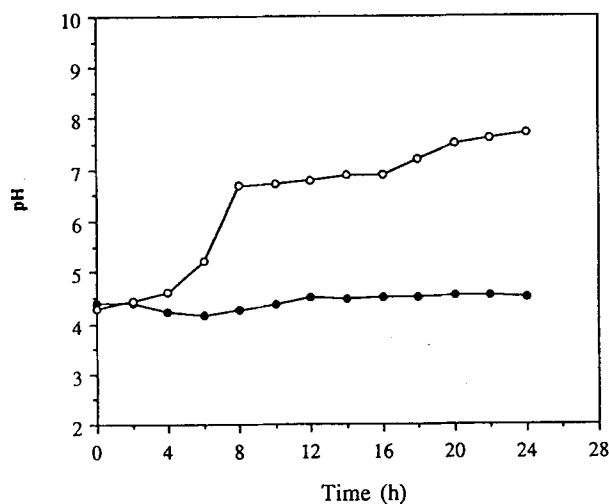
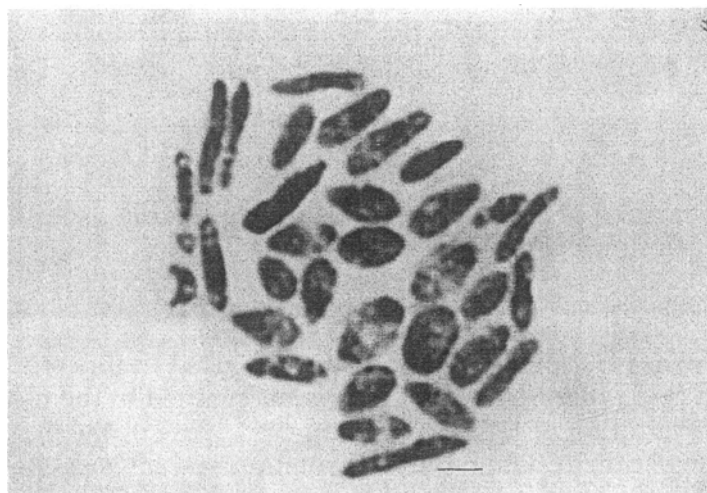Fig. 3.   Batch culture pH. —●— with control; —○— without control.

Fig. 4.   Elongated clustered yeast cells.

The growth curve of *K. fragilis*, determined while maintaining the pH at 4.5, is shown in Fig. 5. The important feature of this curve is that the four principal phases encountered in the history of a microbial culture grown under a batch operation can be clearly recognized. These are: the lag phase, which represents the time for the yeast cells to acclimatize themselves to the new environment; the exponential growth phase, during which the growth rate had a constant maximum value; the stationary phase, during which the growth rate was 0; and the death phase, during which the yeast cells die faster than new cells are produced.
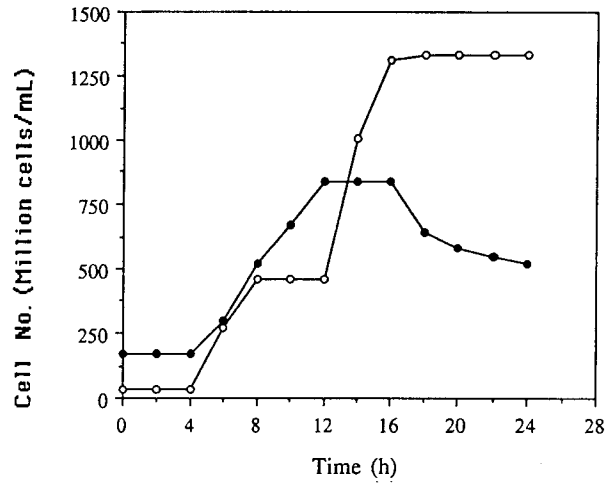
Fig. 5. Batch culture active cell number —●— with pH control; —○— without pH control.
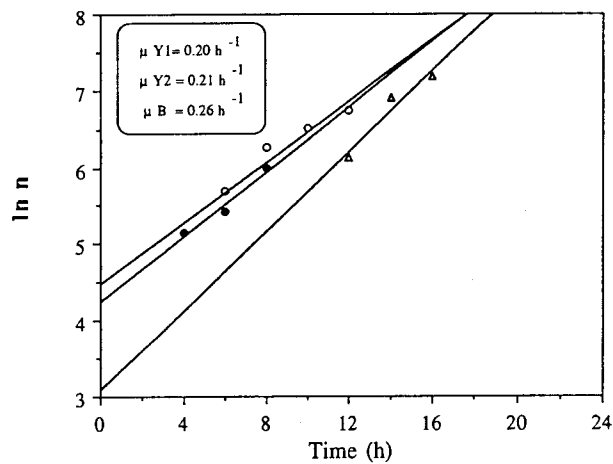

Fig. 6. Determination of specific growth rate of yeast and bacteria. ○ yeast with pH control; ● yeast without pH control; and △ bacteria without pH control.

The natural logarithm of the number of active cells in the exponential growth phase was plotted against the time as shown in Fig. 6. This was done to linearize the exponential portion of the growth curve. The specific growth rate (μ) of the *K. fragilis* population was calculated from the slope of the linear portion of the exponential growth phase using the recognition procedure of the statistical package MINITAB. The value of μ was found to be 0.20 h^{-1}. The duration of the lag phase was found to be approx 4.0 h. Vananuvat and Kinsella (9) studied the production of yeast protein from lactose using *K. fragilis* and observed a lag phase of

about 4.0–5.0 h. Garibay, Gomez-Ruiz, and Barzana (10) used K. fragilis grown in whey with pectin under aerobic condition to produce single-cell protein and pectinase simultaneously. The length of the lag phase observed in their study was about 5.0–6.0 h. Ghaly, Kok, and Ingrahm (11) stated that the length of the lag phase usually depends on the extent to which the new media and environmental factors, such as dissolved oxygen, pH, and temperature, are different from those under which the inoculum was prepared.

The growth curve of K. fragilis determined without pH control also is shown in Fig. 5. The initial lag phase was followed by a period of diauxic growth. Diauxic growth is manifested by two phases of exponential growth separated by a transient lag phase. According to Ghaly, Kok, and Ingrahm (11), this phenomenon occurs when a mixed population of microorganisms is grown in a single medium (or a single microorganism is grown in a complex medium). In the first lag phase, the yeasts were adapting to the environment and producing the necessary enzymes required for the metabolism of the available nutrients. After 4 h of fermentation (lag period), yeast cells multiplied vegetatively by budding, and the population increased at an exponential rate. The specific growth rate for the yeast was 0.21 h^{-1}, which was similar to that obtained with pH control.

Because of the yeast metabolic activity and the breakdown of organic nitrogen to ammonium, the pH of the medium increased with time, reaching 6.7 after 7 to 8 h from the start of the experiment. As the conditions became totally unfavorable (inhibitory) for the growth of the yeast, some of the yeast cells died, whereas others transformed themselves to highly resistant cells, known as spores, in order to survive the new environmental condition (high pH). The size of the yeast population started to taper off thereby, reaching a stationary growth phase (second lag phase). Staining the cells obtained from the samples taken from the system during the stationary phase showed the existence of bacterial cells among the yeast spores (Fig. 7). This indicated that the pasteurization process was not effective in killing all contaminants in the fermentation medium. The period of stationary phase was then considered to be the lag period for the growth of the bacterial cells. The length of this period was approx 4.0 h.

The pH continued to rise, with a corresponding increase in temperature, reaching 8.37 at the end of the experiment. This condition favored the growth of the bacterial cells. The gram reaction at this time showed a number of dead yeast cells. The yeast reproduced by sporulation; that is, repeated divisions of the nucleus were formed into spores. The dead mother cells appeared pink. A greater number of small bacterial cells were found in the specimens. Because a sufficient substrate for the growth and metabolism of the bacteria was still available in the system, the bacterial population increased at an exponential rate until all the substrate was utilized. At this point, the growth rate slowed down, and finally, cell growth
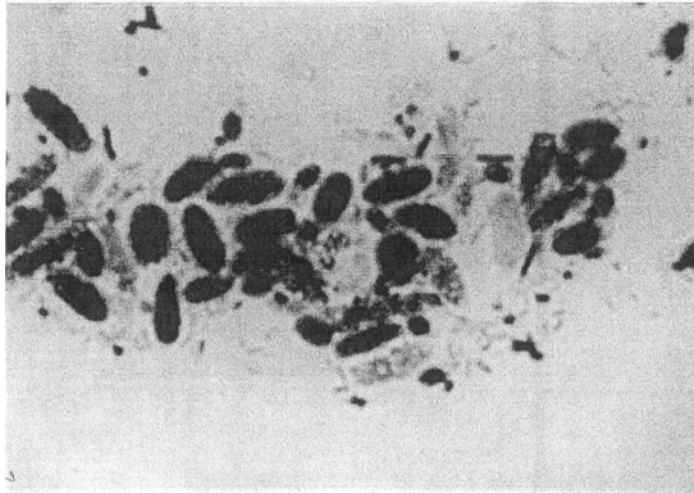
Fig. 7. A yeast culture contaminated with bacteria.

remained constant over a period of time: that is, the final stationary phase. The specific growth rate of the bacteria was found to be a little higher than that of the yeast (0.26 h^{-1}).

The results showed that maintaining the pH between 4.0 and 5.0 is very essential for the growth and survival of *K. fragilis*. It also has been recognized by Singh and Ghaly (*12*), Bernstein, Izeng, and Sisson (*13*), Bernstein and Everson (*14*), and Wasserman (*15*) that keeping the pH at 4.5 eliminates possible contamination by lethal bacteria that grow at pH levels above 6.0. Thus, running fermentation systems for single-cell protein production using *K. fragilis* without controlling the pH may provide a very good chance of contamination by other microorganisms originally present in the cheese whey. Pasteurizing the cheese whey at a higher temperature resulted in the denaturization of the protein, and the heating (70°C) and cooling (1°C) processes did not achieve total destruction of lethal, spore-forming bacteria originally found in the cheese whey.

Lactose Reduction

The lactose concentrations in the fermenter operating with and without pH control are graphically presented in Fig. 8. The initial value of lactose was 5.0%, which was reduced to 0.05% after 16 and 20 h, without and with pH control, respectively. This resulted in a reduction of about 99.0% of the lactose initial found in the reactor.

With pH control, the reduction of lactose displayed three distinct stages that corresponded to the lag, exponential growth, and stationary phases of the yeast growth curve obtained with pH control. During the first stage, there was a slow reduction in the lactose concentration. About
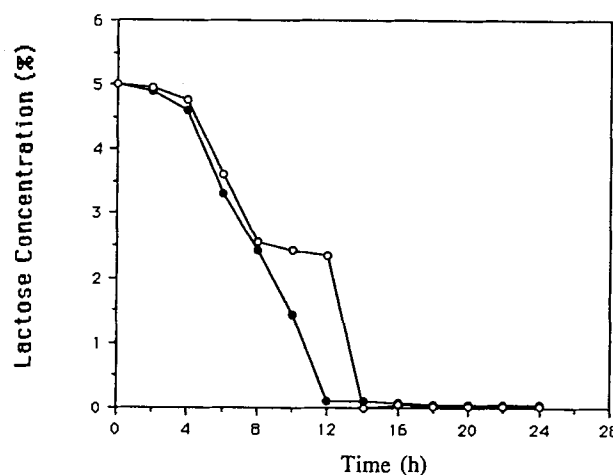
Fig. 8. Batch culture lactose concentration (%). —●— with pH control; —○— without pH control.

8% of the lactose was removed from the system by the yeast cells during this stage. This was utilized mainly for cell respiration and cell endogenous growth (individual cell growth). The second stage was a period of rapid lactose reduction. During this phase, about 90% of the lactose was removed from the system. This was utilized by the yeast for endogenous cell growth (cellular cell growth) and cell mass growth (cell multiplication) as well as cell respiration. In the third stage, the concentration of lactose was very low (0.05–0.12%), and thus, an insignificant reduction of lactose was achieved during this period. This stage lasted approx 8.0 h, in which less than 2.0% of initial lactose was removed from the system. As a result, the microbial growth was kept stationary because of the lack of substrate. This resulted in the death and/or sporulation of the vegetative cells of the yeast.

Without pH control, the reduction of lactose displayed five distinct stages that corresponded to the first lag, first exponential growth, second lag, second exponential growth, and stationary phases of the diauxic growth curve. The reduction in the lactose concentration was slow in the first stage. About 4.6% of the lactose was removed by the yeast cells during this stage. This was utilized for cell endogenous growth as well as cell respiration. The second stage was a period of rapid lactose reduction. During this phase, about 22.0% of lactose was removed from the system. This was utilized by the yeast mainly for cell mass growth. The third stage was a period of slow reduction in the lactose concentration that lasted only 4 h, during which less than 2.0% of initial lactose was removed from the system. As a result, the yeast cell mass was kept stationary because of the change in the environmental condition (high pH).
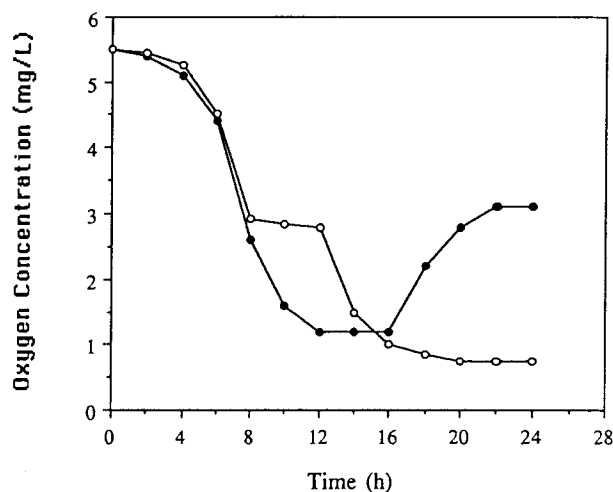
Fig. 9. Batch culture oxygen concentration (L). —●— with pH control; —○— without pH control.

At this time, some bacterial cells were present in the system. Therefore, this period corresponded to the lag period of the bacterial cells. Then, a period of rapid lactose consumption was observed again. As the pH continued to rise and the environmental condition became very favorable to the bacterial cells (unfavorable to the yeast cells), the population of the bacterial cells increased at an exponential rate. As a result, the lactose concentration dropped very sharply from 2.33 to 0.1% in only 2 h. About 45.0% of the initial lactose was removed from the system during this period. In the fifth stage, the concentration of lactose was very low (0.04–0.05%), and the reduction of lactose was insignificant during this period.

Dissolved Oxygen

Initially, the cheese whey was saturated with oxygen before the inoculum of the *K. fragilis* was introduced. The observed dissolved oxygen concentration (at saturation) of the yeast-free cheese whey was 5.5 mg/L. The values of dissolved oxygen measured during the experiment are graphically presented in Fig. 9.

With pH control, the measured values of dissolved oxygen displayed four distinct stages. The first stage corresponded to the lag phase of the yeast growth curve. During this period, the dissolved oxygen decreased slowly (almost linearly) as the number of the yeast cells was kept constant and oxygen was mostly required for cell respiration as well as endogenous cell growth. The second stage corresponded to the exponential growth

phase of the yeast growth curve. In this phase, the cells increased exponentially, and as a result, the oxygen concentration in the fermenter decreased rapidly until it reached a constant minimum value of 1.2 mg/L. The third stage corresponded to the stationary growth phase of the yeast population during which the number of cells was constant. The oxygen concentration in the reactor remained constant as a temporary steady state condition was achieved. During this steady-stage period, the amount of oxygen added to the system by the aeration equipment was equivalent to that consumed by the yeast. The fourth stage corresponded to the death phase of the yeast growth curve. In this period, the number of cells was decreasing with time, and because of this, the oxygen concentration in the system was continuously increasing.

Without pH control, the oxygen concentration displayed five distinct stages that corresponded to the first lag, first exponential growth, second lag, second exponential growth, and stationary phases of the diauxic growth curve. During the first stage, there was a slow reduction in the oxygen concentration as the number of yeast cells was constant and the amount of dissolved oxygen utilized was very small; this was used for the respiration of the yeast cells. As a result, oxygen concentration decreased from 5.5 to 5.25 mg/L in 4 h. In the second phase, the dissolved oxygen concentration in the fermenter decrease sharply because the yeast population increased exponentially. As a result, the dissolved oxygen concentration decreased rapidly from 4.5 to 2.9 in only 2 h. The third stage was a period of slow reduction in the oxygen concentration. In this phase, the oxygen added to the system was almost equivalent to that consumed by the yeast and/or bacterial cells. In the fourth phase, the bacterial cells increased exponentially, and as a result, the oxygen concentration decreased sharply until it reached 0.85 mg/L. In the fifth stage, the concentration of oxygen was constant at 0.75 mg/L because the population size remained constant.

The minimum dissolved oxygen values observed in this study appear to be higher than that of 0.3 mg/L reported by several authors (16–18) as being the critical value above which the oxygen uptake rate by microorganisms is dependent on the oxygen concentration of the medium.

Temperature

The initial temperature value of the cheese whey was 22.0°C with pH control. This was changed during the course of the experiment, as shown in Fig. 10. There was four distinct stages of the temperature change observed during the experiment. The first stage corresponded to the lag phase of the yeast growth curve. During this stage, the temperature increased slowly by 1°C (from 22.0 to 23.0°C) over a 4-h period. The second stage corresponded to the exponential growth phase of the yeast growth curve. During this stage, the temperature increased sharply by 10°C
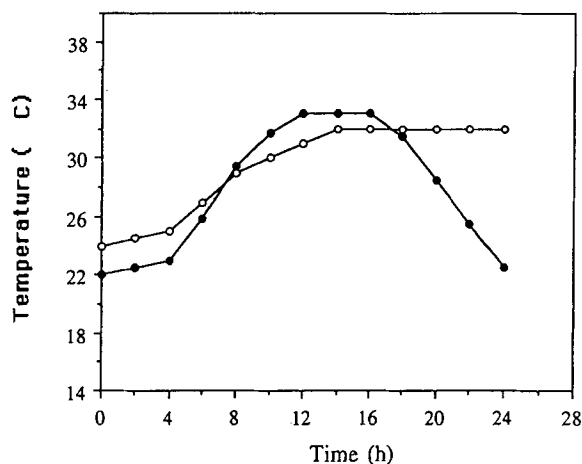
Fig. 10. Batch culture temperature (°C). —●— with pH control; —○— without pH control.

(from 23.0 to 33.0°C) over a period of 8 h. The third stage corresponded to the stationary growth phase of the yeast growth curve. Since the number of yeast cells remained constant during this phase, the temperature leveled off and remained constant during this stage at 33.0°C for about 4 h. The fourth stage corresponded to the death phase. During this stage, the temperature decreased ʾs the number of yeast cells decreased continuously because of the lack of substrate. The temperature decreased by 10.0°C (from 33.0 to 22.0°C) over a period of 8 h.

Without pH control, the initial temperature value of the cheese whey was 24.0°C. This was changed during the course of the experiment. There were three distinct stages of temperature change observed during the experiment. Since the number of yeast cells in the first stage was almost constant, the temperature increased slowly by 1°C (from 24.0 to 25.0°C) over a 4-h period. During the second stage, the temperature increased sharply by 7.0°C (from 25.0 to 32.0°C) over a period of 10 h. During the third stage, the temperature was constant until the end of the experiment.

CONCLUSION

Pasteurizing the cheese whey did not eliminate all the contaminant bacteria originally present. Maintaining the pH at 4.5 is essential for the growth and survival of the yeast *K. fragilis*. Without pH control, the pH of the media continued to rise, resulting in the death and/or sporulation of the yeast cells and the growth of contaminant bacteria. Diauxic growth was observed without pH control, showing the successions of yeast and bacterial growth. The maximum specific growth rate of *K. fragilis* used in

this study with and without pH control was 0.20 h⁻¹, and the length of the lag period was 4.0 h. The maximum specific growth rate of the contaminant bacteria was 0.26 h⁻¹, and the length of the lag period was 4.0 h. The oxygen concentration, lactose consumption, and temperature were all affected by the size and type of microbial population in the reactor.

REFERENCES

1. Ghaly, A. E. & Singh, R. K. (1989), Pollution potential reduction of cheese whey through yeast fermentation. *Appl. Biochem. Biotechnol.* **22(3)**, 181–203.
2. Albert, A. (1985), *Selective Toxicity: The physico-chemical basis of therapy*, 7th Ed., Chapman and Hall, London.
3. Fiechter, A., Kapplei, O., and Meussdoerffer, F. (1987), Batch and continuous culture, *The Yeasts*, Rose, A. H. and Harrison, J. S., Eds., Academic, London.
4. Ben-Hassan, R. M., Ghaly, A. E., and Mansour, M. H. (1991), A computer based pH measurement and control system for fermentation processes. *Appl. Biochem. Biotechnol.* **30**, 233–245.
5. Perry, R. H., Green, D. W., and Maloney, J. O. (1984), *Perry's Chemical Engineer's Handbook*, 6th Ed., McGraw-Hill, New York.
6. APHA (1980), The Standard Methods for Examination of Water and Wastewater, American Public Health Association, New York.
7. Messer, J. W.,. Behney, H. M., and Leudecke, L. O. (1985), Microbiological count methods, *Standard Methods for the Examinatin of Dairy Products*, 15th Ed., Richardson, G. H., ed., American Public Health Association. Washington, DC.
8. Pelczar, M. J. and Chan, E. C. S. (1972), *Laboratory Exercises in Microbiology*, McGraw-Hill, New York.
9. Vananuvat, P., and Kinsella, J. E. (1975), Production of yeast protein from crude lactose by *Saccharomyces fragilis:* Batch culture and continuous culture studies. *J. Food Sci.* **40(2)**, 336–341, 823–825.
10. Garibay, M., Gomez-Ruiz, L., and Barzana, E. (1987), Simultaneous production of single cell protein and pectinase from cheese whey, *Proceeding Food Processing Waste*, Atlanta, GA.
11. Ghay, A. E., Kok, R., and Ingrahm, J. M. (1989), Growth rate determination of heterogeneous microbial population in swine manure. *Appl. Biochem. Biotechnol.* **22**, 59–77.
12. Singh, R. K. and Ghaly, A. E. (1984), Single-cell protein production from cheese whey, ASAE Paper No. 84-6528, St. Joseph, Michigan, IL.
13. Bernstein, S., Tzeng, C. H., and Sisson, D. (1977), The commercial fermentation of cheese whey for the production of protein and/or alcohol. *Biotechnol. Bioeng. Symp.* **7**, 1–9.
14. Bernstein, S. and Everson, T. C. (1973), Protein production from acid whey via fermentation. Proceedings of the National Symposium on Food Process Waste, 4th Environmental Protection Agency Technology Series, No. EPA66012-73-031, College Park, MD.

15. Wasserman, A. E. (1960), The rapid conversion of whey to yeast. *Dairy Eng.* **77**, 374–379.
16. Porges, N. L., Jasewicz, L., and Hoover, S. K. (1953), A microbiological process report: Aerobic treatment of dairy waste. *Appl. Microbiol.* **1(2)**, 202–208.
17. Longmuir, I. S. (1954), Respiration rate of bacteria as a function of oxygen concentration. *Biochemistry* **57(1)**, 81–87.
18. Winzler, K. J. (1941), The repiration of baker yeast at low oxygen tention. *J. Cellular Comparative Physiol.* **17(3)**, 264–271.