ENHANCED LACTIC ACID PRODUCTION FROM CHEESE WHEY WITH NUTRIENT SUPPLEMENT ADDITION

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ABSTRACT

Continuous mix batch bioreactors were used to investigate the effects of various concentrations (0, 5, 10 and 15 g/L) of two nutrients (Yeast extract and Lactamine AA) on the growth of *Lactobacillus helveticus* (ATCC 15009) and the production of lactic acid from cheese whey. The experiments were conducted under controlled pH and temperature of 5.5 and 42^{o}C, respectively. The results indicated that yeast extract at a concentration of 10 g/L gave the highest cell growth, lactose utilization and lactic acid yield. The use of nutrients at low concentrations significantly decreased the lag period whereas lactic acid inhibition was observed when both nutrients were added at concentrations above 10 g/L. The results from this study showed that yeast extract was superior to Lactamine AA in its influence (improved yield and conversion efficiency) when used as nutrient during batch fermentation experiments of lactic acid production from cheese whey. The specific lactose utilitization and lacitc acid production rates were calculated for the lag, growth, stationary, and death phases under nutrient supplemented and non-supplemented conditions. The cells inability to deplete the residual lactose in the medium was an indication of lactic acid inhibition.

Keywords: Batch fermentation; cell growth; cheese whey; inhibition; lactic acid; *Lactobacillus helveticus*; lactamine AA; nutrient addition; yeast extract.

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INTRODUCTION

Cheese whey is the liquid effluent generated during the cheese making process. It is produced at a rate of 2.72×10^6 metric tons per year in Canada, of which about half is currently disposed of as a waste thereby causing serious pollution problems (Ghaly and Ramkumar, 1999). However, cheese whey contains about 5% lactose, which is a suitable substrate for the production of value added products using biochemical conversion processes. Lactic acid is one such a product that has numerous applications in chemical, pharmaceutical, and food industries. It is used as a substrate for the production of some organic acids, de-icing and anti-icing agents, and biodegradable plastics (Lipinsky and Sinclaire, 1986; Yang et al., 1992; and Tango and Ghaly, 1999).

Industrial fermentation of lactic acid may be limited by the availability of micro and macronutrients, which are required by lactic acid producing microbes for cellular growth and maintenance. Micronutrients are predominantly metallic ions, which are required in trace quantities as cofactors in enzymatic reactions, whereas macronutrients include nitrogen, phosphorus, potassium, sodium and sulfur and are needed mainly for the synthesis of cellular material (Amrane and Prigent, 1998). Roy et al. (1987) and Aeschlimann and Von Stocker (1990) showed the need for a complex of nutrients for *Lactobacillus helveticus* for the growth and product formation and the need to supplemant cheese whey with some commercially available growth supplements. Several Studies showed that lactic acid productivity of most *Lactobacilli* is significantly improved by the addition of yeast extract, amino acids, protein concentrates, hydrolysates, vitamins and inorganic compounds such as $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ (Amrane and Prigent, 1998; Demirci et al., 1998; Champagne et al., 1992; and Cheng et al., 1991). Other studies showed the need to supplement cheese whey with some commercially available growth supplements such as corn steep liquor, yeast extract, casamino acids, peptone, neopeptones, cane molasses and trypticase (Cheng et al., 1991; Gupta and Gandhi, 1995; and Roy et al., 1986).

Nutrient supplements such as yeast extract, corn steep liquor, and Lactamine AA (Casein hydrolysate) can improve the nutritional quality of the medium, because they contain growth promoting compounds, in addition to organic nitrogen, and carbonecious compounds. However, the use of these nutrient supplements in large quantities is very expensive and can reach as high as 32 % of the total lactic acid production cost (Norton et al., 1994). There is, therefore, a need to develop an industrially attractive process that considers productivity, residual lactose and economic levels of nutrient supplements.

The main aim of this study was to investigate the effect of nutrient supplements on the amelioration of lactic acid production. The specific objectives of this study were: (a) to investigate the effects of various concentrations (0, 5, 10 and 15 g/L) of two commercially available nutrients (yeast extract and lactamine AA) on cell growth rate, lactose utilization and lactic acid production during cheese whey fermentation using *Lactobacillus helveticus* under batch conditions, and (b) to determine the optimum concentration of the most effective nutrient supplement (as measured by fermentation time and lactic acid yield).

MATERIALS AND METHODS

Experimental Setup

The experimental apparatus is shown in Figure 1. Four 5 L batch bioreactors, each constructed from a plexiglas cylinder of 5 mm thickness, were used. Each bioreactor has four vertical baffles (positioned at 90^o apart) made from plexiglas. Provisions were made on the cover for mounting the temperature probe, the pH probe and the mixing shaft, and two ports for sample collection and pressure release. The agitation speed (150 rpm) was maintained at 150 rpm by a mixing system which consisted of an electric motor (Model 4Z142, Dayton Electric MFG Co., Chicago, IL, USA) with a speed controller and a mixing shaft. The mixing shaft has two flat-bladed impellers of 75 mm diameter, mounted at 148 mm apart (the bottom impeller being 30 mm from the bioreactor floor).

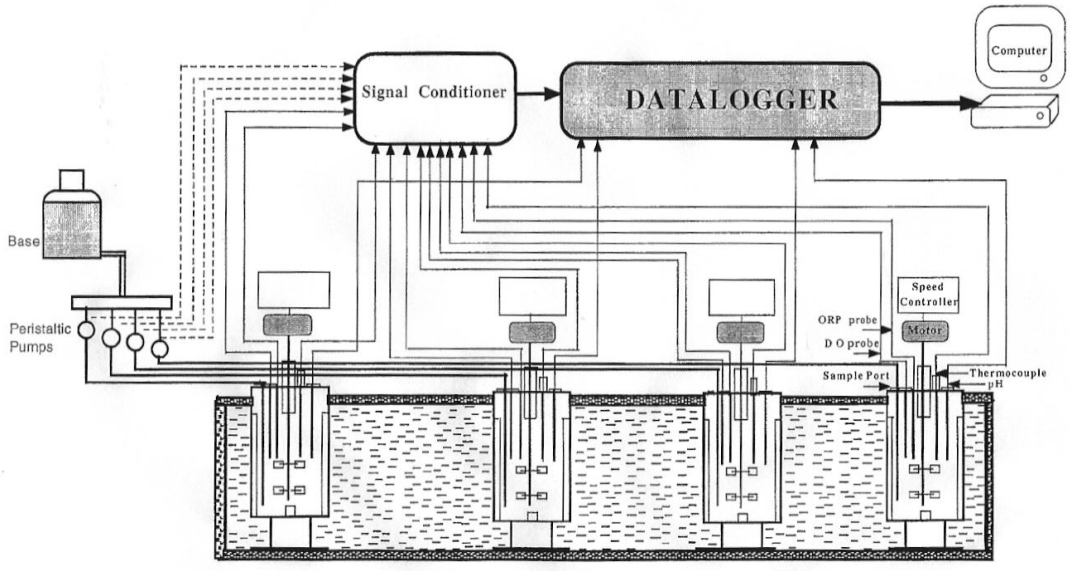


Figure 1. Experimental Apparatus

The fermentation temperature was maintained at 42^{o}C using a specially designed well insulated water bath. Water flow rate within the water bath was controlled by a submersible pump (Model No. 1- M AT, Tecumseh Products Co., Oklahoma City, OK, USA) inserted in the water bath. A uniform distribution of water to the heating unit was facilitated by holes around a steel tube inside which a 2.0 KW heating element (Chromalox Canada Inc., Rexdale, Ont, Canada) is inserted. A temperature sensor (Model No. T675A2100, Honeywell, North York, Ont, Canada) inserted in the water bath was used to monitor the water temperature. The pH of the broth was maintained at 5.5 $\pm$ 0.1 using a pH control system, which consisted of a base tank (30 L), four peristaltic pumps (Model 70 16-52, Cole-Parmer, Chicago, IL, USA), the associated tubing connections, and the pump control unit. The data acquisition system consisted of a data logger, pH probes, and thermocouples. The data logger (Model No. 525, SYSCON International Inc., Los Angeles, CA, USA) was connected to the signal conditioning unit and IBM PS2 personal computer through a

serial communication port. Four thermocouples (Cole Parmer, Chicago, IL, USA) were connected to the data logger, whereas four pH probes (Model No. 13-620-104, Fisher Scientific, Montreal, Quebec, Canada) were connected to the data logger through the signal conditioning unit. A Quick Basic environment was used to develop the software and operate the data acquisition system.

Substrate Collection and Preparation

Cheese whey was obtained from the Farmers Cooperative Dairy Plant in Truro, Nova Scotia, Canada and kept at a cold storage facility (Associated freezers of Canada, Dartmouth, Nova Scotia, Canada) at -25^{o}C to minimize microbial and enzymatic degradation. The characteristics of cheese whey used in this study are shown in Table 1. The solid, chemical oxygen demand, and nitrogen analyses were performed according to the procedures described in the Standard Methods for the Examination of Water and Wastewater (APHA, 1985). The lactose concentration was determined using sugar analyzer (YSI Model 27, Yellow Springs, OH, USA) whereas the lactic acid concentration was determined using glucose/L-lactate analyzer (YSI Model 2000, Yellow Springs, Ohio). Phillips Analytical, Dartmouth, Nova Scotia, Canada, performed the elemental analyses. The technique developed by Ghaly and El-Taweel (1995) was used to sterilize the whey. The pasteurization process involved heating the cheese whey at 70^{o}C for 45 minutes, followed by cooling it suddenly in the ice bath at 0^{o}C for 30 minutes and then kept at room temperature (20^{o}C) for 24 hours for spores to germinate. The process of alternating heating and cooling was repeated three times. No microbes were observed under the microscope. This technique was used to avoid denaturing of protein at high temporatures in the autoclave.

Nutrient Supplements

Two complex nutrients (yeast extract and lactamine AA) were added to cheese whey as growth supplements. The yeast extract was obtained from Difco (Difco Laboratories, Detroit, MI, USA). The lactamine AA was obtained from Champlain Industries Company, Mississauga, Ont, Canada. Tables 2 show the composition of yeast extract and lactamine AA, as provided by their manufacturing companies. Both nutrients contain various amino acids. Yeast extract contains various vitamins and Lactamine AA contains various elements.

Inoculum preparation

Lactobacillus helveticus (ATCC 15009) was obtained from the American Type Culture Collection (Rockville, MD, USA). The bacteria was revived and maintained in tomato juice-yeast extract (TJ-YE) broth (ATCC medium 17). The rehydrated bacterial culture was placed in incubator (Series 25, Incubator, New Brunswick Scientific Co. Inc., NJ, USA) at 37 ^{o}C for 3 days on TJ-YE supplemented with 15 g/L agar. The visible colonies of the bacterial culture were scooped from the surface of the agar in two Petri dishes using a sterile loop and transferred into 150 mL of pasteurized cheese whey in a 250 mL sterile Erlenmeyer flask. A total of 34 flasks were then capped with non-absorbent cotton plug and mounted on a controlled environment-reciprocating shaker (Series 25, Incubator Shaker, New Brunswick Scientific Co. Inc., Edison, NJ, USA). The shaker was operated at 250 rpm for 48 h. The bacterial culture were collected from the flasks and stored in refrigerator at 4^{0}C until needed.

Table 1. Some characteristics of the cheese whey.

Characteristic	Concentration (mg/L)
Total solids	68250
Fixed solids	6750
Volatile solids	61550
Suspended solids	25160
Fixed solids	230
Volatile solids	24930
Total Kjeldahl nitrogen	1560
Ammonium nitrogen	260
Organic nitrogen	1300
Total chemical oxygen demand	81050
Soluble chemical oxygen demand	68050
Insoluble chemical oxygen demand	13000
Lactose	48200
Lactic Acid	2200
Potassium	1670
Chlorine	950
Calcium	880
Phosphorus	480
Sodium	435
Sulfur	150
Magnesium	90
Iron	1

pH = 4.9

Table 2. Composition of yeast extract and Lactamine AA

Constituent	Yeast extract	Lactamine AA
Composition (%)		
Moisture	30.0	5.0
Total nitrogen	8.8	13.5
Protein[+]	55.0	84.0
Amino Nitrogen	---	5.4
Ash		6.0
pH (6 % solution)		7.0
Solubility (mg/L)@ 30^{o}C	3 000-10 000	250.0
Amino acids (% of total)		
Alanine	3.4	0.16
Aminobutyric acid	0.1	---
Arginine	2.1	0.20
Asparagine	3.8	0.37
Cystine	0.3	0.01
Glutamic acid	7.2	1.18
Glycine	1.6	0.10
Histidine	0.9	0.15
Isoleucine	2.0	0.27
Leucine	2.9	0.48
Lysine	3.2	0.43
Methionine	0.5	0.14
Ornithine	0.3	---
Phenylalanine	1.6	0.24
Proline	1.6	0.57
Serine	1.9	0.23
Threonine	1.9	0.22
Tryptophan	---	0.04
Tyrosine	0.8	0.11
Valine	2.3	0.39
Elemental profile (%)		
NaCl	<1	
Copper	---	<0.50
Iron	---	23.40
Magnesium	---	0.01
Potassium	---	0.10
Sodium	---	2.70
Vitamin content (ppm)		
Thiamine	20-30	---
Riboflavin	50-70	---
Pyridoxine	25-35	---
Niacinamide	600	---
Pontothenic acid	200	---

[+] Protein = 6.25* (Total nitrogen).

Experimental Protocol

Each bioreactor and its components (including electrodes) were chemically sterilized using 2 % potassium metabisulphite, thoroughly cleaned with hot distilled water and then placed in sterilized plastic bags until needed for use. The corresponding amount of nutrient supplement needed was weighed (24, 48 and 72 g for 5, 10 and 15 g/L) and placed in the bioreactor. The bioreactor was filled with pasteurized cheese whey (4.32 L) and immediately inoculated with 480 mL inoculum to achieve the working volume of 4.8 L. Each bioreactor was covered and the mixing motor was turned on (speed set at 150 rpm). The data acquisition system and the computer were turned on and the computer program was activated. All the experiments were conducted in three replicates.

Samples collection and analyses

Samples of about 25 mL each were collected throughout the experiment for cell number, lactose and lactic acid analyses. The samples were collected every 2 h for a period of 18 h, every 6 h for the next 30 h and then every 8 h until the end of the experimental run. The cell concentration was determined using the dehydrogenase activity measurement procedure described by Tango and Ghaly (1999). The weight of bacterial cell given as $1.2x10^{-11}$ g/cell by Schuler and Kargi (1992) was then used as a conversion factor to calculate the cell mass concentration. The lactose concentration was determined using sugar analyzer (YSI Model 27, Yellow Springs, Ohio), and lactic acid concentration was determined using glucose/L-lactate analyzer (YSI Model 2000, Yellow Springs, Ohio). All the analyses were performed on the samples in doublicate.

RESULTS AND DISCUSSION

Cell Growth

The effects of nutrients supplements on the cell growth of the *L. helveticus* during the batch fermentation of cheese whey are illustrated in Figure 2. Table 3 shows the effects of the nutrient supplements on maximum cell number and specific growth rates of *L. helveticus,* and the effective fermentation time. The lag period and specific growth rate were determined graphically by the procedure described by Ghaly et al. (1989). In this study, the effective fermentation time is the total length of the lag, exponential, and stationary phases. This concept was adopted because no significant amounts of lactic acid were produced after these phases for both nutrients.

The maximum cell number obtained without the addition of nutrient supplements was 116 x 10^6 cells/mL. The addition of nutrient supplements substantially increased the maximum number of cells. Increasing the nutrient supplement concentration up to 10 g/L, increased the number of cells to 282 x 10^6 cells/mL (by 143 %) when lactamine AA was added and to 228 x 10^6 cells/mL (by 96 %) when yeast extract was added. Further increases in the concentration of nutrient supplements (15 g/L) only increased the cell number to 138 x 10^6 cells/mL (by 18 %) for lactamine AA and to 144.3 x 10^6 cells/mL (by 24 %) for the yeast extract. Aeschlimann and von Stockar (1990) observed that supplementation of whey with lower concentrations of nutrients greatly enhances the fermentation by *L. helveticus*, but higher concentrations diminish the cell concentration as a result

of toxicity. Marshall and Earle (1975) reported that the increase in bacterial concentration caused by supplement

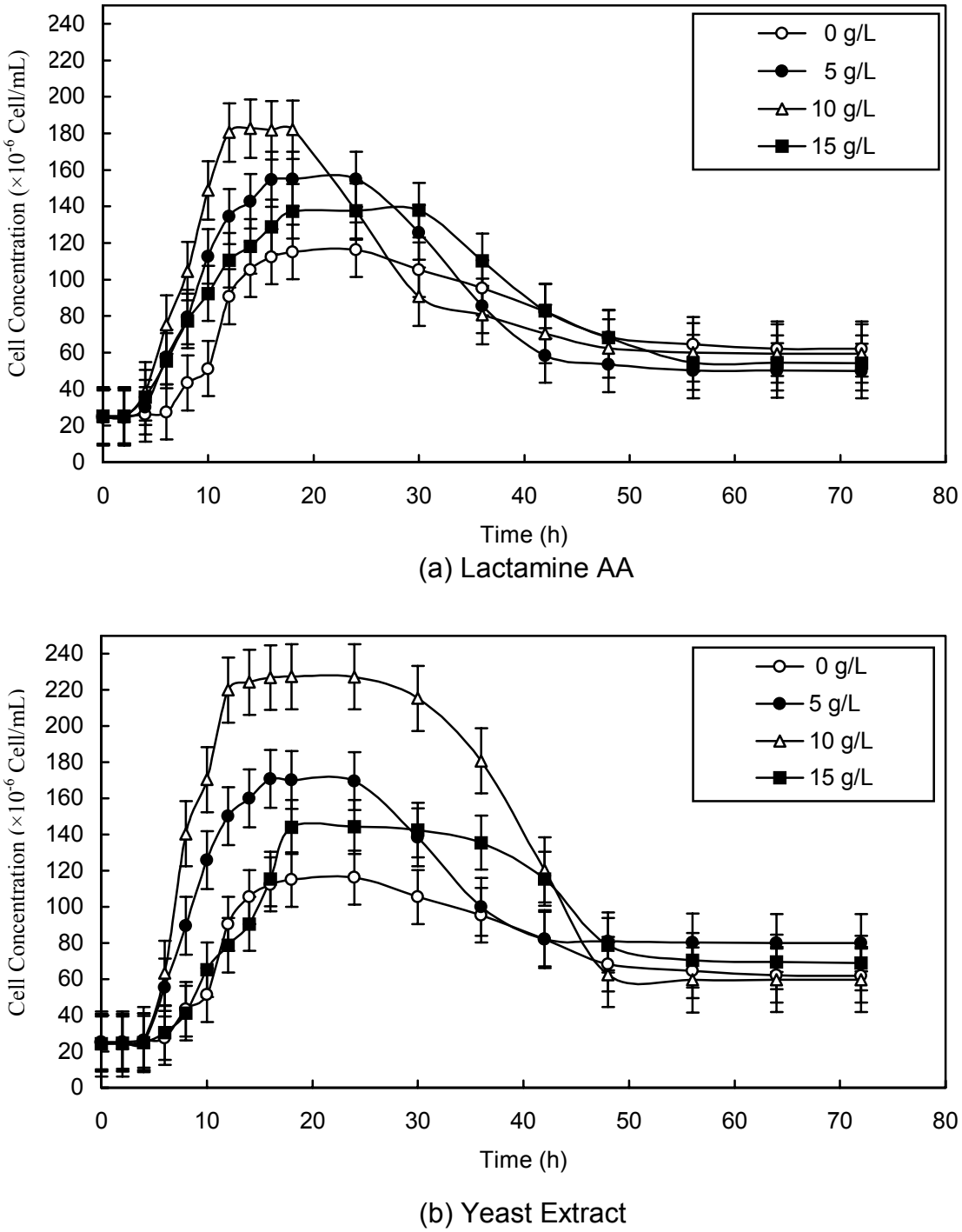


Figure 2. Effect of nutrient supplement concentration on the growth of *L. helveticus*

Table 3. Kinetic parameters for batch fermentation at different nutrient supplement concentrations.+

Nutrient Supplement	Supplement Concentration (g/L)	Maximum Cell Number (10^6 cells/mL)	(%)*	Specific Growth Rate (h^{-1})	(%)*	Effective Fermentation Time: Lag Total (h)	Exponential (h)	Stationary (h)	(h)
None	0	116 ±15	100	0.14±.02	100.0	4.2±0.2	17.8±0.5	8.0±0.3	30.0
Lactamine AA	5	155 ±15	133	0.14±.02	106.0	2.0±0.1	16.0±0.5	6.0±0.2	24.0
	10	182 ± 16	157	0.20±.03	150.0	0.8±0.1	11.2±0.4	6.0±0.2	18.0
	15	138 ± 15	118	0.14±.02	103.0	1.3±0.1	18.7±0.5	12.0±0.4	30.0
Yeast extract	5	171 ± 16	147	0.17±.02	128.0	3.3±0.1	13.2±0.4	14.0±0.5	29.5
	10	228 ± 18	196	0.28±.03	206.0	1.8±0.1	6.7±0.2	11.0±0.3	19.5
	15	144 ± 15	124	0.12±.02	90.0	3.5±0.2	15.5±0.5	13.0±0.4	30.0

* Percentage of value with no supplement

\+ Values are the average of three replicates ± standard deviation.

addition would be advantageous in batch fermentation, since the maintenance acid production component would be increased, and this accounts for most of the lactic acid produced in batch culture.

A specific growth rate of 0.14 h^{-1} was obtained without the addition of nutrient supplements. The addition of 10 g/L of lactamine AA and yeast extract increased the specific growth rate by 50 % and 106 %, respectively. Further increases in lactamine AA and yeast extract concentrations (15 g/L) only increased the specific growth rate by 3 % for lactamine AA and decreased it by 10 % for the yeast extract. The addition of nutrient supplements gave rapid cell growth due to the presence of most bacterial growth factors including amino acids, lipids, nucleotides (purines and pyrimidines), and vitamins (in case of yeast extract) which promote rapid propagation of the cells, enhance metabolism and stimulate the physiological activity of the cells.

The observed lag period without the addition of nutrient supplement was 4.2 h. The addition of nutrient supplements substantially reduced the lag period. Using 10 g/L nutrient supplements resulted in a shorter lag period of 0.8 h for the lactamine AA and 1.8 h for yeast extract. Bailey and Ollis (1986) reported that the length of the lag phase is affected by the nutrient concentration in the medium, whereas Ghaly et al. (1989) stated that the length of the lag

phase depended on the extent to which the medium (nutrient concentration) and the environmental factors (such as pH and temperature) were different from those under which the inoculum was prepared.

The effective fermentation time without the addition of nutrient supplement was 30 h. The addition of nutrient supplement with concentration upto 10 g/L reduced the effective fermentation time, with lactamine AA being more effective than yeast extract. A higher concentration of 15 g/L of each nutrient supplement increased the effective fermentation time over that without nutrient supplements by 2 h. Microorganisms require micronutrients and macronutrients only in trace quantities, as excess amounts result in retarding the synthesis of cellular material and enzymatic reactions. Table 4 shows the mineral elements available in acid cheese whey and their role and concentration of minerals required for cell functions. The addition of lactamine AA and yeast extract supplemented the mineral requirements for different functions of the bacterial cells. With yeast extract addition, only sodium chloride was supplemented (at 18 %), whereas with the addition of lactamine AA, Copper, Iron, Magnesium, Potassium and Sodium were supplemented at 0.5, 23.4, 0.01, 0.1 and 2.7 ppm, respectively.

Cox and MacBean (1977) and Reddy et al. (1976) reported that the addition of growth supplements to cheese whey significantly reduced fermentation time. Aeschlimann and von Stockar (1990) were able to shorten the fermentation time by adding various commercially available growth supplements. In this study, the enhanced viability of the bacteria and the increase in the specific growth rate decreased the effective fermentation time by 35 - 40 %. Such a decrease in the fermentation time has a dramatic reflection on the cost of the fermentation process and thus has a significant impact on the economics of the lactic acid production.

Lactose Utilization

Figure 3 shows the trends of lactose concentration during the fermentation process with the addition of nutrient supplements. The lactose concentration initially decreased slowly (during the lag period), then decreased rapidly (during the exponential growth), and finally decreased slowly (during the stationary and death phases). With no nutrient supplement added to the cheese whey, 92 % of lactose was utilized within 50 h of fermentation. When nutrient supplements were added at concentrations of 5, 10 and 15 g/L, the same percentage of lactose (92.0 %) was utilized after 40, 24 and 32 h, for lactamine AA and after 47, 39 and 49 h for yeast extract, respectively. Aeschlimann and von Stockar (1990) reported that increasing yeast extract concentration (0.0 to 3.0 g/L) decreased the residual lactose concentration from 2.5 to 0.1 g/L. Vahvaselka and Linko (1987) obtained a residual lactose of 3 g/L using yeast extract at concentration of 5 g/L. Reddy et al. (1976) reported only 1.7 % residual lactose concentration (about 1 g/L) when cheese whey was supplemented with yeast extract at 2 g/L. This study has shown that the addition of nutrient supplements are required for complete lactose utilization, but these nutrient supplements may be added to enhance lactose consumption rate and thus reduce the effective fermentation time thereby minimizing the fermentation operating cost.

Table 4. Macronutrients and Micronutrients available in acid cheese whey and their role in cell growth (Jones and Greenfield, 1984).

Element	Concentration (mg/L)	Role
MACROELEMENT		
Nitrogen $(N)^-$	1560	Is an important ionic growth factor determining the rate of fermentantion. It controls the synthesis of protein and nucleic acid.
Phosphorus $(P)^-$	480	Is essential for the growth of cell as well as enhancing the rate of fermentation. It plays an important part in carbohydrate metabolism.
Sulphur $(S)^-$	150	Used as a constituent in proteins such as amino acids (cysteine and methionine) and co-enzymes such as carboxylases.
Potassium $(K)^+$	1670	Enhances tolerance to toxics. Involved in control of intracellular pH. Stabilizes the optimum pH for fermentation. K^+ excretion is used to counter balance uptake of essential ions such as Zn^{2+} and Co^{2+}.
Magnesium $(Mg)^{2+}$	90	Buffers the cell against adverse environmental effects. Involved in activating sugar uptake. Levels of Mg^{2+} are regulated by divalent cation transport system.
MICROELEMENT		
Calcium $(Ca)^{2+}$	880	Is essential for the growth of the cell and stimulates growth and fermentation
Iron $(Fe)^{2+,3+}$	1	In the active site of many cell proteins.
Chlorine $(Cl)^-$	950	Passively diffuses into cells. Stimulates the uptake of some sugars.
Copper $(Cu)^-$	---	Extremely toxic to the cell. Used in the synthesis of enzymes and proteins. Used in the electron transport system.
Sodium $(Na)^+$	435	Acts as a counter ion in the movement of some positive ions.

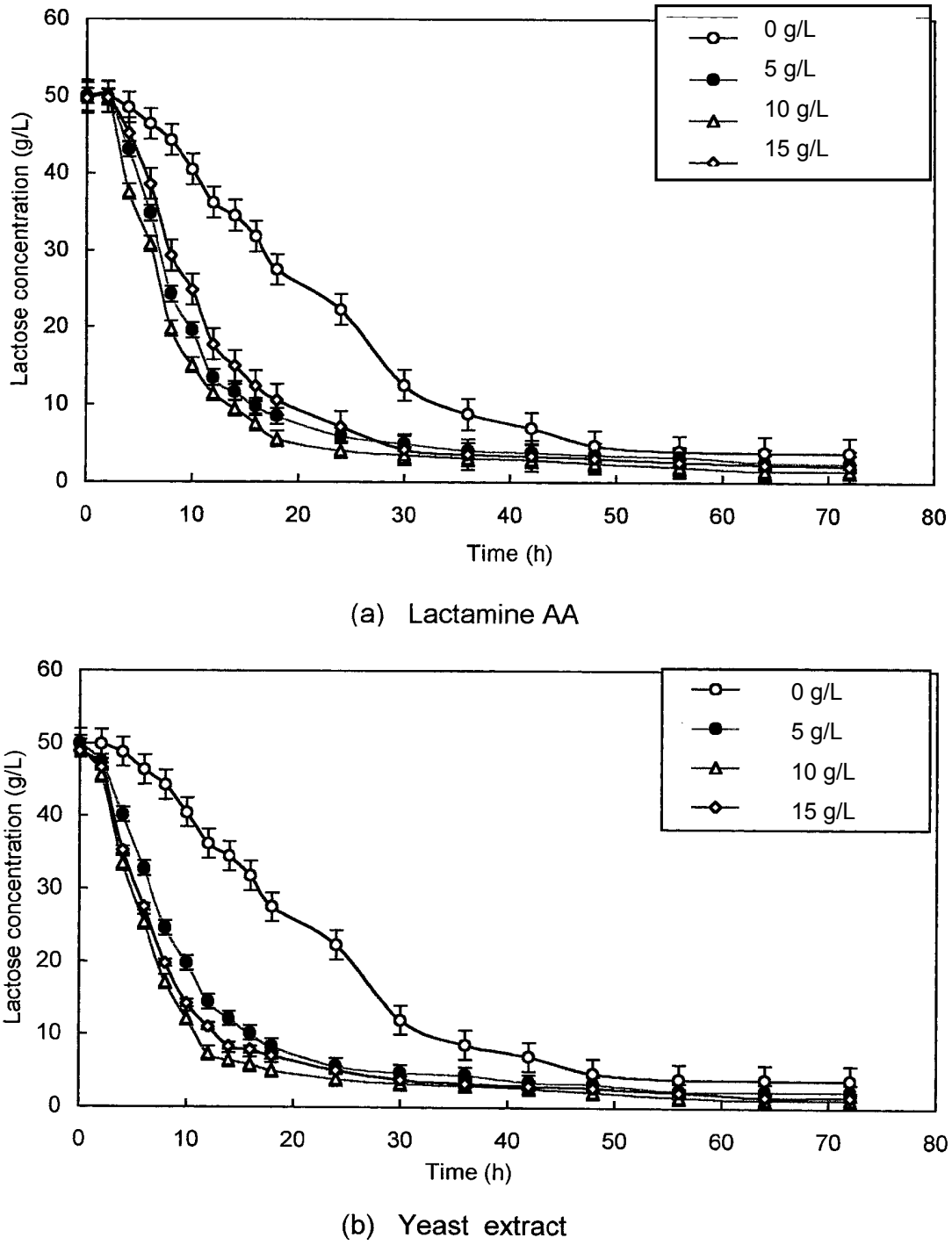


Figure 3. Effect of yeast extract concentrations on lactose utilization by *L. helveticus*

Table 5 shows the effect of nutrients supplements on the average specific rate (g/L/h) of lactose consumption by *L. helveticus* during the various growth phases. Increasing the nutrient supplement concentration (up to 10 g/L), increased the rate of lactose consumption during the exponential growth phase from 1.35 to 3.25 g/L/h for lactamine AA and to 4.15 g/L/h for yeast extract. Further increases (upto 15 g/L) of the nutrient supplement concentration, only increased lactose consumption to 2.16 g/L/h for lactamine AA to 2.32 g/L/h for yeast extract. Norton et al. (1994) and Roy et al. (1987) obtained similar results using yeast extract and corn steep liquor.

Table 5. Rates of lactose consumption (g/L/h) by *Lactobacillus helveticus.*

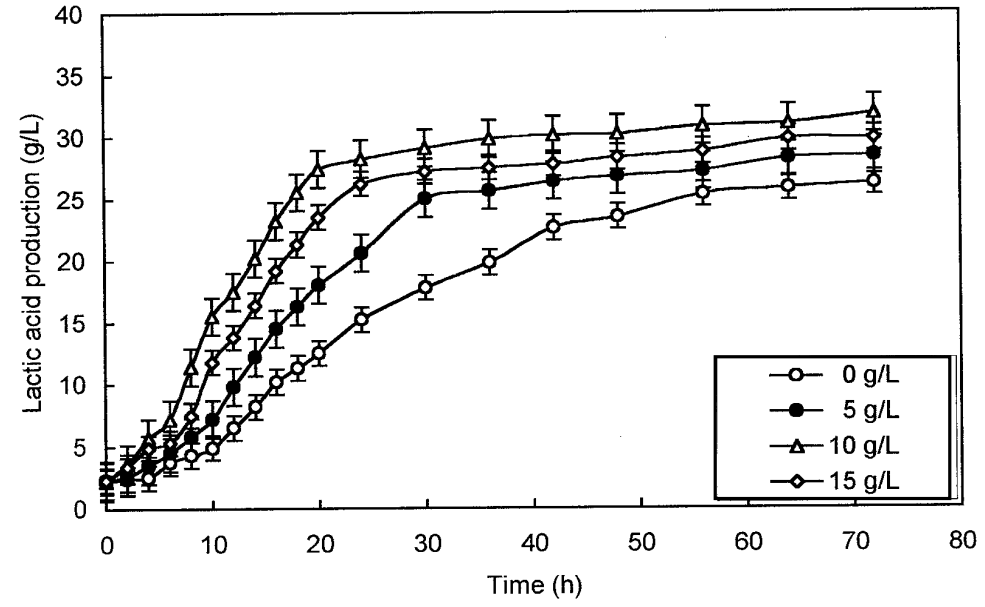
Growth phase	Supplement concentration (g/L)						
	0	5		10		15	
		LAA	YE	LAA	YE	LAA	YE
Lag phase	0.36	0.45	1.39	0.52	2.42	0.38	1.70
Exponential phase	1.35	2.59	2.74	3.25	4.15	2.16	2.32
Stationary phase	1.25	0.41	0.55	0.52	0.59	0.45	0.46
Death phase	0.52	0.09	0.10	0.17	0.17	0.06	0.07

LAA = Lactamine AA
YE = Yeast Extract

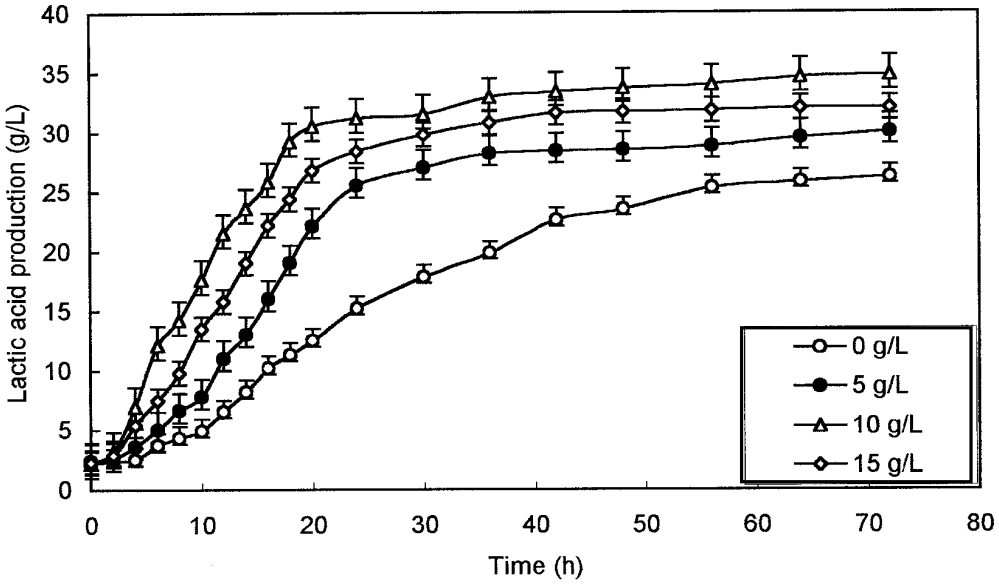
Lactic Acid Production

Figure 4 shows the effect of nutrient supplements on the lactic acid concentration. For all treatments, the lactic acid production increased with the fermentation time. The highest lactic acid concentration was attained at the corresponding maximum cell number. Lactic acid was produced during both growth and non-growth phases. Earlier work by Luedeking and Piret (1959) illustrated these kinetics and Roy et al. (1987) proposed kinetic models to illustrate this phenomenon.

The maximum lactic acid produced from cheese whey without supplements was 15.2 g/L. With the addition of nutrient supplements to the cheese whey at concentration of 5, 10 and 15 g/L, the maximum lactic acid production at the end of the exponential period was 18.0, 23.2 and 23.5 g/L for lactamine AA; and 22.1, 34.8 and 26.8 g/L for yeast extract, respectively. Acschliman and Von Stockar (1990) reported enhanced lactic acid fermentation by *L. helveticus* when using yeast extract.



(a) Lactamine AA



(b) Yeast extract

Figure 4. Effect of nutrient supplement concentration on lactic acid production by *L. helveticus*

Table 6 shows the specific rate of lactic acid production at different nutrient supplement concentrations. Increasing the supplement concentration (upto 10 g/L), increased the rate of lactic acid production during the exponential growth phase to 1.34 g/L/h for Lactamine AA and to 1.85 g/L/h for yeast extract. Further increases (upto 15 g/L) of the nutrient supplement concentration, only increased the rate of lactic acid production to 1.10 g/L/h for Lactamine AA and to 1.44 g/L/h for yeast extract. Ohleyer et al. (1985) and Aeschlimann and von Stockar (1989) observed improved lactic acid production when upto 15 g/L of yeast extract was added to cheese whey. Vahvaselka and Linko (1987) obtained a better acid production rate with yeast extract (when compared with casein hydrolysate, corn steep liquor and malt sprout extract) at 5 g/L. Cox and MacBean (1977) and Boyaval et al. (1987) achieved lactic acid production rate of upto 10.8 g/L/h using 10 g/L of yeast extract during whey permeate fermentation, whereas Roy et al. (1986) and Aeschlimann and von Stockar (1989) reported volumetric productivity of 2.7 and 3.7 g/L/h for medium supplemented with 15 g/L yeast extract, respectively.

Table 6. Rates of lactic acid production (g/L/h) by *L. helveticus.*

Growth phase	Supplement concentration (g/L)						
	0	5		10		15	
		LAA	YE	LAA	YE	LAA	YE
Lag phase	0.14	0.15	0.11	0.38	0.91	0.46	0.54
Exponential phase	0.54	0.87	0.99	1.34	1.85	1.10	1.44
Stationary phase	0.55	0.68	0.94	1.33	0.97	0.33	0.27
Death phase	0.30	0.28	0.11	0.16	0.12	0.06	0.08

LAA = Lactamine AA
YE = Yeast Extract

Conversion Efficiencies

Tango and Ghaly (1999) stated that during lactose fermentation, lactose is utilized by *L. helveticus* (homofermenter) for the cell growth and maintenance, energy release and production of lactic acid according to the following Equations:

(a) Cell Growth (Synthesis):

$$\mathbf{5}\ C_{12}H_{22}O_{11} + \mathbf{12}\ NH_4^+ \xrightarrow{\textit{Bacteria}} \mathbf{12}\ C_5H_7NO_2 + \mathbf{31}\ H_2O + \mathbf{12}\ H^+ \qquad (1)$$

(b) Product formation and respiration:

$$\mathbf{45}\ C_{12}H_{22}O_{11} + \mathbf{45}\ H_2O \xrightarrow{\textit{Bacteria}} \mathbf{180}\ C_3H_6O_3 + \mathbf{Energy} \quad (2)$$

The net biochemical reaction of the above two Equations can be written as follows:

$$\mathbf{50}\ C_{12}H_{22}O_{11} + \mathbf{12}\ NH_4^+ + \mathbf{14}\ H_2O \xrightarrow{\textit{Bacteria}} \mathbf{180}\ C_3H_6O_3 + \mathbf{12}\ C_5H_7NO_2 + \mathbf{12}H^+ + \mathbf{Energy} \quad (3)$$

From equation (3), the stoichiometric lactic acid yield ($Y_{P/S}$) was estimated to be 0.95 g lactic acid/g lactose and the cell yield ($Y_{X/S}$) was found to be 0.08 g cell/g lactose. A small proportion of lactose is also used for the cell maintenance and release of energy.

Table 7 shows the effects of the nutrient supplements on the cell yield (cells / g lactose), actual lactic acid yield (g lactic acid / g lactose) and conversion efficieny. The lowest lactic acid yield (0.57 g lactic acid/g lactose) was obtained with no supplements added. This was increased to 0.65 and 0.70 g lactic acid / g lactose when lactamine AA and yeast extract were used at a concentration of 10 g/L.

Table 7. Effect of nutrient supplement concentration on the yield and conversion efficiency for the cell and lactic acid.

Supplement	Concentration	Cell		Lactic Acid	
	(g/L)	Yield** (g cells/g lactose)	Conversion efficiency (%)	Yield* (g lactic acid/g lactose)	Conversion efficiency (%)
None	0	0.030	37.5	0.57	60.0
Lactamine AA	5	0.039	48.7	0.60	63.2
	10	0.045	56.3	0.65	68.4
	15	0.035	43.7	0.63	66.3
Yeast extract	5	0.042	52.5	0.63	66.3
	10	0.057	71.3	0.70	73.7
	15	0.037	46.2	0.68	71.6

* Stoichiometric lactic acid yield = 0.95 g lactic acid/ g lactose
** Stoichiometric cell yield = 0.08 g cell/ g lactose

Conversion Efficiency = (Actual Yield/ Stoichiometric Yield) x100

The lactic acid conversion efficiency (experimental yield/theoretical yield) obtained in this study was 60.0 % for cheese whey with no supplement. However, the highest conversion efficiencies were 68.4 and 73.7 % with lactamine AA and yeast extract (at 10 g/L) respectively. The lactic acid conversion efficiencies obtained in this study are comparable to those reported by other researchers. Aeschlimann and von Stockar (1990) and Siebold et al. (1995) reported the maximum lactic acid efficiencies of 72 and 97 %, respectively.

The cell yield was 0.030 g cells/g lactose for cheese whey with no supplement. The highest cell yield of 0.057 g cell/g lactose was achieved when 10 g/L yeast extract was added to the cheese whey, indicating a 90 % increase in conversion efficiency. This cell conversion efficiency (71.3 %) appears low due to the product inhibition.

The cells inability to deplete the residual lactose in the medium was an indication of lactic acid inhibition, even though the maximum lactic acid concentration (34.8 g/L) obtained in this study was below the inhibition level of 40 g/L reported by Ohleyer et al. (1985).

CONCLUSION

With no nutrient added to the cheese whey, 92 % of the lactose was utilized after a fermentation time of 50 h, whereas about 92 and 94 % of the initial lactose concentration was utilized after 24 and 40 h when lactamine AA and yeast extract were used at the concentration of 10 g/L, respectively. Increasing the amount of both yeast extract and lactamine AA above 10 g/L, resulted in slight decreases in the maximum cell number. Each of the supplements significantly increased the cell growth; with yeast extract being more effective than lactamine AA. Using yeast extract at a concentration of 10 g/L enhanced viability of the bacteria, increased the specific growth rate (by 106%) and decreased the total fermentation time by 33 % and produced the highest lactic acid (34.8 g/L). Cell growth inhibition was observed with the high concentration (15 g/L) yeast extract and lactamine AA.

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