NONASEPTIC CONTINUOUS DIALYSIS FERMENTATION OF CONCENTRATED WHEY TO PRODUCE RUMINANT FEEDSTUFF AND LACTIC ACID

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ABSTRACT

NONASEPTIC CONTINUOUS DIALYSIS FERMENTATION OF CONCENTRATED WHEY TO PRODUCE RUMINANT FEEDSTUFF AND LACTIC ACID

By

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A continuous dialysis fermentation was developed to convert concentrated waste whey into a crude protein feedstuff for ruminant animals and ammonium lactate. The feasibility was predicted by a mathematical model simulated on a digital computer. These predictions then were tested experimentally. Dried sweet whey was reconstituted to almost 5 times the concentration of normal liquid whey. The concentrated whey (23% lactose) plus 0.8% yeast extract was charged into a 5-liter fermentor and a continuous culture of *Lactobacillus bulgaricus* was maintained at 44 C and pH 5.3. The whey lactose was converted into lactic acid, which was neutralized by ammonia to ammonium lactate. A series of steady-state conditions was managed nonaseptically for 76 days to study the kinetics and parametric variables of the process. Subsequently, the process was operated at more or less optimum conditions for 18 days to determine efficiency and productivity. As the fermentation progressed with time, there occurred an actual increase in efficiency of the conversion. At optimum conditions the substrate was converted essentially completely to bacterial cells and ammonium

lactate, with less than 0.1% of other compounds. With an optimum retention time of 18.5 hr and an optimum ratio of fermentor effluent flow rate to reservoir effluent flow rate of .405, there resulted a residual lactose concentration of 0.2% in the fermentor and 0.05% in the reservoir, corresponding to lactate concentrations of 7.9% and 2.5% respectively. The process yielded two product streams: the fermentor effluent contained ammonium lactate, bacterial cells and residual whey proteins, which could be used as nitrogenous foods for ruminants; the reservoir effluent contained only ammonium lactate, which could be used for producing lactic acid and ammonium sulfate for industrial and agricultural uses. Continuous dialysis fermentation proved to be at least 4 times more efficient than an ordinary continuous fermentation in the rate of substrate conversion and to yield not only useful feedstuff but a source for pure lactic acid.

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1. INTRODUCTION

Whey utilization is a large and worsening problem to the dairy industry. American cheesemakers annually discard about 13 billion pounds of liquid whey because of its limited market value. Much is spread on fields and dumped in quarries and streams. Since whey contains 6 to 7% solids, about half of those in milk, its waste is not only an environmental problem but a severe economic and food loss (43). The problem is increasing: the U.S. Department of Agriculture estimates that there will be an additional 750 million pounds of whey solids within the next few years (19).

Much research has focused on the development of methods and uses for dried or concentrated whey (54). The processing of whey by pressure filtration through semipermeable membrane systems to obtain milk protein concentrates is promising. Both pilot-scale and production plants for such ultrafiltration of whey have operated for several years in recovering 40 to 70% protein concentrates (24, 44, 53). The drawback of this process, however, is the concurrent production of large volumes of lactose-containing ultrafiltrate, which is as much a pollution problem as the whole whey. Possible uses of the whey or lactose permeate (ultrafiltrate) are: production of chemical products; fortification of skim milk; hydrolysis to glucose-galactose syrup; and fermentations to yeast protein, alcohol, vinegar, vitamins or lactic

acid (24, 76). Researchers have recently reported wine fermentations using hydrolyzed whey permeate syrups (65).

A prime goal in agriculture is the production of animal feed that is noncompetitive with human food, and microbial fermentations can increasingly be used for this purpose. A model for such fermentations and a potential solution to the whole whey and lactose permeate problems exists in the conversion of these wastes into a feed supplement containing a high concentration of crude protein (N X 6.25), which can be used for ruminant animals such as cattle. The conversion has recently been shown to be economically feasible, and the safety and efficacy of the crude product has been demonstrated (20, 22). The conversion process essentially involves the homofermentative bacterial conversion of whey lactose into lactic acid under nonaseptic conditions, with neutralization by ammonia. The primary purpose of the fermentation is to trap ammonia into a salt form so it can be tolerated when fed to cattle, and thereby so the crude protein content of the feed can be increased. The product has been proven comparable to soybean meal in nutritional quality and better than urea with respect to toxicity (23). Patent applications have been filed by Michigan State University for batch and continuous operation of the process (21, 31).

A rationale similar to that for recycling whole whey can be applied not only to whey ultrafiltrate but also to other natural waste products, although with different organisms and different acid products. For example, colleagues at MSU have demonstrated the

feasibility of converting cattle manure into ammonia-enriched feedstuff for ruminants (63).

Although whey is not the cheapest substrate for industrial lactic acid fermentations (19), this potential use of whey should be considered because lactic acid is a valuable primary industrial chemical for which synthetic production costs are high (42). Lactic acid has many uses in the food, pharmaceutical and chemical industries (41). Furthermore, a promising new technique exists for recovering lactic acid from a fermentation that is neutralized with ammonia (61). The method involves the use of an ion exchange system and a by-product of the method is $(NH_4)_2SO_4$, which may be used as a fertilizer (41).

The practical usefulness of these conversions lies in the ability to conduct the fermentation as efficiently as possible, with substrate and product solids concentrated as much as possible, and desirably with the products separated for alternative uses.

The impetus for further research laid with the idea of improving these goals of the whey fermentation by the application of membrane technology. A modified mathematical model supported this idea. Accordingly, the primary objective of the present research was to determine the theoretical and practical feasibility of continuous dialysis fermentation for converting whey into a source of crude protein for cattle and of pure lactate for industry. It was also hoped that the work would provide a demonstration and analysis of the operational parameters and the biological performance of continuous dialysis fermentation. The following elements of continuous dialysis fermentation were evaluated experimentally: retention time in

fermentor, rate of water through the reservoir, culture stability under nonaseptic conditions, and product quality.

2. HISTORICAL BACKGROUND

2.1 Ammonium Lactate as a Ruminant Feed

Researchers have long recognized that whey and similar materials can be converted by fermentation into sources of crude protein for ruminants. Several reports and patents exist for such fermentation processes, as reviewed recently by Keller (29). In the last few years, because of the increasing whey problem and costs of protein supplements, colleagues at Michigan State University have developed a simple and economical process for the conversion of cheese-whey to fermented, ammoniated, condensed whey ("Bactolac") and researched its value as a protein supplement (20, 22, 23). In two feeding trials, they found ammonium lactate is essentially equal to soybean meal and superior to urea with an improved feed efficiency as a source of supplemental protein for 144 yearling steers. The steers were able to consume twice as much ammonium lactate nitrogen as urea nitrogen before toxic levels were reached. Urea accounted for 75% of the crude protein content of liquid supplements manufactured and sold in 1973 (20).

2.2 Continuous Lactic Acid Fermentations

A continuous process for the production of lactic acid from whey was developed and operated on both laboratory and plant scale by

Whittier and Rogers (78). The appealing characteristic of this process is that sterilization of equipment and substrate are not required to prevent contamination by deleterious organisms. The restrictive conditions, 44 C and pH 5.0-5.8, maintain the lactic fermentation free of contamination. However, problems resulting from the crystallization of residual sugar in the product impede recovery of the acid.

Variations of this method have been used with limited success for the continuous cultivation of starter cultures for the dairy industry (3, 33, 35, 36, 37, 38, 45, 56). Only Lewis firmly adhered to the restrictive conditions and thus was able to keep a viable population of cells with no contamination for a useful length of time (33). She managed a continuous flow yogurt starter culture of mixed populations of *Lactobacillus* and *Streptococcus* for periods up to one month. In 1974, MacBean demonstrated the feasibility of this mixed culture fermentation for the continuous production of yogurt (40).

Recently, Keller and Gerhardt showed that continuous fermentation was a simple and efficient improvement for converting whey into a source of crude protein for cattle (30). The method was run non-aseptically for 6 weeks with excellent product quality and with an actual increase in conversion of the whey lactose to ammonium lactate.

2.3 Dialysis Culture

Dialysis culture is the growth of microorganisms in an environment at least partially enveloped with a semipermeable membrane. Solutes in the immediate vicinity of the microorganisms, whether metabolic products or nutrients, are able to diffuse through the membrane in the direction of and in proportion to the concentration gradient.

The concept of dialysis culture has been extensively reviewed and refined by Schultz and Gerhardt (67). The review details the several methods of dialysis culture operation, the theory of dialysis operation, and the many microorganisms studied in dialysis culture. A thesis by Humphrey gives a thorough presentation and analysis of the operational parameters and the biological performance of dialysis culture (27). He used a new and specifically designed dialyzer for dialysis; the same dialyzer was used in the present project.

Much earlier, Gerhardt and Gallup had noted the greater growth and lactic acid production of *Lactobacillus acidophilus* in dialysis culture (15). This observation was explained by the diffusion of lactic acid from the bacterial culture to the reservoir, thereby relieving the product inhibition effect. This was confirmed and extended by Friedman and Gaden, who also managed a dialysis lactic acid fermentation (12).

Dialysis culture can be used to facilitate recovery of a product (16). Abbott and Gerhardt determined the extent to which dialysis could increase product formation in a model semicontinuous fermentation process, the conversion of naphthalene to salicylic acid by *Pseudomonas fluorescens* (1). The dialysis fermentation was calculated to be 2.6-fold more productive and enabled production based mainly on maintenance metabolism, so relatively little substrate was wasted upon bacterial growth. Altogether, these previous studies gave promise of applying dialysis culture to the whey fermentation.

Current reports which concern dialysis culture have been few. Preparation of concentrated dairy starter cultures of lactic acid

bacteria by use of dialysis was recently patented (55). The cultures increased to populations of at least 5 X 10^{10} organisms per ml. Hsu and Daly used a dialysis technique to study the repression of polygalacturonic acid lyase in a culture of *Aeromonas liquefaciens* (25). Their dialysis culture method produced high enzyme yield at low substrate cost. Lane in Australia presently is developing a dialysis whey ultrafiltrate fermentation for the production of valuable yeast protein (32).

Colleagues in Gerhardt's laboratory have recently developed novel systems for in vivo hemodialysis culture of microbial and mammalian cells (2, 18, 48, 62). This application of dialysis culture enables a culture to grow on nutrients from the blood stream of a living animal, yet remain separate from the macromolecular and cellular defense mechanisms of the blood. Moreover, study can be made of the toxic effect to the host of dialyzable metabolic products of the parasite cells.

3. MATERIALS AND METHODS

3.1 Bacterial Culture

Lactobacillus bulgaricus strain 2217 (Chris Hanson's Laboratory, Milwaukee, Wis.), obtained from Reddy (22), was used as the inoculum for the first fermentation. The culture was maintained in a sterile medium of 10% skim milk powder and 90% tap water contained in 25-ml screw-top test tubes. Each week the culture was transferred to fresh medium. Inoculated tubes were incubated at 44 C for 8 to 12 hr, till coagulation of the milk occurred. After coagulation, the cultures were stored at 4 C.

3.2 Substrate

Sweet dairy whey powder was obtained from Galloway West Co., Fond Du Lac, Wisconsin. The whey was reconstituted by adding 6,000 g of tap water and 61 g of yeast extract to 1,600 g of whey powder in a plastic carboy. The carboy was shaken and stored at 4 C for 24 hr to completely dissolve the powder. The whey solution consisted of 23% lactose and 0.8% yeast extract (approximately 28% total solids).

3.3 Fermentation Equipment

As the fermentations progressed, improvements were made in some of the component parts of the whole fermentation system (Figure 1).

Fig. 1. Photograph of continuous dialysis fermentation system used in the experimental investigation.

Only the equipment comprising the final fermentation set-up is described here. Figure 2 is a schematic diagram of the system.

The fermentations were carried out in a 5-liter bench-top fermentor with automatic temperature control (Microferm Model MF 105, New Brunswick Scientific Co., Inc., New Brunswick, N.J.). The pH was controlled by an automatic pH control module (Model pH-22, New Brunswick Scientific Co., Inc., New Brunswick, N.J.).

The fermentation was conducted by continuously pumping the whey solution from an unsterilized feed reservoir into the fermentor and allowing the product (containing bacterial cells) to overflow continuously into a product reservoir, thus maintaining a constant fermentor volume. Concurrently, the culture in the fermentor was continuously circulated through a dialyzer, where solutes were exchanged against a continuously circulating stream of reservoir tap water. Fresh tap water was continuously pumped into the reservoir circuit and reservoir product (free of bacterial cells) was allowed to overflow continuously into a second product reservoir. A reservoir vessel was not utilized for purposes of modeling and to minimize the chance of dialysate contamination. The reservoir circuit consisted only of tubing, a circulating pump, and the reservoir side of the dialyzer.

Nitrogen was continuously purged into the fermentor and served as a device to force the product up and out of the fermentor to the fermentor product reservoir (29).

Concentrated NH_4OH (approximately 58%) was used to neutralize the acid produced during the fermentations.

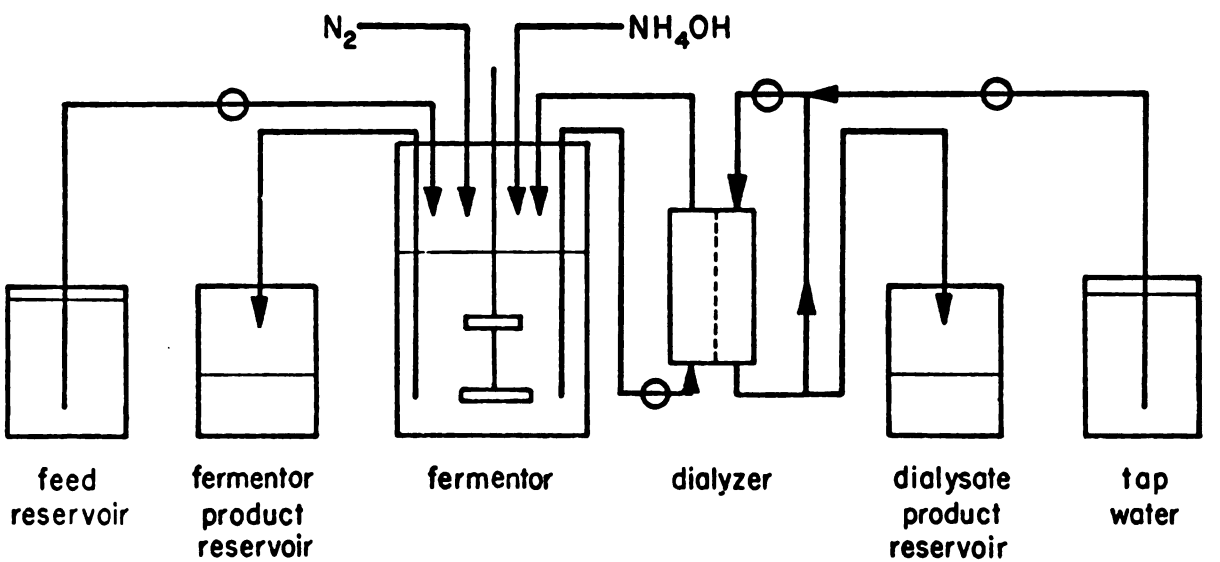

Fig. 2. Schematic diagram of continuous dialysis fermentation system used in the experimental investigation.

The feed reservoir was a 5-liter glass fermentor jar. A stirring motor (Arthur H. Thomas Co., Philadelphia, PA) was used to prevent settling of the whey solution solutes in the jar. The reservoir was kept in an insulated cooler which was replenished daily with ice. The temperature of the feed reservoir never exceeded 6 C.

A plate-and-frame dialyzer was used; it was designed and constructed by Gerhardt and co-workers (17, 27, 67). It is a multi-membrane dialyzer especially suitable for dialysis culture and other biological applications. The dialyzer consists of two end plates that compress an alternating series of thin metal frames, gaskets, sheet membranes (Visking Regenerated Cellulose, Union Carbide Corp., Chicago, Ill.), and molded silicone rubber separators. Eight membranes were used providing .23 m^2 of total surface area.

Finger-type peristaltic pumps (Model T 8 and Model T 6S, Signamotor Company, Middleport, N.Y.) controlled the flow rates of feed and water to the fermentor and reservoir circuits, respectively. Polyurethane tubing, obtainable from the pump manufacturer, was used for the section of line that passed through the pumps.

Positive displacement variable speed pumps (Maisch Biological Metering Pump, Model HQDCC, 11029NM, Tuthill Pump Co., Chicago, Ill.) were inserted in the fermentor circuit and in the reservoir circuit, circulating the fluids from their respective vessels through the dialyzer and back.

3.4 Procedure for Continuous Fermentation

To begin the fermentation, the fermentor was filled with 2.9 liters of the concentrated whey solution. Next, the temperature (44 C) and pH (5.3) were adjusted and 100 ml of inoculum were added. The fermentation was allowed to proceed batch-wise for approximately 18 hr. At this time the fermentor was connected to the dialyzer and reservoir circuit and circulation of fluids were begun. Concurrently, 0.6 liters of fresh whey were added to the fermentor because of the larger volume of the fermentor circuit. For equilibrium purposes, the connected compartments were permitted to proceed batch-wise. After 5 hr the continuous feed and continuous tap water were started. Approximately every 12 hr, samples were taken from a glass "T" inserted in the circuit between the fermentor and dialyzer and from the effluent port of the reservoir circuit.

Usually the fermentor retention time was manipulated by adjusting the fermentor volume. But the feed flow rate was also a significant factor in the reported retention times. The process parameter ϕ was varied by adjusting the flow rate of tap water into the reservoir circuit.

The continuous dialysis fermentation system was in service for a total of 94 days. The process was interrupted at various times for either dialyzer cleaning, fermentor cleaning, or installation of a better component for the system. During these interruptions, the fermentor contents were stored at 4 C. The culture was never reinoculated at the times when it was put back to work.

3.5 Fermentor Measurements

The pH of a sample from the fermentor was regularly checked on a separate pH meter which was calibrated against buffers of known pH. If necessary, the appropriate correction was made.

The temperature of the fermentor contents was continuously recorded and never varied more than ±0.5 C. Periodically, a thermometer was inserted through the inoculation port of the fermentor to verify the recorded temperature.

Fermentor and reservoir samples were quickly placed in a freezer and kept there until the time of analysis.

All input and output solution volumes of the system were measured by use of a graduated cylinder to determine the respective flow rates.

3.6 Lactose Determination

Lactose determinations were made by the soluble carbohydrate analysis of Dubois et al. as modified by Montgomery (9, 50).

One ml of sample was diluted with water so that the lactose concentration was between 5-50 ug/ml. Next, a 1 mg of lactose/ml stock solution was diluted 1:9 to 100 ug/ml. Using 1, 2, and 4 ml of stock solution and sufficient water to bring the volume to 10 ml, three working standards then were made: 10 ug/ml, 20 ug/ml, and 40 ug/ml. Aliquots of 2 ml of each of the standards, each of the diluted samples, and one aliquot of water (zero blank) were transferred to 20 X 150 mm test tubes. To each tube was added 1.0 ml of phenol reagent (25 g of phenol crystal to 500 ml of distilled water) and, as soon as possible, 5.0 ml of concentrated H_2SO_4 were added

directly and rapidly to each solution (the solutions should bubble on addition of the H_2SO_4). The tubes were then vortexed and left standing at room temperature for approximately 1 hour. At 485 nm, a Bausch and Lomb Spectronic 20 zeroed with the water blank was used to determine the O.D. of each sample and standard.

3.7 Ammonium Determination

The ammonium concentrations in the fermentor and reservoir circuits were used as an index of lactic acid production and as an indication of the efficiency of the dialyzer in exchanging solutes. The concentrations were determined by a modification of a Nesslerization colorimetric method reported by Johnson (28).

Samples of 1 ml were diluted with water so that the ammonium concentrations were between 4-40 ug/ml. One ml of each diluted sample was placed in a spectrophotometer cuvette. Standards were prepared from a 1 mg of $(NH_4)_2SO_4$/ml stock solution. The solution was first diluted 1:9 and then 0.1, 0.3, 0.5, 0.7 and 1.0 ml of the diluted standards and sufficient water were added to a cuvette to bring the volume to 1.0 ml. To the sample and standard cuvettes were added 2.0 ml of Nessler's reagent and 3.0 ml of 2N NaOH. The Nessler's reagent contained (per liter) 4.0 g of KI, 4.0 g of HgI_2 and 1.75 g of gum ghatti. The contents of the cuvette were mixed and allowed to develop color at room temperature for 15 min. The absorbance was read at 490 nm. Blanks contained 1.0 ml of distilled water in place of the 1.0 ml sample.

3.8 Lactic Acid Determination

Product quality was examined and lactic acid concentrations were determined by the gas chromatographic procedure of Carlsson (5). One ml of a sample was applied on 1 ml of packed cation-exchange resin (Dowex 50W-X8, 100-200 mesh, H-form, washed in water; Bio-Rad Laboratories, Richmond, Calif.) on glass wool in a Pasteur pipette. After the sample drained through the resin,* the resin was then washed twice with 0.5 ml of redistilled water. All the fluid was collected from the pipette and a 2.0 ul sample was directly analyzed in a gas chromatograph (Model 810, hydrogen flame detector, F&M Scientific Corp., Avondale, Pa.). The column was a 6 ft. by 1/4 in. O.D. and 2 mm I.D. coiled glass column (HP 810, Type 3, Septum to Flame Detector, Supelco, Inc., Bellefonte, Penn.) prepacked with a porous polymer (Chromosorb 101, 80/100 mesh, Supelco, Inc., Bellefonte, Penn.). The column was conditioned overnight with the carrier gas at 250 C and then run isothermally at 220 C. The injection port temperature was 250 C and the detector temperature was 230 C. The carrier gas was 10 ml per min. of nitrogen, and the respective hydrogen and air pressures were 7.5 and 11.0 psig.

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*One ml of a fermentor sample would not drain through the resin because of the high cell concentration. Accordingly, a 5 ml fermentor sample was centrifuged (Sorvall Superspeed RC-2B, Ivan Sorvall, Inc., Norwalk, Conn.) for 10 min at 12,000 X g to remove the cells. The supernatant was decanted, measured by graduated cylinder, and 1 ml was then allowed to drain through the resin. The rest of the procedure followed as above. The chromatographic results were corrected for the loss in volume of cells to get the reported values.

4. THEORY

A mathematical model describing growth and product formation in the microbial fermentation of glucose to sodium lactate was developed two decades ago (39). Recently, the model was modified for the continuous fermentation of whey lactose to ammonium lactate (30). A model also exists for dialysis fermentation (67). Together, these models served as the basis for the theoretical study of this project.

Figure 3 is a schematic diagram for a generalized continuous dialysis fermentation system corresponding to that of Schultz and Gerhardt (67). The symbols are those used previously (30, 67). The assumption is made that high mixing and recycling rates insure homogeneity throughout the system, that turbulence and excess surface insure insignificant fouling and permeability limitation of the dialyzer membranes for a useful period of time, and that bacterial metabolic rates remain constant. A reservoir vessel for dialysate was essentially eliminated, and the volume of the reservoir circuit was assumed to be negligible relative to the fermentor volume for purposes of modeling ($V_r = 0$).

By use of the standard conservation method of formulation, a set of mathematical relationships were developed analogous to those of Schultz and Gerhardt (66). Balances of substrate, product, and bacterial mass of the system can be stated as:

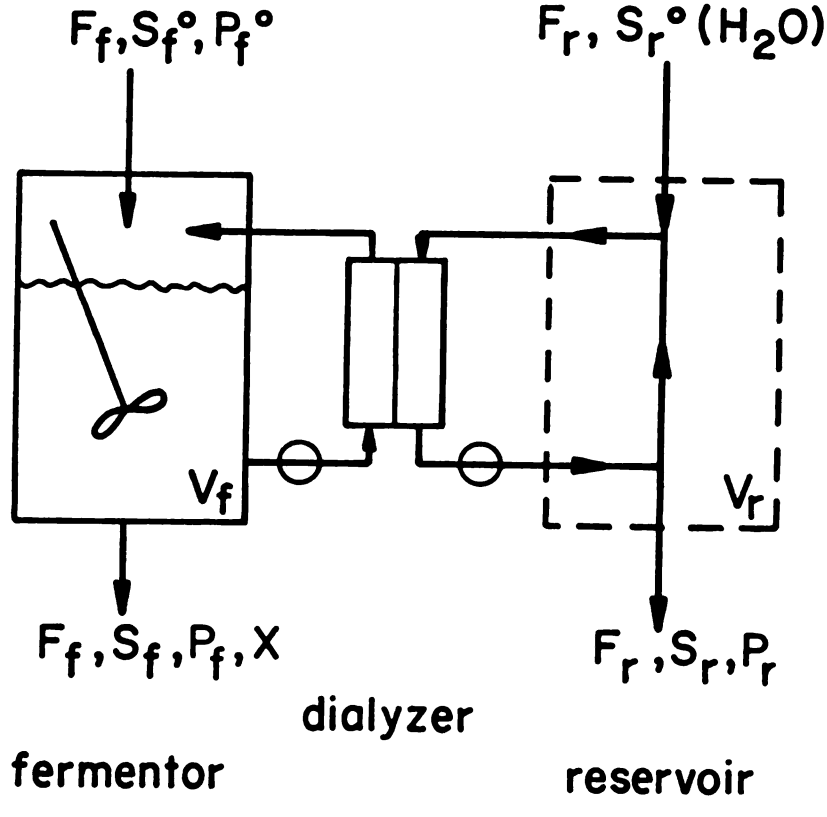

Fig. 3. Schematic diagram of a completely continuous dialysis fermentation system. The symbol F is the flow rate, S the substrate concentration, P the product concentration, V the volume and X the cell concentration into, within and out of the fermentor and reservoir vessels.

Rate of Feed	+	Rate of Production	=	Rate of Withdrawal	+	Rate of Accumulation

For the fermentor chamber, the differential equations are:

$$F_f S_f^{o} + V_f r_s = F_f S_f + P_{ms} A_m (S_f - S_r) + V_f \frac{dS}{dt}f \quad (1)$$

$$F_f P_f^{o} + V_f r_p = F_f P_f + P_{mp} A_m (P_f - P_r) + V_f \frac{dP}{dt}f \quad (2)$$

$$0 + V_f r_g = F_f X_f + V_f \frac{dX}{dt}f \quad (3)$$

For the dialysate chamber, the relationships are:

$$P_{ms} A_m (S_f - S_r) + 0 = F_r S_r + 0 \quad (4)$$

$$P_{mp} A_m (P_f - P_r) + 0 = F_r P_r + 0 \quad (5)$$

The terms, $P_{ms} A_m (S_f - S_r)$ and $P_{mp} A_m (P_f - P_r)$, describe the diffusion of the respective solute across the dialyzer membrane. They are based on Fick's law of diffusion which states that the rate of diffusion through a uniform film of liquid is related to the permeability through the film, the area of the film, and the concentration gradient across the film. An assumption was made that the ratio of the permeabilities for substrate and product solutes were inversely proportional to their molecular weight ratio (i.e., $P_{mp} = .25\ P_{ms}$).

The fermentation rate relationships are accepted forms. The rate of product formation is related to substrate utilization by Monod's yield coefficient (49):

$$r_p = -\gamma r_s \quad (6)$$

TABLE 1. Glossary of symbols.

Symbol	Definition	Units
F_f	flow rate into and out of the fermentor	ml/min
F_r	flow rate into and out of the reservoir	ml/min
S_f^o	substrate concentration in the feed stream	mg/ml
S_f	substrate concentration in the fermentor	mg/ml
S_r	substrate concentration in the reservoir	mg/ml
P_f^o	product concentration in the feed stream	mg/ml
P_f	product concentration in the fermentor	mg/ml
P_r	product concentration in the reservoir	mg/ml
X_f	cell mass concentration in the fermentor	mg/ml
V_f	volume of liquid in the fermentor	ml
$-r_s$	rate of substrate utilization due to growth	mg/min
r_p	rate of product formation due to growth	mg/min
r_g	rate of growth of the organism	mg/min
P_{ms}	permeability of the membrane to substrate	$mg/min/cm^2$
P_{mp}	permeability of the membrane to product	$mg/min/cm^2$
A_m	area of membrane	cm^2
t	time	min

As suggested previously, the substrate utilization rate includes growth and maintenance terms (39):

$$-r_s = \alpha r_g + \beta X_f \tag{7}$$

where α and β are empirical constants. The first term on the right states that the rate of substrate utilization is proportional to the growth rate of the culture. The second term states that the substrate utilization rate is also a function of the biomass of the culture.

The rate-of-growth expression was selected to include both substrate limitation and product inhibition (26):

$$r_g = \mu_m \left(\frac{S_f}{K_s + S_f}\right)\left(\frac{1}{1 + P_f/K_p}\right) X_f \tag{8}$$

where: μ_m = maximum specific growth rate of the organism

K_s = Michaelis-Menten saturation constant

K_p = product inhibition constant

The former is analogous to an enzyme reaction and relates bacterial growth to the substrate concentration. Although substrate limitation was not considered significant in previous work (30), it was believed that higher populations and higher conversion rates resulting from dialysis may cause substrate concentrations to occur at limiting levels. The product inhibition term is based on reversible noncompetitive enzyme inhibition kinetics. The value of dialysis fermentation greatly depends on the magnitude and form of this effect.

The retention time T_f equals V_f/F_f and is the reciprocal of the specific growth rate μ or r_g/X_f of the organism. Qualitatively,

the retention time is the average time a cell remains in the continuous fermentor.

Because of the many variables so far mentioned, Dr. George Coulman of the Department of Chemical Engineering, Michigan State University, developed process parameters to reduce the complexity of the system. The dimensionless relationships are:

$$P_m = \frac{P_{ms} A_m}{F_f} \tag{9}$$

and

$$\phi = F_f / F_r \tag{10}$$

In the theoretical study, osmosis of water across the membrane was not considered. Consequently, in programming the conservation of mass equations, the feed flow rate equaled the fermentor effluent flow rate and the flow rate of tap water into the reservoir equaled the reservoir effluent flow rate.

5. RESULTS

In this paper the term *efficiency* is used to describe the ability of the dialysis process to completely convert lactose to products. *Productivity* refers to the amount of lactate per product volume the process produces.

5.1 Computer Simulation of the Process

A mathematical model was modified from previous work (30) and accepted as a valid representation of growth and product formation for the dialysis whey fermentation. Dr. George Coulman, Michigan State University, applied the kinetic Equations 6, 7, 8 to the fermentation balance Equations 1, 2, 3, 4, 5 and then programmed them on a digital computer to obtain the process simulation. The purpose of the simulation was to determine the feasibility of continuous dialysis culture and its greater efficiency, if any, over conventional continuous culture. Positive computer output encouraged experimental work, which was then guided by more computer simulations. However, since computer output was needed before experimental results were available, first approximations were necessary for the constants of the mathematical model. Thus, the equations were manipulated to eliminate some constants by producing independent and dimensionless variables. Other constants were approximated from the literature.

The material balance equations were programmed at steady state conditions (the time derivatives are zero). Absolute results were not considered important, but significance was given to the general trends of the output.

The value $\mu_m T_f$ ($T_f = V_f/F_f$) was chosen as an appropriate independent variable for general study. This manipulation eliminated the need for giving values to the constants α and β. Values for the various other constants and initial conditions are given in Table 2. The program was executed beginning at $\mu_m T_f$ equal to 1 to eliminate the problem of simulating bacterial washout from the system. In plotting the output of the process simulations in this paper, μ_m was given a value of .27 (determined from hindsight) so that more continuity would exist between the computer and experimental results.

Table 2. Values used for the simulations of the continuous dialysis fermentation. Exceptions are noted in the simulation figures.

Symbol	Value
S_f^o	180 mg/ml
P_f^o	10 mg/ml
P_{ms}	.001 mg/min/cm^2
P_{mp}	.003 mg/min/cm^2
A_m	2,300 cm^2
K_s	0.2 mg/ml
K_p	30 mg/ml
γ	.9

A process simulation comparison of the efficiencies of substrate conversion of a continuous dialysis culture and a conventional continuous culture is shown in Figure 4. It is apparent that the dialysis system can readily attain the desired residium of 0.5% lactose. Moreover, the form of the results further validates the mathematical model and predicts much greater efficiency with dialysis, thus encouraging more computer simulations and experimental work.

5.2 Kinetic Sensitivity

Next, simulations were made of the important kinetic parameters. A plot of the residual lactose concentration in the fermentor, S_f, versus the retention time, T_f, (Figure 5) illustrates the effect of K_p (product inhibition parameter) on dialysis culture operation. No other parameter had as significant an effect on the slope of this curve. The remaining kinetic parameter, K_s, appeared to have less effect on the performance of the system.

5.3 Parametric Sensitivity

Important in guiding the experiments were simulations illustrating the effects of the process parameters, P_m and ϕ. Examination of the dimensionless permeability, P_m, indicates a significant effect (Figure 6). Larger values of P_m shorten the retention required to ferment the whey. Note that P_m and T_f are coupled through F_f but can be manipulated independently. Figure 7 illustrates that the necessary retention time is further dependent on the feed rate when ϕ is constant. Accordingly, with a .23 m^2 membrane, constant ϕ, and constant V_f/F_f, the system is more efficient at a F_f of 0.5 ml/min

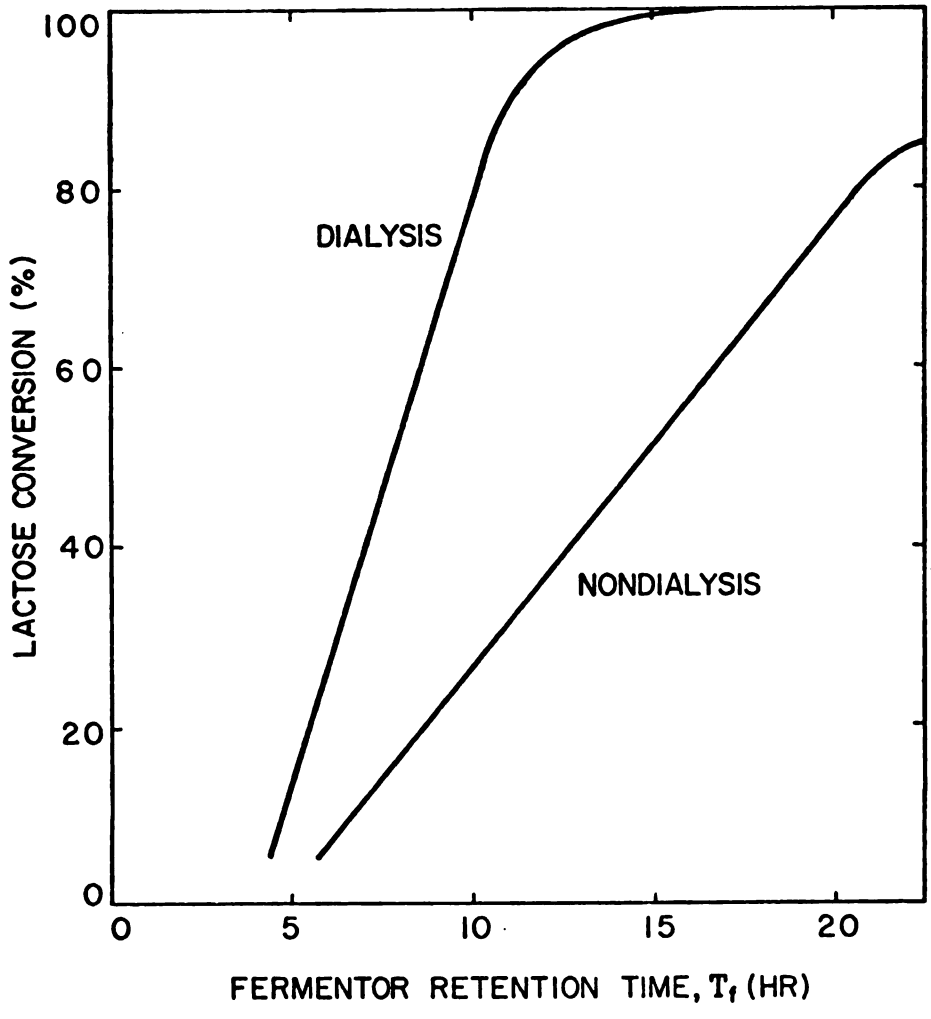

Fig. 4. Comparison of efficiency of continuous lactate fermentation in a dialysis and nondialysis mode as predicted by computer simulation. In each case the substrate contains 5% lactose. Data are from Coulman, MSU.

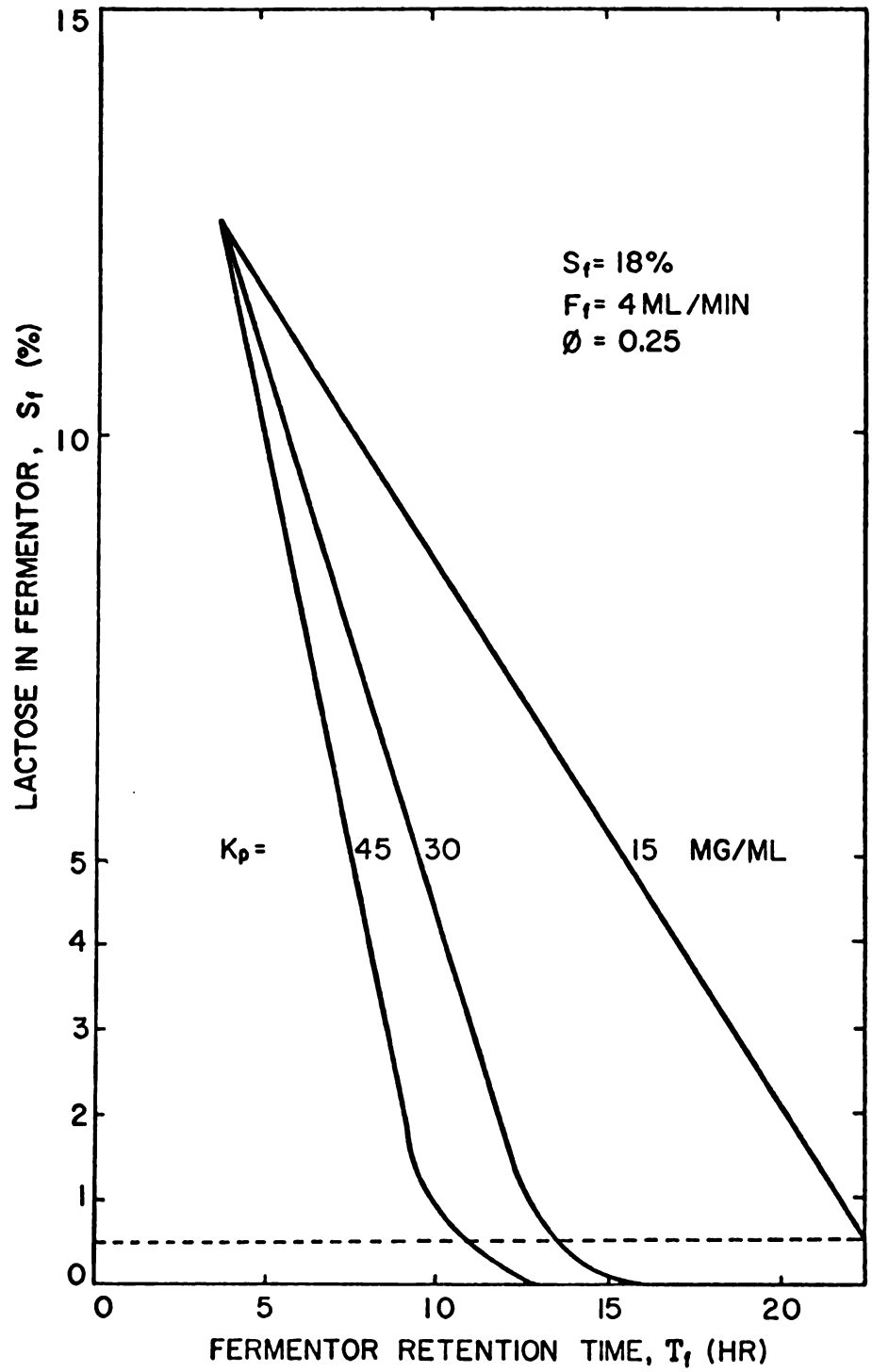

Fig. 5. Effect of product inhibition on operation of continuous dialysis fermentation as predicted by computer simulation. The objective is to maintain S_f below the dashed line. Data are from Coulman, MSU.

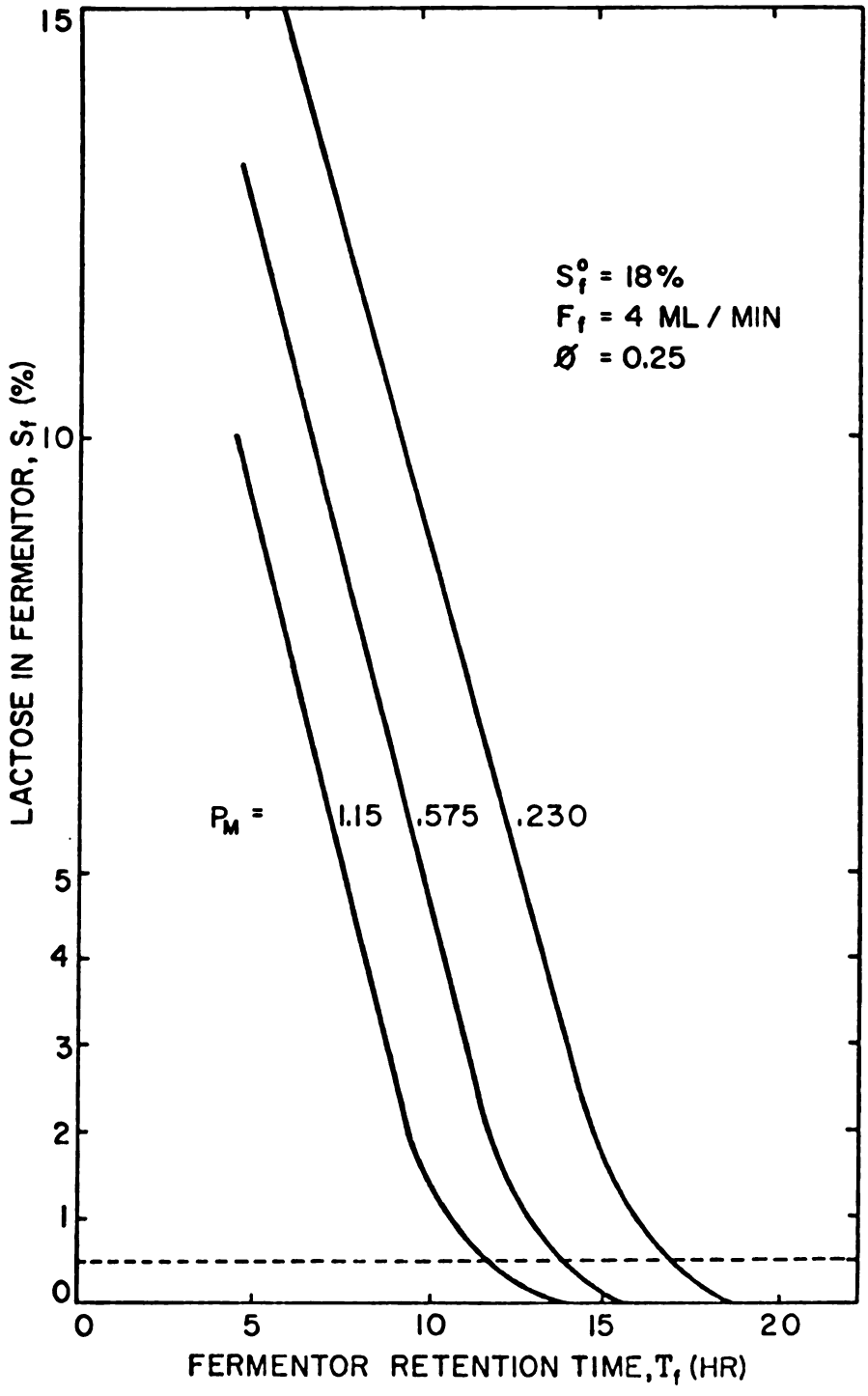

Fig. 6. Effect of permeability process parameter on operation of continuous dialysis fermentation as predicted by computer simulation. The objective is to maintain S_f below the dashed line. Data are from Coulman, MSU.

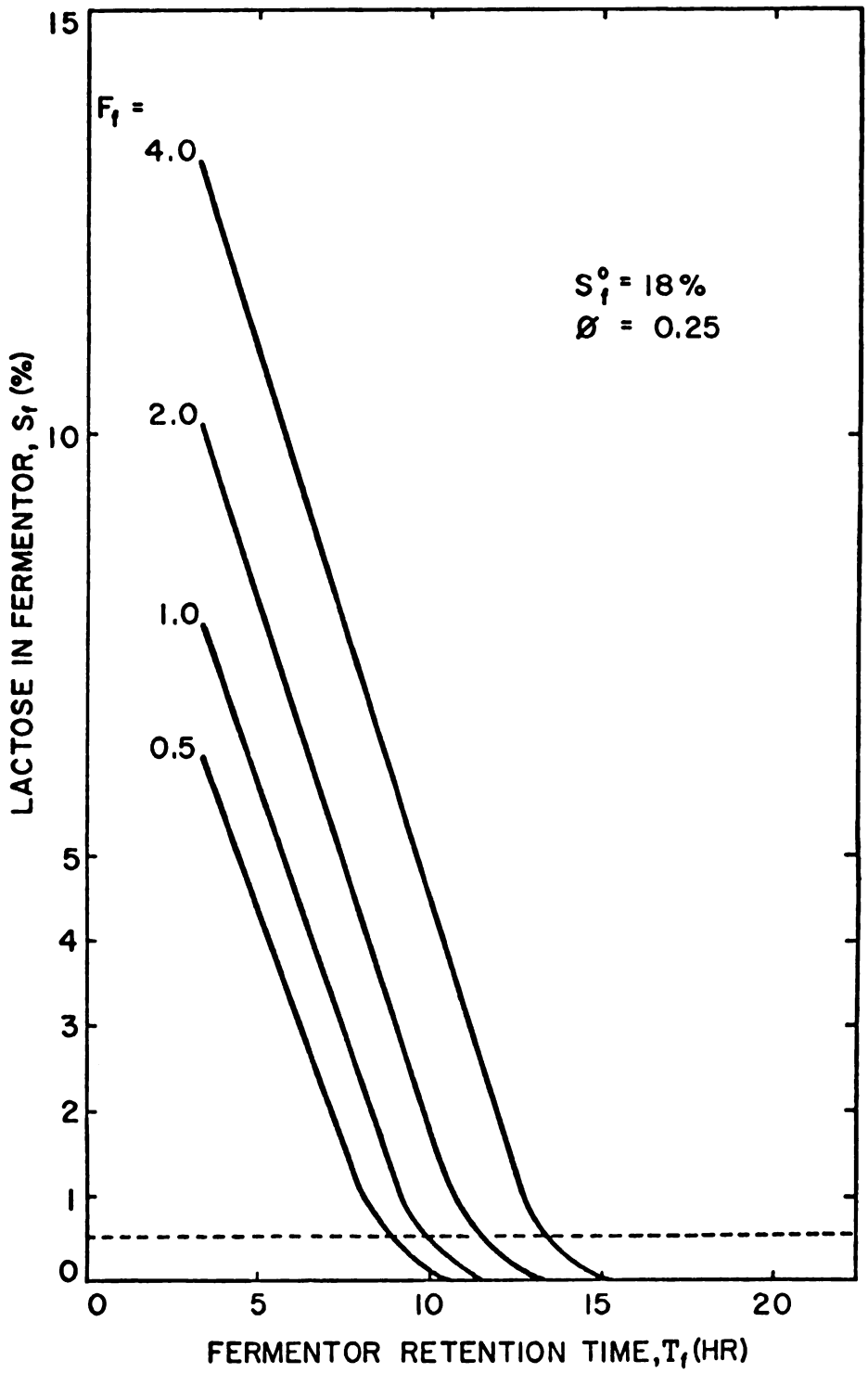

Fig. 7. Effect of feed flow rate on operation of continuous dialysis fermentation as predicted by computer simulation. The objective is to maintain S_f below the dashed line. Data are from Coulman, MSU.

than 4.0 ml/min. F_r was found to have the similar effect (not illustrated). The simulation of the ratio of whey feed into fermentor to water into reservoir, ϕ (F_f/F_r), shows its importance (Figure 8). Higher values of ϕ increase the product concentration in the fermentor and reservoir, but lengthen the needed fermentor retention time. The reverse also applies. With each of the parametric variables studied, their individual effect is shown mostly at the lowest retention time at which the maximum rate of substrate utilization occurs.

5.4 Retention Time

The first experimental work determined the effect of fermentor retention time on the process system. The retention time was varied by manipulating the fermentor volume. Therefore, other important parameters (P_m, ϕ, F_f) remained unchanged. Values used for the process parameters were determined by examining the parametric simulations and considerations of the limits of the available equipment. Results and projections are depicted in Figure 9 and correlate well with the kinetic simulation of the mathematical model (see Figure 5). The projections (throughout this paper) are based on the process simulations. The two illustrations also show the decline in the residual lactose concentration in the fermentor with increasing retention time during two different time periods. Each figure shows a point at which a further increase in retention time results in no significant decrease in the residual lactose concentration.

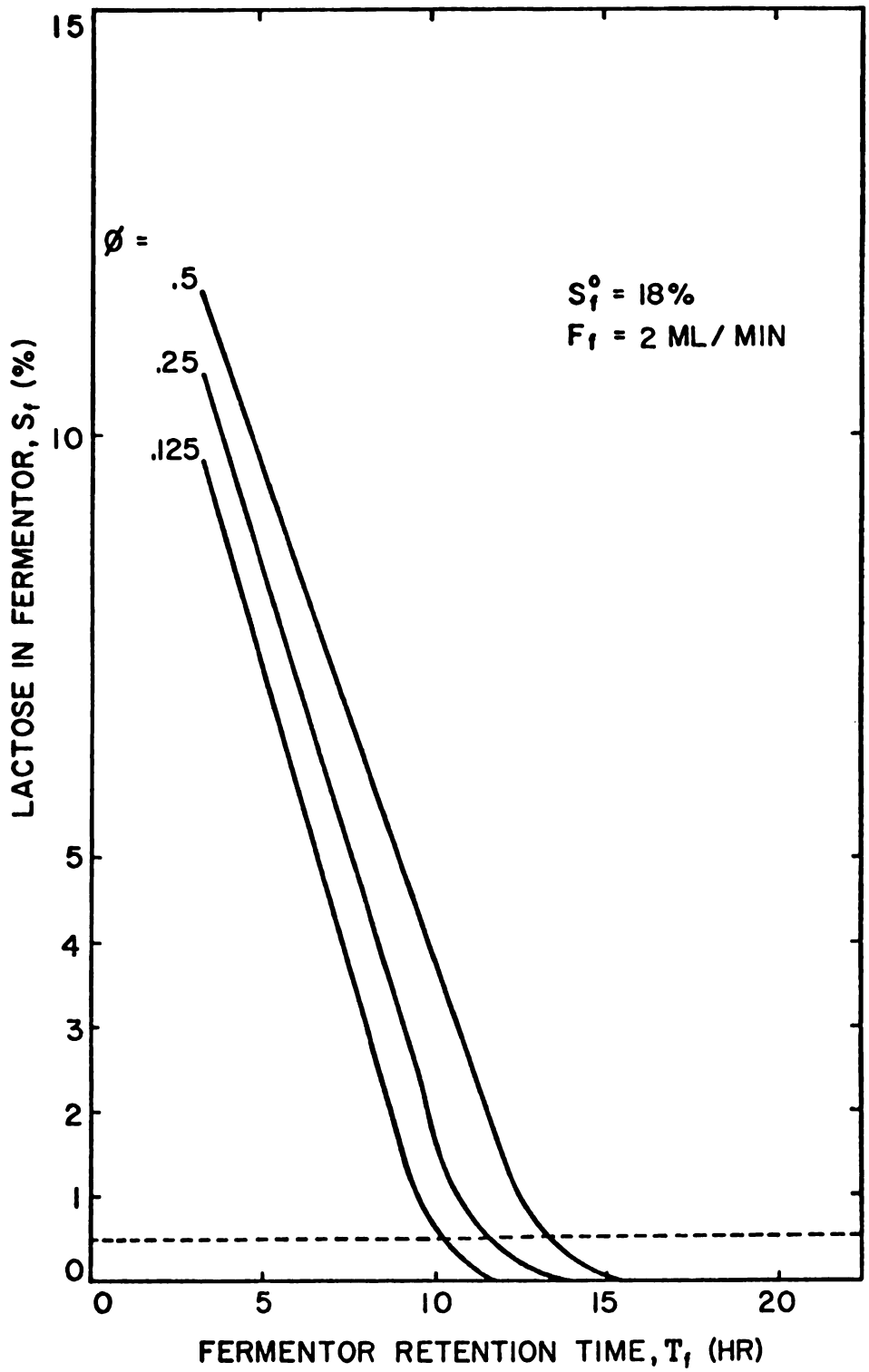

Fig. 8. Effect of ϕ process parameter, F_f/F_r, on operation of continuous dialysis fermentation as predicted by computer simulation. The objective is to maintain S_f below the dashed line. Data are from Coulman, MSU.

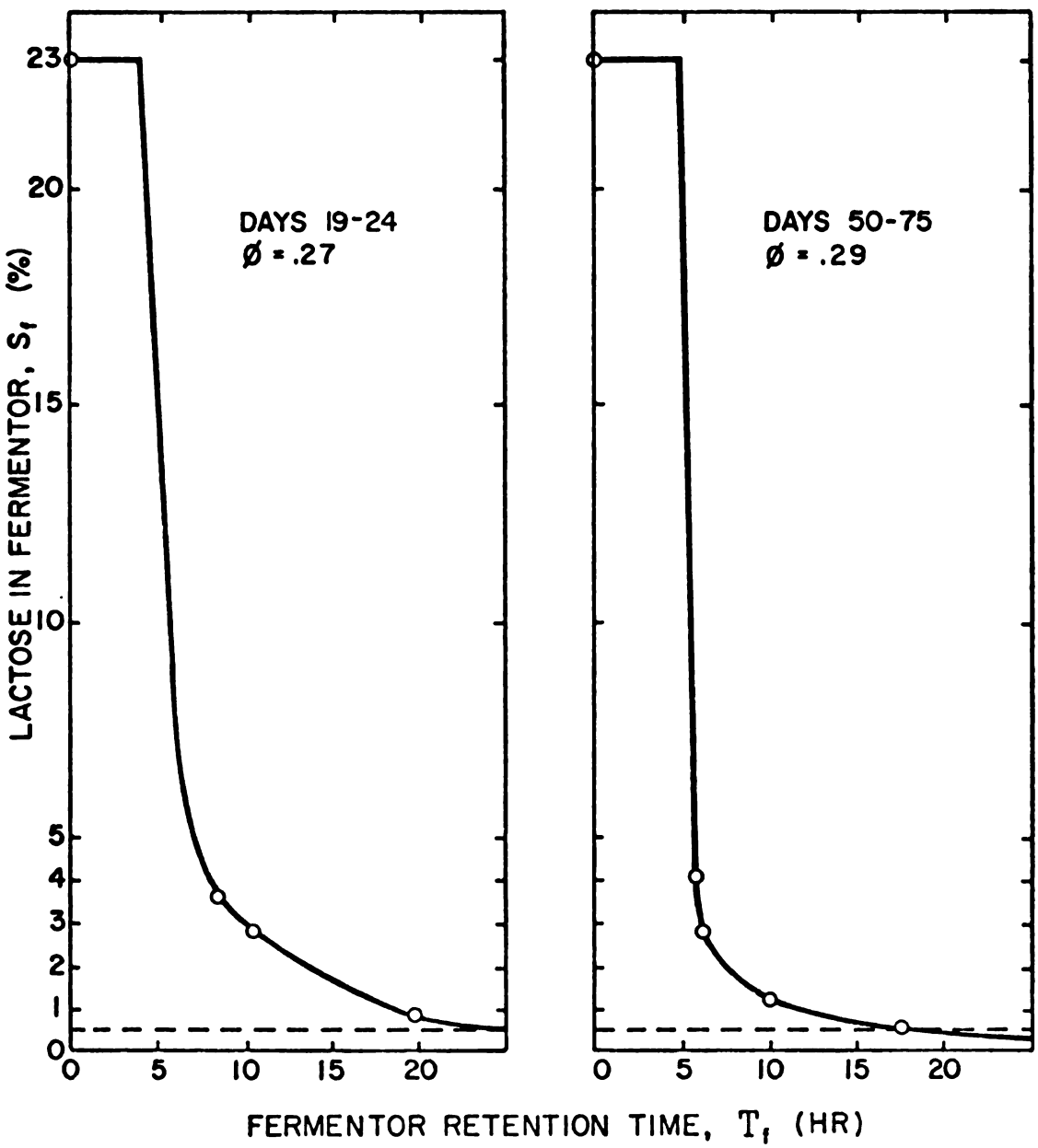

Fig. 9. Experimental effect of fermentor retention time on conversion efficiency during the continuous dialysis fermentation. Projections are based on computer simulations. The objective is to maintain S_f below the dashed line.

5.5 Cultural Adaptation

A comparison of the two illustrations in Figure 9 shows an adaptation of the continuous culture. The efficiency of whey conversion by the dialysis culture increased substantially by day 50 of the fermentation. After day 50, the culture was able to reduce the residual lactose to a given point with a shorter retention time than at the start of the fermentation.

The adaptation can be shown in quantitative terms. Assume P_f is small in comparison to K_p (i.e., $1 + P_f/K_p = 1$). Then Equation 8 can be rearranged and a Lineweaver-Burk plot can be made (Figure 10). Results indicate that between day 25 and 50 the maximum specific growth rate increased from .18 hr^{-1} to .27 hr^{-1}. The K_s for each time period is 20.8 g/l, which is very high; this will be discussed later.

5.6 Product Tolerance

Again assume P_f is small and recall that when S_f is much greater than K_s, μ is the reciprocal of T_f at steady state conditions. Consequently, μ can be obtained as a function of P_f (Figure 11). The maximum specific growth rate in this figure is taken from Figure 10. A higher concentration of product decreases the specific growth rate of the culture and, accordingly, the system requires a longer retention time to reduce the substrate to the desired 0.5% lactose. The figure also compares the same function to that obtained from a nondialysis whey fermentation. At a given specific growth rate, observe that the dialysis culture is able to withstand a much greater concentration of lactate than the nondialysis culture.

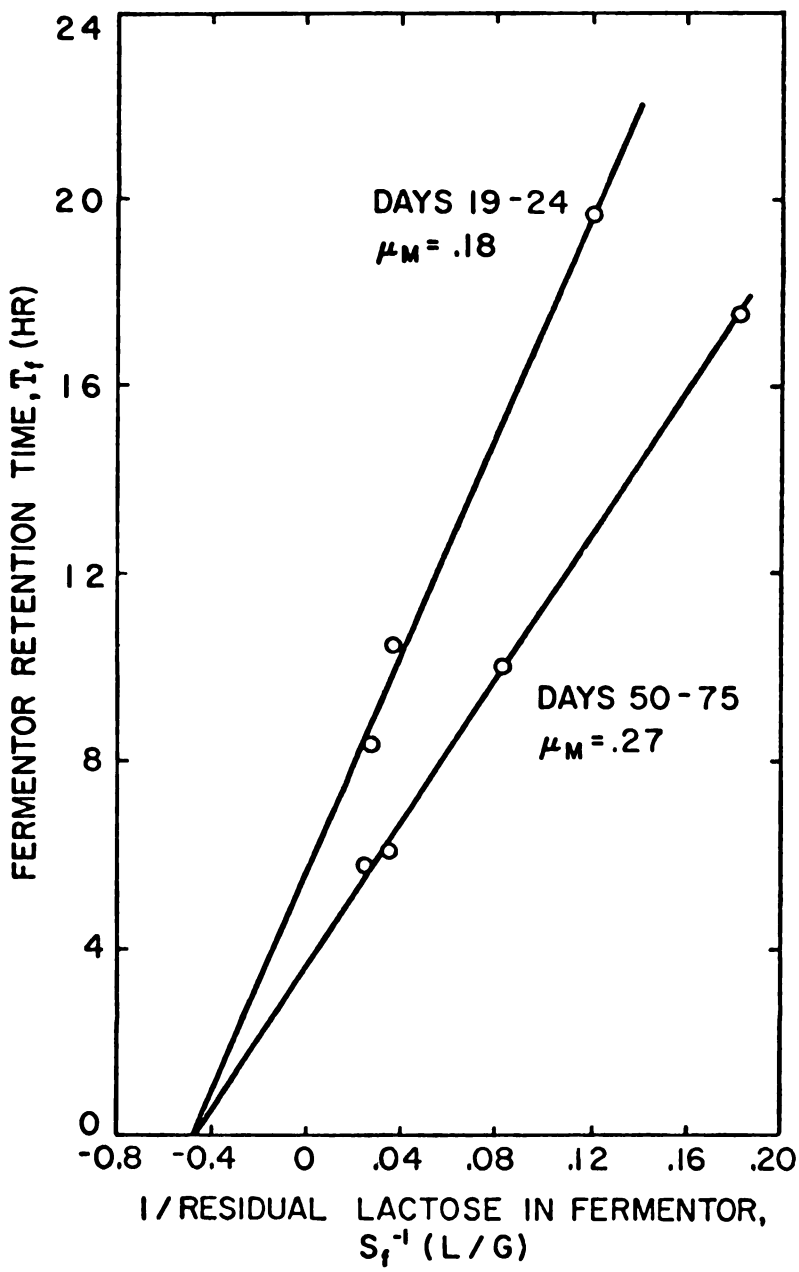

Fig. 10. Lineweaver-Burk plot which compares the maximum specific growth rate of the culture at two different time periods during the experimental continuous dialysis fermentation. K_s *as determined from the abscissa-intercept is 20.8 g/l for each time period.*

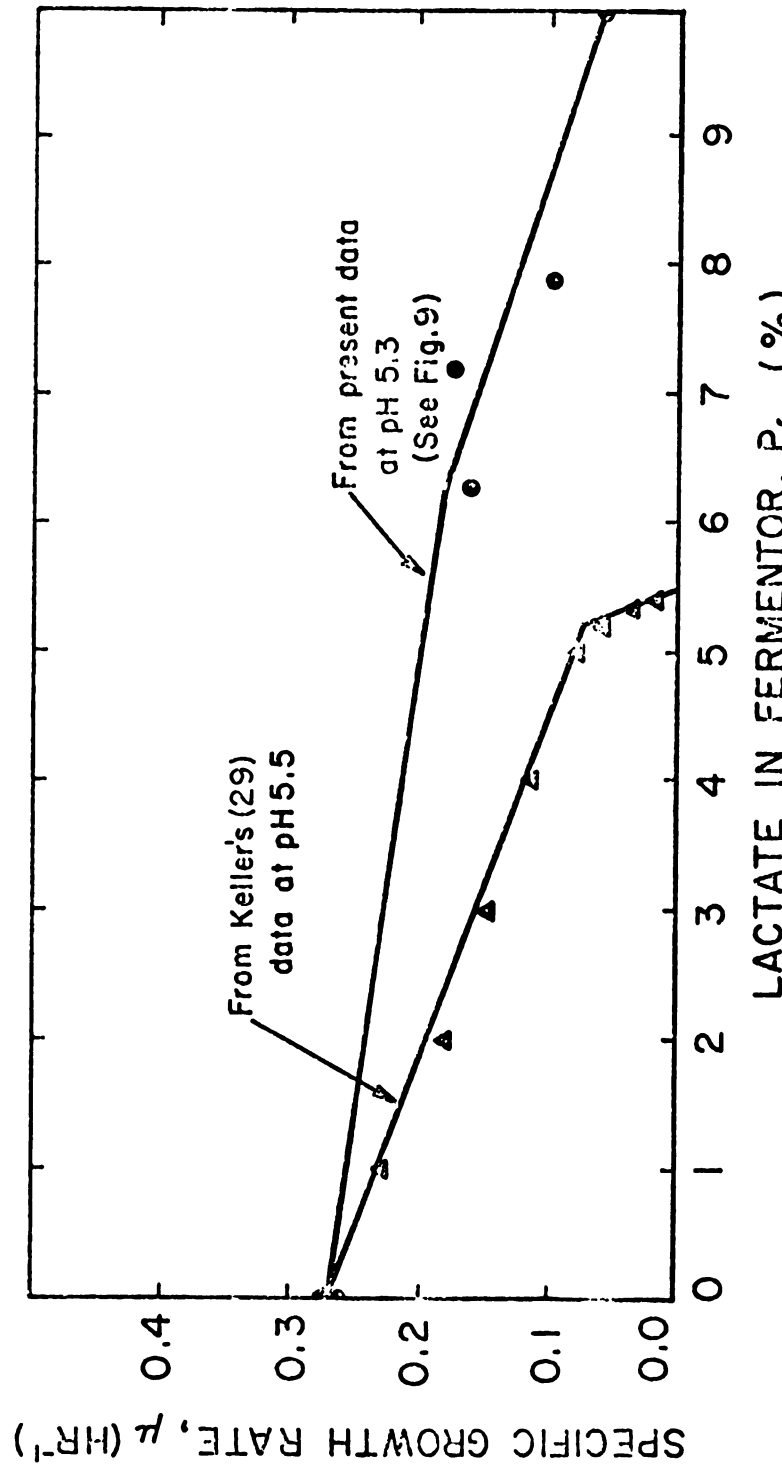

Fig. 11. Experimental effect of lactate concentration on the specific growth rate in dialysis (Sf^o = 23% lactose) and non-dialysis (S_f^o = 5% lactose) continuous fermentations.

5.7 Process Parameter ϕ

The next major experiment determined the effect of the parameter ϕ (F_f/F_r) on the dialysis system. The dimensionless ratio was manipulated by varying the reservoir water flow rate, F_r. The fermentor retention time, therefore, remained unchanged. Results are represented in Figures 12 through 16. Figure 12 shows the experimental data and projected theoretical changes in the residual lactose in the fermentor as a function of the flow rate of water dialysate through the reservoir side of the dialyzer, with constant fermentor retention time. At an average retention time of 16.4 hr, it was apparent that the desired 0.5% lactose could be readily attained in the system, i.e., if F_r = 13 ml H_2O/min.

Ammonium was used as an indicator of the product concentration in the fermentor and reservoir. Figure 13 shows the increase in residual lactose as the ammonium in the fermentor increases. The maximum ammonium concentration, 44.5 mg/ml, was achieved when the reservoir water flow rate equaled zero (nondialysis).

Lactate as a function of increasing dialysis is shown in Figure 14. As the reservoir flow rate increases and ϕ becomes small (increasing dialysis) the lactate concentration first increases to a maximum (F_r = 6.2, ϕ = .68), then decreases to the range where the residual lactose is less than 0.5%. Again, the dialysis culture is able to withstand a greater concentration of lactate than nondialysis culture. This unexpected result will be discussed later.

Figure 15 with similar coordinates as Figures 12 and 13, shows the experimental data and projected theoretical changes in the

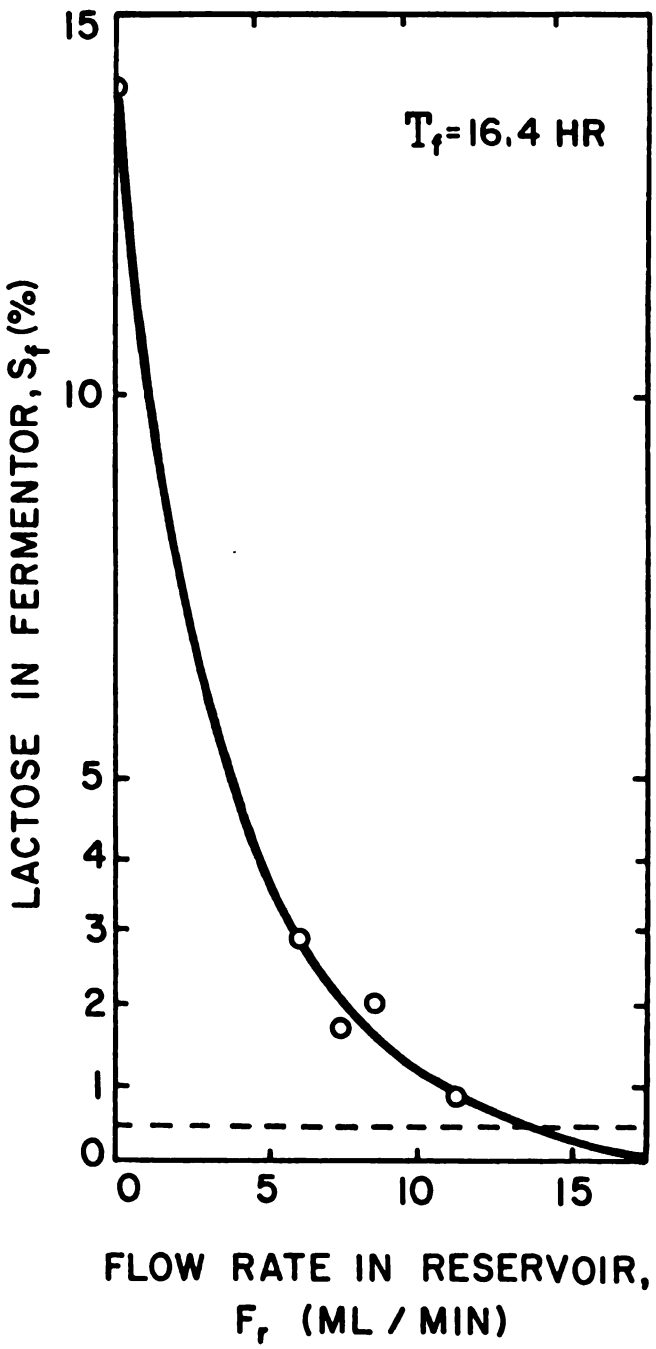

Fig. 12. Experimental effect of reservoir dialysate flow rate on conversion efficiency in the fermentor during continuous dialysis fermentation. The objective is to maintain S_f below the dashed line.

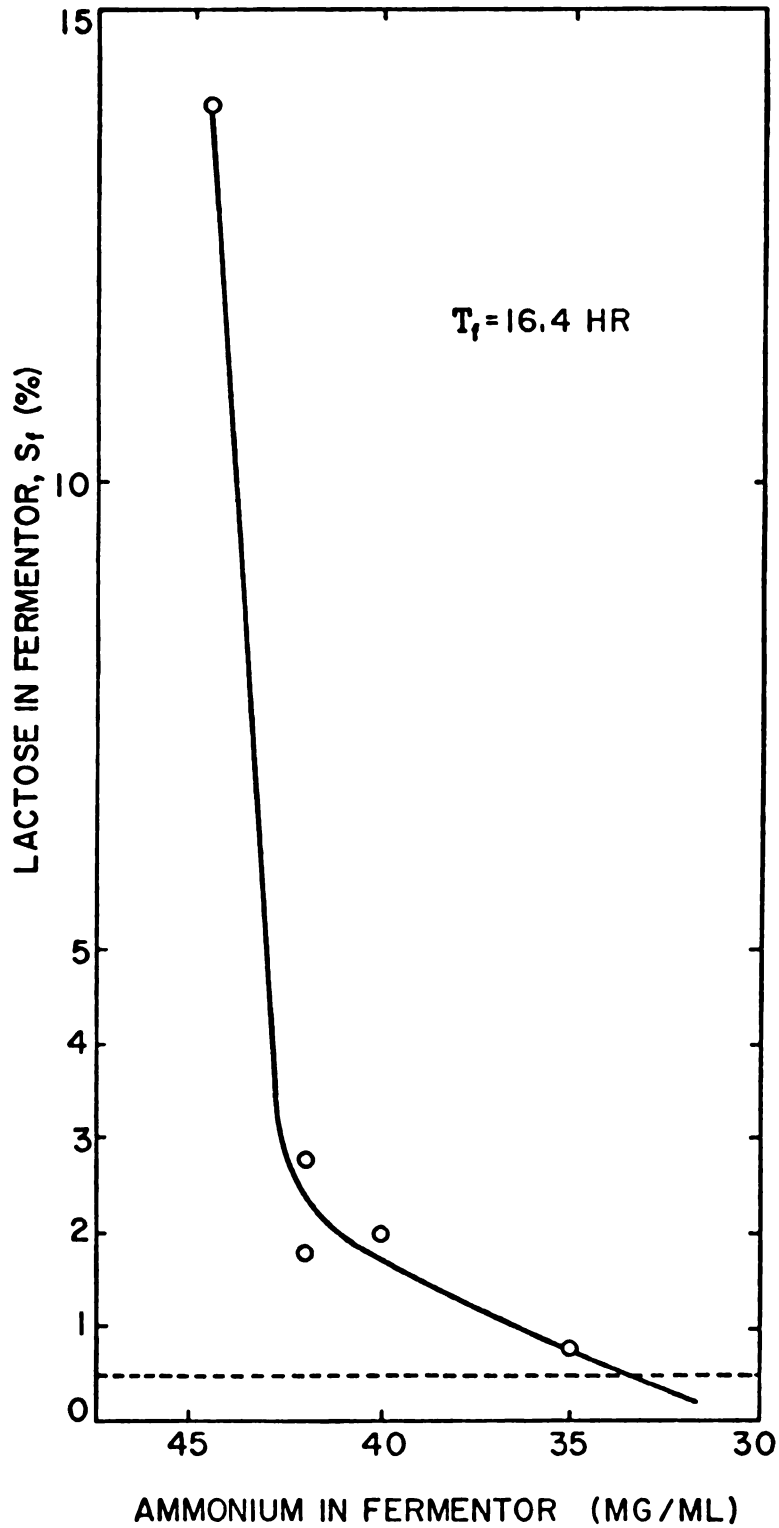

Fig. 13. Experimental effect of ammonium (an indicator of the product concentration) in the fermentor on conversion efficiency during continuous dialysis fermentation. The objective is to maintain S_f below the dashed line.

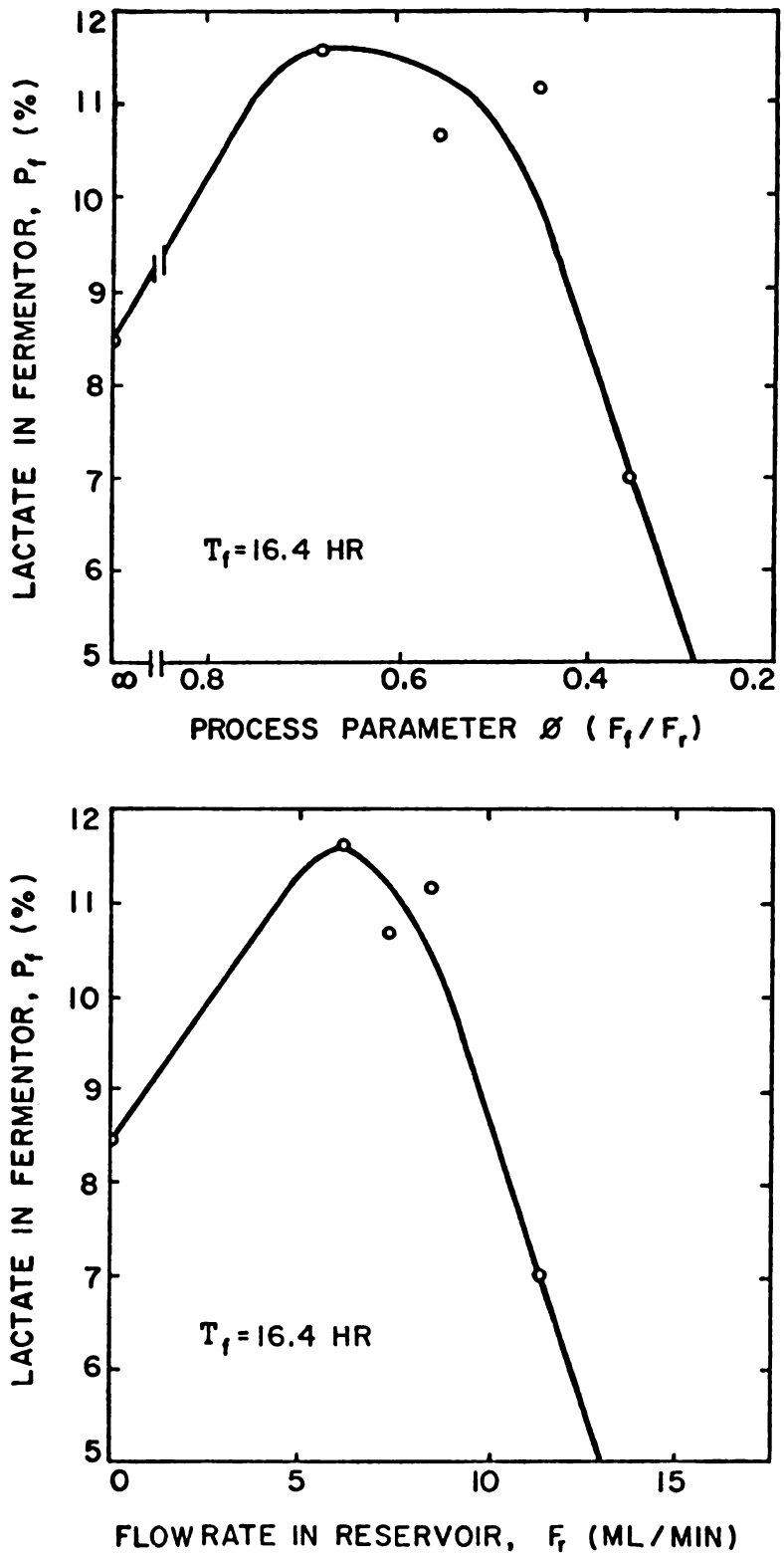

Fig. 14. Experimental effect of dialysis on the lactate concentration in the fermentor. Dialysis increases as the reservoir flow rate increases from 0.0 ml and as ϕ decreases from ∞.

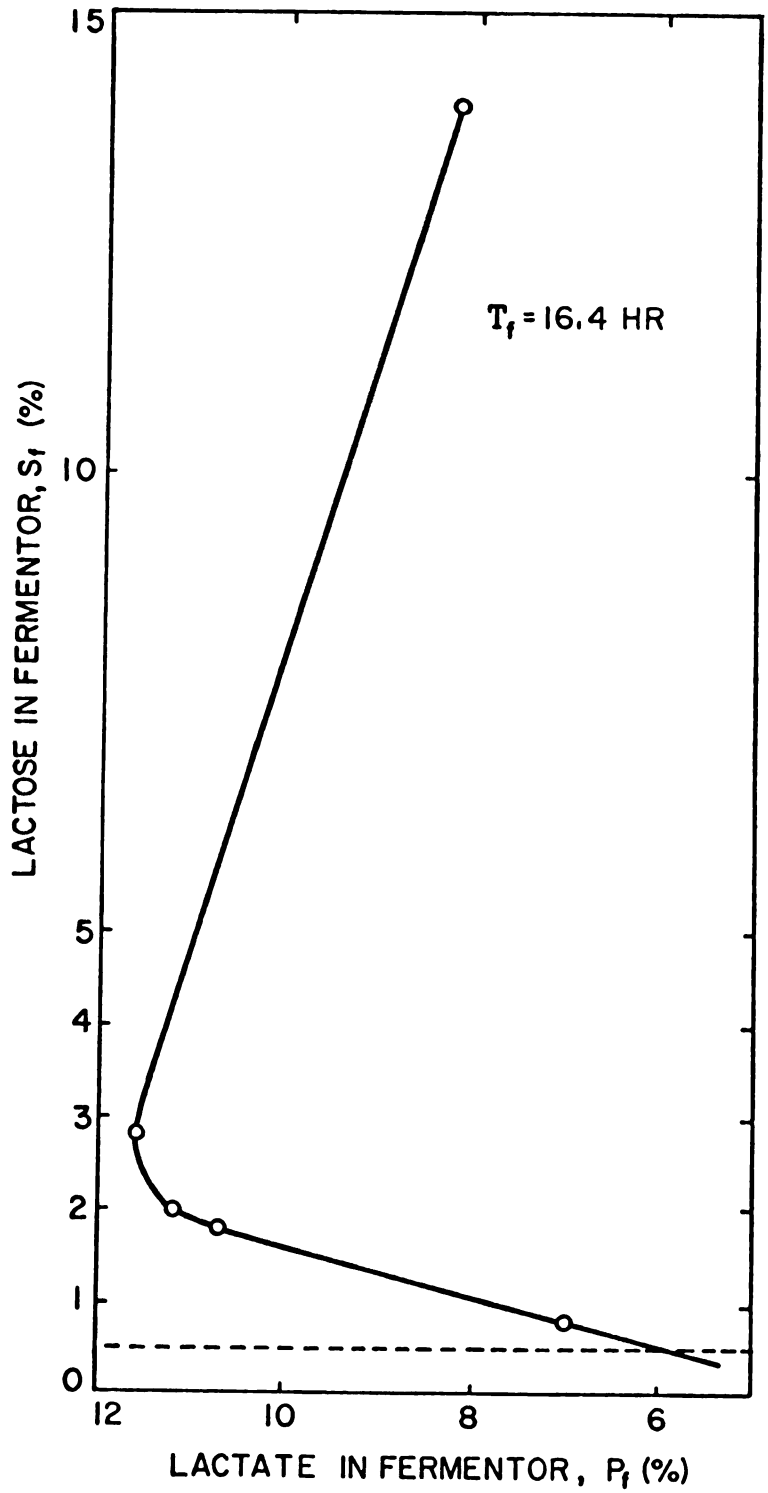

Fig. 15. Experimental effect of lactate concentration in the fermentor on conversion efficiency during continuous dialysis fermentation. The objective is to maintain S_f below the dashed line.

residual lactose as a function of the lactate in the fermentor. At a retention time of 16.4 hr, the desired 0.5% residual lactose level is reached when the lactate is less than 6.0% in the fermentor. Much of the form of this curve is a result of a net transfer of water across the membrane, from the reservoir, diluting the fermenting culture.

Sufficient information can be obtained from Figure 15 to make a material balance of substrate and product for the fermentor chamber. The percent residual lactose plus the percent lactate in the fermentor should approximately equal the sum of their initial percent feed concentrations. However, this is not the case. Dilution of the fermentor contents by reservoir water occurs. A plot of the sum of S_f and P_f versus F_r shows the increasing dilution of the fermentor contents as the reservoir flow rate increases (Figure 16).

5.8 Dialyzer Feasibility

The preceding experimental work took place during a 76 day continuous fermentation. The continuous operation was interrupted twice, once each on days 30 and 40 to clean the plate-and-frame dialyzer. The fermentor compartments of the dialyzer were thoroughly clogged (concentration polarization) on days 30 and 76 with only thin channels open to allow fermentor-dialyzer circulation of culture. The reservoir compartments of the dialyzer stayed clean throughout the experiments. Dialysis was halted from day 34 to 40 because of breakdown of two circulating pumps. Continuous fermentor operation with concentrated whey was maintained during this period and non-dialysis data were collected. The dialyzer was cleaned on day 40

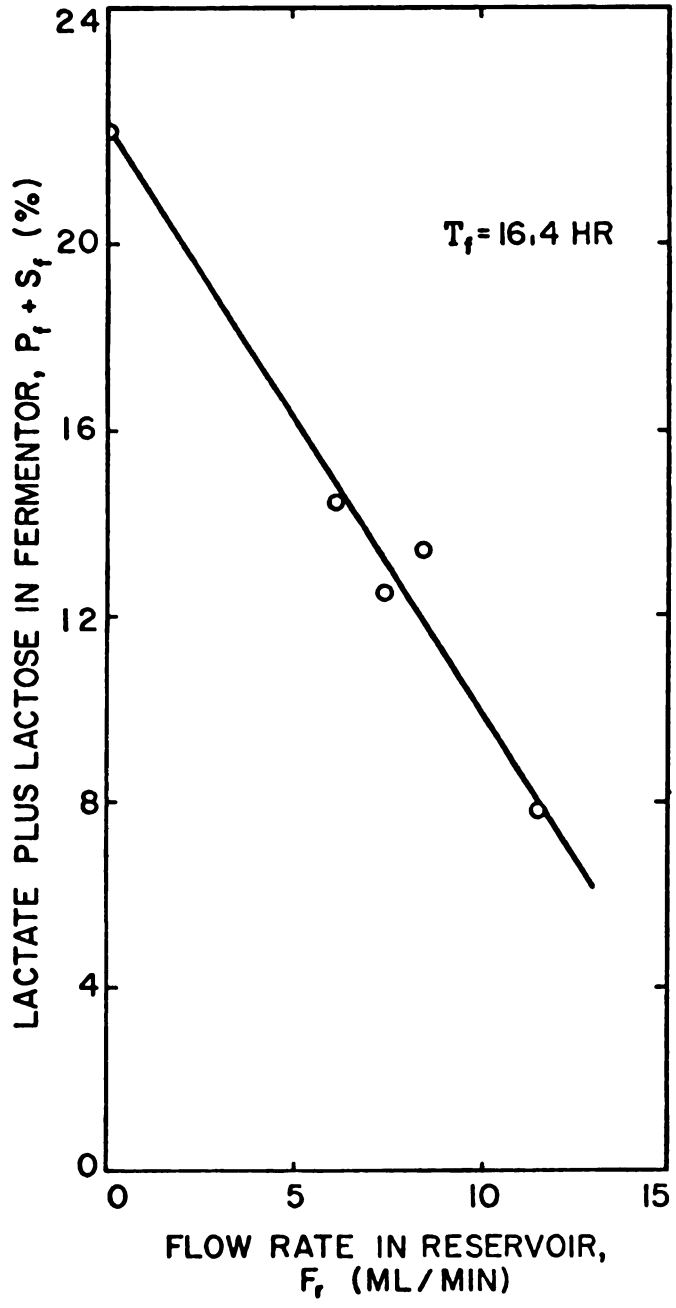

Fig. 16. Dilution of the culture by water from the reservoir circuit as indicated by the decreasing sum of the lactate and residual lactose concentrations as the reservoir flow rate increases.

because of this inactivity, but the effort was unnecessary. Altogether, the plate-and-frame dialyzer did not need cleaning for periods up to 4 weeks.

The same dialysis membranes, made of regenerated cellulose by the Visking process, were used throughout the 76 day fermentation. They proved durable.

Two earlier trial fermentations tested the feasibility of using a Cordis Dow hollow-fiber artificial kidney (Model 2, Cordis Corporation, Miami, Fla.) for dialysis whey fermentation. The fermenting culture was circulated outside the hollow-fiber membranes, through the dialyzer jacket. The water dialysate flowed through the regenerated cellulose hollow-fibers. However, in each trial the dialyzer proved undesirable because of rapid clogging (5 days). The design was inadequate to meet the necessary turbulence requirements at the membrane-cell culture interface.

5.9 Model Dialysis Fermentation

The next objective was to operate a dialysis fermentation, with the adapted culture, at optimum conditions to establish the efficiency and productivity of continuous dialysis culture for fermenting whey. The results are shown in Figures 17 and 18. It was found that a retention time of 19 hr and ϕ of 0.4 readily maintained the residual lactose below 0.5% in the fermentor and 0.06% in the reservoir and respective ammonium above 30 mg/ml and 10 mg/ml. The optimum lactate was found to be 7.9% in the fermentor and 2.5% in the reservoir at these conditions. The fluctuations in residual lactose were due mostly to inadvertent changes in the speeds of the

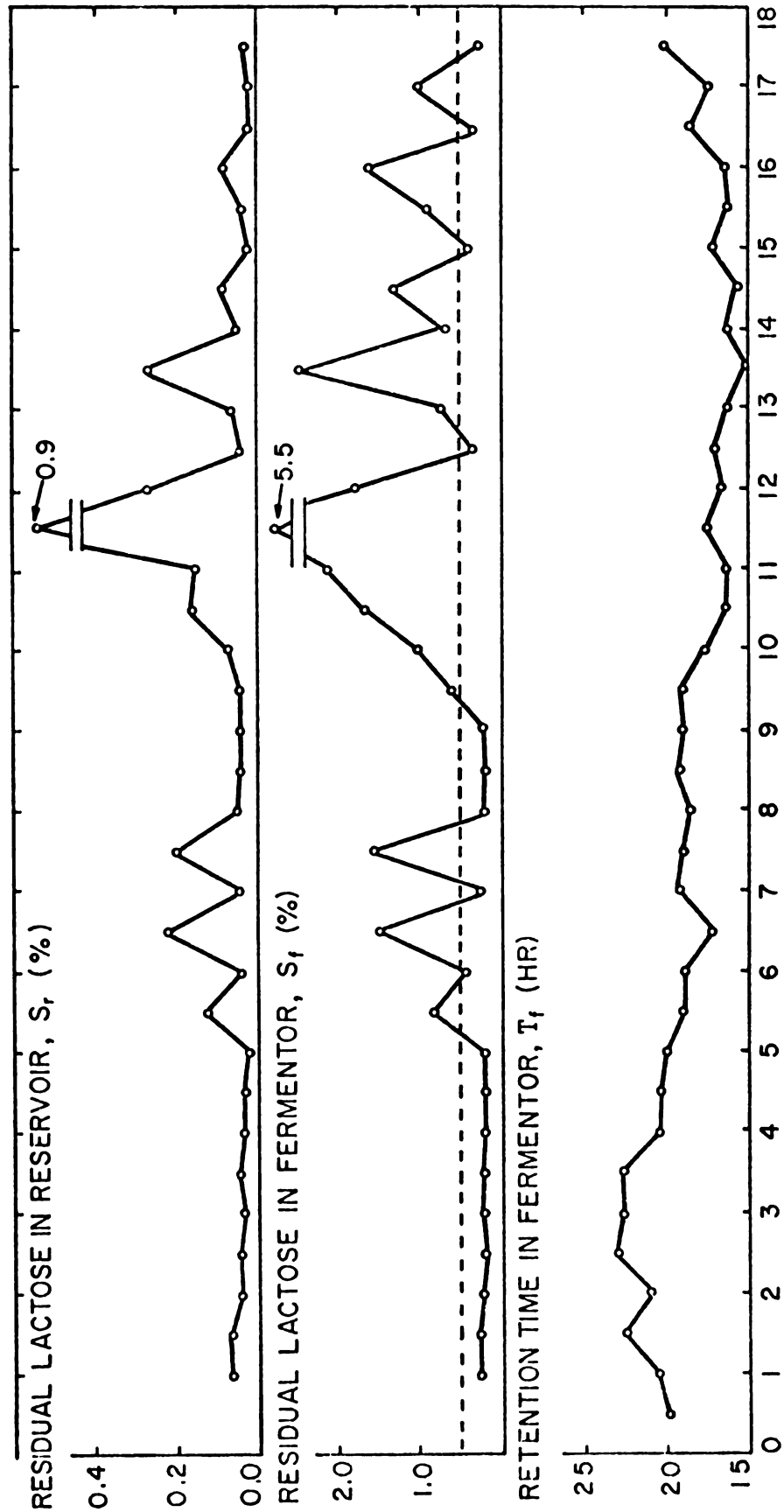

Fig. 17. Experimental effect of retention time on the concentration of residual lactose in the fermentor and reservoir during the model continuous dialysis fermentation. The effect is also a function of the process parameter ϕ (see Figure 18). The objective is to maintain S_f below the dashed line.

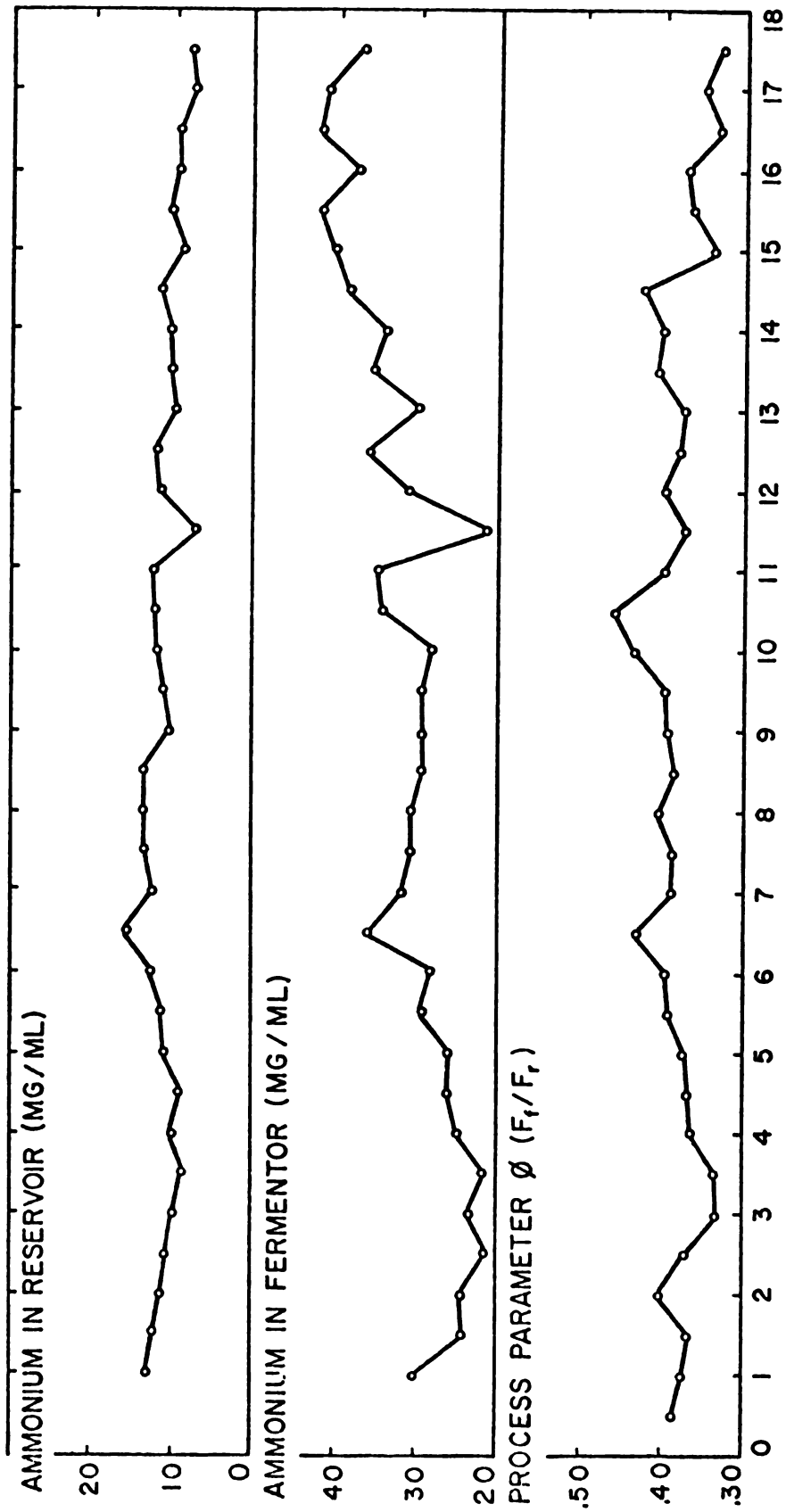

Fig. 18. Experimental effect of process parameter ϕ on the concentration of ammonium (an indicator of product concentration) in the fermentor and reservoir during the model continuous dialysis fermentation. The effect is also a function of fermentor retention time (see Fig. 17).

peristaltic pumps; note the varying retention time in Figure 17. The failure of a circulating pump caused the large fluctuation on days 11 and 12.

Since the previous fermentation indicated a net transfer of water across the membrane, water balances were made of the model dialysis fermentation (Table 3). Table 3 is a list of the flow rates of feed, tap water, and ammonium hydroxide into the system and the rates of fermentor and dialysate effluents out of the system. The second from the last column is an input minus output balance which should equal zero. The last column in the table is the percent fermentor dilution, which is the amount of fermentor effluent product that came from the water reservoir circuit. The mean fermentor dilution for 17 days was calculated to be 35.3%. Notice that as the fermentation progresses the percent fermentor dilution becomes consideraly less.

Table 4 lists representative substrate and product material balance data for the fermentation. The last column is a calculated value of S_f^o using flow rates from Table 3 and a conservation-of-mass equation. The mean calculated S_f^o (22.3%) agrees well with the experimental value of 23%. Accordingly, no significant portion of the substrate was lost to products other than lactic acid.

5.10 Product Quality

The fermentation products were analyzed by gas chromatography to determine the presence of atypical metabolic products (Figures 19 and 20). These chromatograms are from samples taken after over 80 days of continuous fermentation and show excellent product quality.

TABLE 3. Inputs and outputs of water (ml/min) during the model continuous dialysis fermentation.

Days	A Whey Feed (F_f')	B Tap Water (F_r')	C NH_4OH	D Fermentor Effluent (F_f)	E Dialysate Effluent (F_r)	(A+B+C) -(D+E)	% Fermentor Dilution[a] $(\frac{D-A}{D}) \times 100$
	--	--	--	--	--	--	--
1	↑	9.90	0.23	3.21	8.59	+0.16	43.0
	1.83	9.25	0.18	2.94	8.01	+0.31	37.8
2	↑	9.03	0.22	3.15	7.95	-0.02	41.6
	1.84	8.84	0.20	2.85	7.73	+0.30	35.4
3	1.84	9.70	0.22	2.88	8.65	+0.23	36.1
	1.71	9.67	0.18	2.89	8.66	+0.01	40.8
4	2.07	9.89	0.22	3.22	8.85	+0.11	35.7
	2.00	9.93	0.21	3.26	8.89	-0.01	38.7
5	2.10	10.04	0.24	3.33	8.95	+0.10	36.9
	2.25	9.94	0.25	3.50	8.92	+0.02	35.7
6	2.21	9.95	0.26	3.48	8.80	+0.14	36.5
	2.45	9.99	0.30	3.84	8.86	+0.04	36.2
7	2.01	10.03	0.28	3.43	8.81	+0.08	41.4
	2.24	10.10	0.22	3.50	9.02	+0.04	36.0
8	2.19	9.96	0.29	3.56	8.79	+0.09	38.5
	2.16	9.90	0.22	3.41	8.83	+0.04	36.7
9	2.25	9.94	0.25	3.50	8.88	+0.06	35.7
	2.34	9.80	0.23	3.50	8.81	+0.06	33.1
10	2.50	9.68	0.30	3.76	8.63	+0.09	33.5
	2.66	9.80	0.30	4.02	8.72	+0.02	33.8
11	--	11.40	0.34	4.06	10.21	--	--
	2.60	11.27	0.14	3.80	10.12	+0.09	31.6
12	2.64	10.94	0.32	4.00	10.01	-0.11	34.0
	2.50	11.34	0.30	3.90	10.22	+0.02	35.9
13	2.77	11.47	0.33	4.05	10.62	-0.10	31.6
	3.08	11.58	0.34	4.31	10.58	+0.11	28.5
14	2.58	11.25	0.36	4.05	10.10	+0.04	36.3
	2.90	11.01	0.33	4.22	9.96	+0.06	31.3
15	2.65	12.07	0.35	3.84	11.32	-0.09	31.0
	2.77	12.06	0.32	4.06	11.05	+0.04	31.8
16	2.80	11.83	0.36	4.04	10.84	+0.11	30.7
	2.37	11.64	0.28	3.56	10.67	+0.06	33.4
17	2.64	11.62	0.29	3.80	10.81	-0.06	30.5
	--	10.75	0.25	3.27	9.88	--	--

[a]Mean = 35.3; standard deviation = 3.5.

TABLE 4. Representative material balance for the model continuous dialysis fermentation.

Fermentation Day	S_f (%)	S_r (%)	P_f (%)	P_r (%)	Calculated S_f^o (%)[a,b]
2	0.23	0.04	6.5	2.5	21.6
4	0.20	0.03	6.0	2.2	18.3
8	0.20	0.05	7.9	2.5	22.5
9	0.23	0.04	7.2	2.4	20.7
12 1/2	0.33	0.04	9.4	2.4	24.2
16 1/2	0.35	0.02	11.0	2.3	26.6

[a] $S_f^o = \frac{F_f S_f + F_f P_f + F_r S_r + F_r P_r}{F_f'} - P_f^o$, $P_f^o = .9$. Values for F_f, F_r, and F_f' are taken from Table 3.

[b] Mean = 22.3; standard deviation = 2.9.

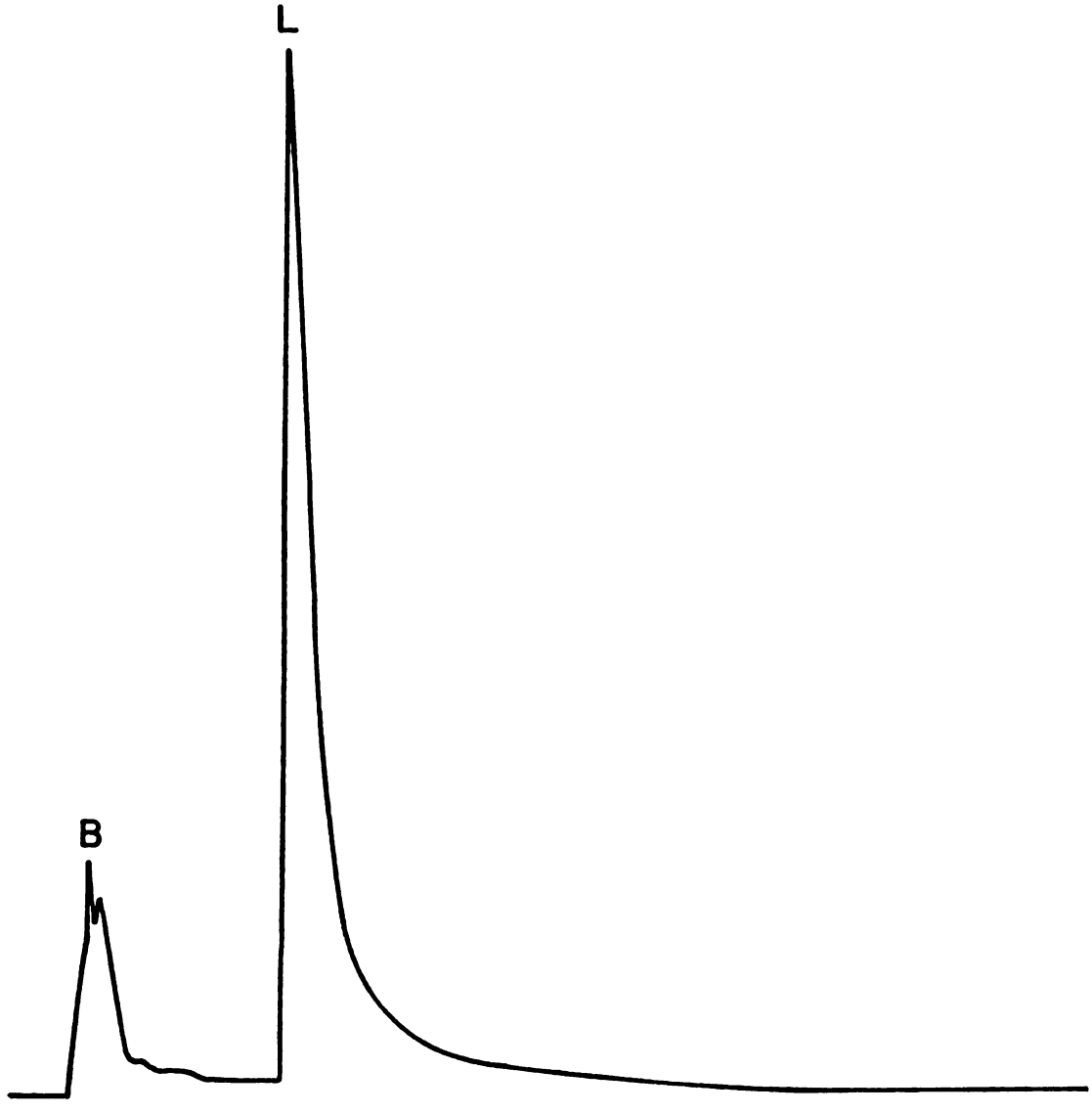

Fig. 19. Gas chromatograph of the overflow from the fermentor (attenuation = 64). Symbols: B, background; L, lactic acid.

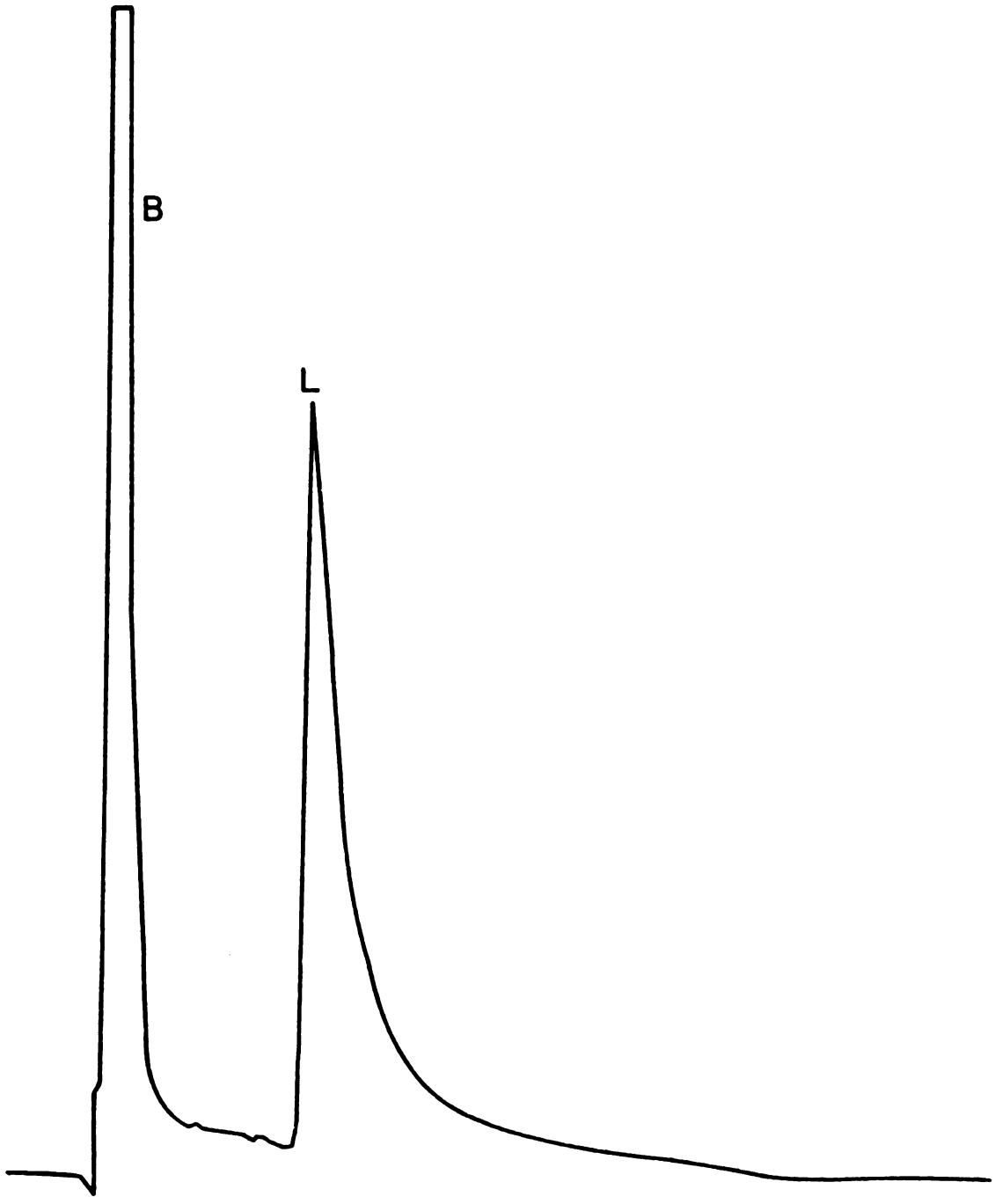

Fig. 20. Gas chromatograph of the dialysate from the reservoir circuit (attenuation = 16). Symbols: B, background; L, lactic acid.

When compounds other than lactate were detected, they accounted for less than 0.1% of the fermentor or reservoir product. The chromatograms are typical of those obtained from samples taken throughout the experiments when the residual lactose was less than 0.5% in the fermentor. At parametric conditions where the residual lactose was greater than 0.5% in the fermentor, other metabolic products were sometimes observed, but they were never in significant concentrations. Thus, the lactic acid fermentation was maintained homofermentative for 94 days.

6. DISCUSSION

6.1 Computer Simulation, a Tool for Fermentation Design and Improvement

The mathematical model successfully predicted the feasibility of a continuous dialysis fermentation for converting whey lactose predominantly into ammonium lactate, with little residual lactose. Much of the molecular products of a whey fermentation was removed from the immediate environment of the bacterial cells, by using the principle of dialysis, and thus dialysis relieved the feedback inhibition by product that normally regulates its production. Consequently, continuous dialysis fermentation enabled the fermentation of a much greater concentration of substrate in a shorter time interval as compared to a conventional continuous fermentation (30).

A comparison of Figures 5 and 9 confirmed that a continuous dialysis fermentation can be effected as predicted by the mathematical model. The right illustration in Figure 9 points out that a ϕ of .29 and a retention time of 18 hr results in 0.5% residual lactose in the fermentor. The fermentor effluent is 10% lactate at these conditions (Figure 11).

The computer simulations in Figures 5 through 8 guided the experiments and were the basis for the projections in the experimental results. Further, they show the means to improve the efficiency of substrate conversion and the product concentration of the fermentation.

The kinetic sensitivity simulation (Figure 5) showed that the rate of the dialysis fermentation is primarily limited by product inhibition. K_p is the only parameter that had a significant effect on the slope of this curve. The importance of product inhibition is experimentally verified in Figure 11. Higher concentrations of product decrease the specific growth rate of the organism; thus the system requires a longer retention time to reduce the substrate to the desired 0.5% lactose. Therefore, measures to improve the product tolerance of the organism are valuable. Since growth supplements are known to increase the product tolerance of *Lactobacilli* (64), the feed was supplemented with yeast extract (0.8%) throughout the experiments.

Examination of the dimensionless permeability, P_m (Figure 6), indicated that a higher value would decrease the needed retention time to attain the desired low residium of lactose in the system. Recall that P_m increases proportionately with the product of the permeability of the molecular species and membrane area and inversely with the feed flow rate (i.e., $P_m = P_{ms} A_m/F_f$). Accordingly, a more permeable membrane and larger membrane area in relation to the influx of feed into a given volume would shorten the required retention time. Both are possible of improvement. However, a problem in utilizing a larger membrane area would be in maintaining much turbulence at the membrane-bacterial culture interface. Figure 7 illustrates that greater reduction of the residual lactose in the fermentor can be effected by lowering the feed flow rate at constant ϕ and retention time. Since $F_f = V_f/T_f$, the effect is due to the larger ratio of membrane area to fermentor volume (i.e., P_m increases).

The manipulation of fermentor effluent flow rate to reservoir effluent flow rate ratio, ϕ, to a lower value would also decrease the necessary retention time (Figure 8). But this decrease in retention time would occur at the expense of the fermentor and reservoir effluent products being more diluted by water. The reverse is also true. A higher value of ϕ would increase the product concentrations and the necessary retention time. At a retention time of 16.4 hr, these trends are experimentally verified between ϕ of .35 and .68 in Figure 14 and the lower half of Figure 15. A useful project would be to determine the form of these curves at other retention times.

6.2 Osmosis and the Dialysis Fermentation

An unexpected result of the dialysis fermentation was the large influx of water from the reservoir to the fermentor, diluting its contents. The reason is because of osmosis, which is a net transfer of solvent (water) across a semipermeable membrane from the region of low solute concentration to the region of higher concentration (52). In the dialysis fermentation, since the solute concentrations on each side of the semipermeable membrane are unequal (i.e., the membrane is more permeable to solvent than solute), osmosis occurs.

The osmotic pressure, π, is the pressure difference across the membrane required to prevent spontaneous flow in either direction through the membrane. Van't Hoff empirically found that $\pi = cRT$, where: c = the concentration of solute in moles per liter, R = the universal gas law constant, and T = the temperature in degrees

Kelvin (72). Thus the osmotic pressure depends on the number of molecules of solute in solution. In the model fermentation, calculations of the osmotic pressure due to solutes show that the net pressure resulting from lactate is greater by a factor of at least 50 than that resulting from the residual lactose. Therefore, the search and examination of other membranes for the dialysis process should primarily concern the permeability of the membrane to lactate.

The membrane used in the dialysis process is permeable to salt ions but not cells and protein. Donnan noted that the osmotic pressure is much greater in this situation, especially at low polymer concentrations, than that predicted by the Van't Hoff equation (51). The reason is that the salt ions are of lesser concentration on the side of the membrane where the polymers are and thus there is an increase in small ions of opposite charge.

As noted in the introduction, it might be profitable on an industrial scale to remove the valuable whey proteins from the whey substrate before fermentation. This would eliminate most of the Donnan effect. The osmotic pressure due to bacterial cells is unknown but certainly less than soluble proteins.

A more permeable membrane may reduce the extent of dilution by allowing greater solute transfer and thus reducing the solute concentration differences on each side of the membrane. The effect on osmosis of employing a greater fermentation retention time would also be an important study.

Ultrafiltration and microfiltration* techniques are promising methods for improving a membrane-assisted fermentation. These methods eliminate the dilution problem by utilizing pressure filtration to separate the fermenting bacterial cells from their surrounding fluids. The operation is one of "reverse osmosis." The processes would produce a permeate product of higher lactate content than that of the dialysis process. Further, these processes are efficient in their inherent use of feedback of organisms. This recycle of cells is also possible in the dialysis process and needs study, which (including ultrafiltration and microfiltration studies) should begin by using computer methods to apply the whey fermentation kinetic model to the conservation of mass equations of the proposed system. The resulting output illustrating the interrelationships between important variables would be valuable in designing validation experiments.

To date, the major amount of ultrafiltration work has dealt with enzymatic reactions and the utility of membranes in enzyme retention. Numerous reports have dealt with the theory and modeling of membrane ultrafilter reactors (6, 8, 11, 14, 34, 60, 66, 71, 75). Several studies have used microfiltration of microbial cell populations for a variety of purposes (10, 46, 58, 59, 70, 73, 74, 77).

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*Ultrafiltration is defined as the separation of solute molecules by means of their unequal passage through a semipermeable membrane because of a pressure gradient.

Microfiltration is defined as the separation of solute molecules from microbial cells by means of the passage only of the molecules through a semipermeable membrane because of a pressure gradient.

6.3 Cultural Adaptation

Keller and Gerhardt previously reported the evolution of a fitter bacterial population during a prolonged continuous fermentation (30). The same result occurred for the continuous dialysis fermentation (Figures 9 and 10). Later in the fermentation the bacterial culture was definitely able to ferment the same amount of substrate in a shorter interval of time than earlier in the fermentation. At similar values of ϕ for example, early in the fermentation a retention time of 19.7 hr resulted in residual lactose of 0.83%; later, a retention time of 17.6 hr resulted in a residium of 0.55% lactose. The maximum specific growth rate of the culture increased by a factor of 1.5 between these time periods (Figure 10). Interestingly, the μ_m of the adapted culture is identical to that obtained by Keller and Gerhardt in an ordinary continuous fermentation (10).

It was not determined whether the fitter population resulted from cultural adaptation, mutation, favorable contamination, or a symbiotic culture. A sample from the fermentor effluent, when plated on nutrient agar or trypticase soy agar, showed three different colony types. As observed by phase microscopy, all three were rods, but distinct morphological differences existed. Inoculation of the types into lactose broth containing Durham tubes and phenol red showed they each fermented lactose to acid without gas production.

The Michaelis-Menten constant, determined in Figure 10, has the same value before and after adaptation. This means the maximum product concentration tolerated by the bacterial culture did not change over the pertaining time periods. But after the adaptation,

the culture was able to grow and metabolize at a faster rate at the maximum product tolerance level.

The value of the Michaelis-Menten constant is very high (20 g/l). Since the product concentration was not considered in determining the value of K_s, most of the term reflects product inhibition and not a limiting substrate concentration. However, higher cell populations are produced by dialysis culture as compared to conventional culture (13, 15). Further, it is known, but not well understood, that K_s depends on the biomass concentration (7, 57). In experimental tests with *Aerobacter aerogenes*, Contois found that the cell mass became gradually less than the predicted value as the cell mass increased (i.e., K_s increased) (7). Again though, the greater K_s value at a higher cell mass concentration may be due to what is mainly an effect of product inhibition.

6.4 Product Tolerance

As compared to the conventional continuous process of Keller and Gerhardt (30) (whey not concentrated) continuous dialysis culture showed the dialysis culture is able to endure more than twice as much lactate at low specific growth rates (Figure 11). Besides the obvious substrate concentration differences, the main factor preventing greater specific growth rates in the conventional culture was that the feed was not supplemented with growth factors. Again, at a given product concentration, the bacterial culture metabolizes at a faster rate when growth factors are added to the feed.

The two illustrations in Figure 14 show that as dialysis increases (i.e., when ϕ decreases and F_r increases) from ϕ of ∞ to .68

and from F_r of 0.0 to 6.2 ml/min, the bacterial culture is able to tolerate a greater concentration of lactate. For example, at $\phi = \infty$ (nondialysis with concentrated whey and growth supplement) the culture grows at a lactate concentration of 8.2% and at $\phi = .68$ the culture grows at 11.6% lactate.

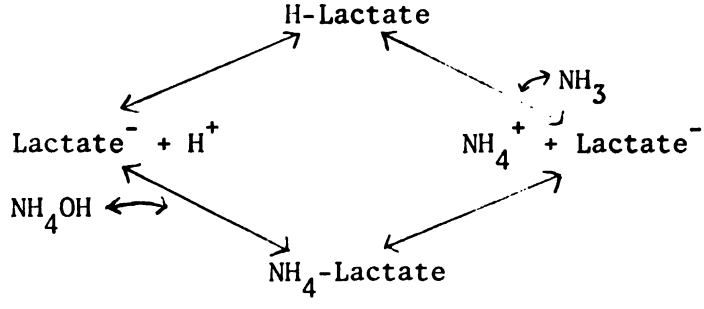
As an attempt to explain this phenomena, recall that the salt solution obtained from an aqueous solution of a weak acid and a strong base is not neutral, but slightly basic. For example, the hydrolysis reaction is:

$$\underset{\text{dissociated acid}}{H_2O + \text{Lactate}^-} \longleftrightarrow \underset{\text{undissociated acid}}{OH^- + \text{H-Lactate}}$$

But a low pH is maintained in the whey fermentation, thus the principle dissociation reaction becomes:

$$\text{H-Lactate} \longleftrightarrow H^+ + \text{Lactate}^-$$

Ammonium hydroxide is used to neutralize the hydrogen ions. The resulting salt of lactate influences the concentration of lactate ion in the system which in turn influences the concentration of the undissociated acid:



Simon and Beevers generalized that it is mainly this concentration of undissociated acid that inhibits the activity of a bacterial culture (68, 69).

The membrane of the dialysis fermentation is more permeable to ammonium than lactate (see page 63). Consequently, dialysis by removing cations from the fermenting culture to a greater extent than the organic compounds, may slightly shift the equilibrium from the undissociated to the dissociated acid. Thus the culture could tolerate more acid under dialysis conditions. Possibly, the cations themselves have an inhibitory effect on the culture.

Rogers and Whittier suggest that peroxides may inhibit lactic acid bacteria (64) and since *Lactobacillus bulgaricus* is known to produce hydrogen peroxide (47), dialysis may also improve the whey fermentation by removing this low molecular weight compound from the culture.

High substrate concentrations are known to inhibit bacterial growth in some fermentations (26, 37). The much higher residual lactose concentration in the nondialysis fermentation (14%) in comparison to the dialysis fermentation (2.8% at ∞ = .68) may, therefore, be a factor that prevents more acid production by the nondialysis culture. However, no growth problems were encountered at the start of the fermentation when *Lactobacillus bulgaricus* was inoculated directly into concentrated whey lactose (23%).

Brown and Halsted suggest that the growth rate reduction of *Trichoderma viride*, caused by increased hydrogen ion concentration (acid products), is related to a membrane diffusion limitation of

the substrate/permease complex (4). They further mention work with *Propionibacterium shermanii* and *Candida utilis* that quantitatively attributes the hydrogen ion effect as an occurrence of noncompetitive inhibition by the hydrogen ions at the cell membrane. Much work remains before the effect of product inhibition is understood.

6.5 Model Dialysis Fermentation

A goal of the dialysis fermentation was to keep the residual lactose less than 0.5% in the fermentor because lactose at greater concentrations may be harmful to ruminants and monogastrics. Furthermore, with more lactose converted more lactic acid is produced. Lactate concentrations were aimed to be comparable to those obtained from batch and conventional continuous processes.

The model fermentation depicted in Figures 17 and 18 shows graphically the operation and characteristics of the dialysis fermentation. Results indicate that a retention time of 19 hr and ∞ of .4 easily maintains the residual lactose below 0.5% and 0.06% and ammonium above 30 mg/ml and 10 mg/ml in the fermentor and reservoir, respectively. Lactate was found to be 7.9% in the fermentor and 2.5% in the reservoir at these conditions.

For comparison, the ordinary continuous fermentation developed by Keller and Gerhardt (30) at optimum parameters provided the following results: feed whey of 5% lactose, retention time of 31 hr, residual lactose of 0.2% and lactate of 6%. Thus more than 4 times more substrate was put through the continuous dialysis fermentation than could be put through the ordinary continuous system with comparable retention times, conversion efficiencies, and

productivities. These results clearly demonstrate the substantially increased efficiency that can be obtained by dialysis with the continuous fermentation of whey.

Figures 17 and 18 also show the relations of substrate and product between the fermentor and reservoir. Early in the fermentation the mean fermentor-to-reservoir ratios of the concentrations of lactose and ammonium are, respectively, 6.1 and 2.3. The lactate ratio is 2.7 (see Table 4). Clearly, the membrane is the major component of the dialysis process that is limiting efficiency of substrate conversion and reservoir product concentration. A more permeable membrane would allow removal of a greater amount of product from the bacterial culture, thereby enriching the reservoir effluent with lactate. Moreover, additional relief from product inhibition would be had, resulting in more efficient dialysis operation. In other terms, greater membrane permeability increases the process parameter, P_m, thus allowing ϕ to be raised without increasing the residium of lactose in the system.

The whey fermentation culture is very capable of adjusting to fluctuations in its environment. For example, the culture quickly adapts to fluctuations in retention time (Figure 17). Fluctuations above a retention time of 20 hr had little effect on the resulting residual lactose in the system and fluctuations below 20 hr required less than a day to return to the desired residium of lactose. On fermentation day 42 of the retention time studies, the pH inadvertently increased to 8 and the next day decreased to 4. The culture survived this wild fluctuation without reinoculation, but 7 days were needed

for the culture to regain its efficiency of substrate conversion. These examples, together with the nonaseptic conditions, indicate the hardy nature of the culture, a valuable quality of the whey fermentation.

The water balance data of all inputs and outputs of the model dialysis fermentation showed the dilution of the fermenting culture by water from the reservoir circuit (Table 3). Interestingly, this effect becomes significantly less as the fermentation progresses. The average fermentor dilution for the last 4 days of the fermentation was 31.7% as opposed to 38.6% for the first 4 days. The rate of feed whey increased and more concentration polarization occurred as the days progressed, but it was not determined whether these factors had any relation to the decreasing osmosis of water.

The substrate and product material balance data indicate that little substrate was lost to products other than lactate (Table 4). The table also shows the increasing lactate concentration in the fermentor as the days progress; this is a result of the increasing concentration polarization and decreasing membrane permeability, which are indicated by the greater S_f/S_r and P_f/P_r ratios. The lactate concentration remains constant in the reservoir circuit, rather than decreasing, because the osmotic pressure increases due to the greater fermentor lactate concentration.

6.6 Product Quality

The substrate for the dialysis whey fermentation is in concentrated form (23% lactose). Although there is cost to concentrating normal liquid whey, this disadvantage may be offset by the advantages of concentration. The transportation costs would be less for

concentrated whey. Furthermore, by concentrating the whey (i.e., by evaporation) microbial activity in the waste whey would cease, which together with the whey being in concentrated form, would result in a more stable substrate, a good start for obtaining excellent product quality in a fermentation.

The essential purpose of the whey fermentation is to trap ammonia in a salt form so that it can be tolerated by ruminants. It is important, therefore, that the product be predominantly ammonium lactate. Further, a large lactate concentration is wanted so that less energy is needed to concentrate the product for feedlot use. It is also desirable to have a low concentration of lactose in the product. The more sugar substrate that is converted to acid the more ammonia that is trapped. Also, residual lactose may crystallize in a product storage tank and form a troublesome sludge. If the cells produced in the fermentor are to be used as a monogastric protein feed, a low lactose level is desired because of the ill effect of lactose on many monogastrics.

Gas chromatography showed the chemical products of the whey dialysis fermentation to be almost entirely lactate at low residiums of lactose. The model fermentation showed that 97.7% of the substrate could be converted to lactate with less than 0.1% of other metabolic products. Lactate product equal to 7.9% in the fermentor and 2.5% in the reservoir were obtained at a ϕ of .4 and a retention time of 18.5 hr.

The product stream from the reservoir circuit contained protein-free ammonium lactate, which may be an excellent source for

the chemical synthesis of valuable lactic acid and ammonium sulfate. Lactic acid as an acidulent in foods and as a solvent and plasticizer in lacquers and varnishes are just a couple of its many possible uses. Ammonium sulfate may be used agriculturally as a fertilizer.

9. CONCLUSIONS

1. A computer simulation of a modified mathematical model accurately predicted the feasibility of continuous dialysis culture for fermenting whey. Further simulations were a valuable tool in fermentation design and improvement.

2. The process operating at a retention time of 18.5 hr, ϕ of .405, and whey feed of 23% lactose, resulted in residual lactose of 0.2% in the fermentor and 0.05% in the reservoir and lactate of 7.9% and 2.5%, respectively.

3. Improvement rather than degeneration of the conversion efficiency of the bacterial population during extented continuous fermentation was confirmed.

4. Excellent product quality was obtained without sterile and aseptic techniques.

5. A plate-and-frame dialyzer was capable of continuous dialysis for weeks and, when concentration polarization occurred, the dialyzer could be disconnected from the system, cleaned and then reconnected.

6. Membrane permeability and clogging of the dialyzer were the major limitations of the dialysis process; both are possible of improvement.

7. The membrane process yielded two product streams: the fermentor effluent contained ammonium lactate, bacterial cells and residual whey protein, which together could be used for ruminant feed; the reservoir effluent contained only ammonium lactate, which could be used for chemical syntheses.

8. The dialysis fermentation was at least 4 times more efficient than an ordinary continuous fermentation.

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