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ORIGINAL PAPER

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Lactic acid from cheese whey permeate. Productivity and economics of a continuous membrane bioreactor

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Abstract The economics of incorporating membrane modules in several steps in the conversion of whey permeate to lactic acid was studied. Membrane recycle fermenters operating at a cell concentration of 40 g l^{-1} resulted in a productivity of 22.5 g $l^{-1}h^{-1}$ with a lactate concentration of 89 g l^{-1} and a yield of 0.89. The membrane units (reverse osmosis for preconcentrating whey permeate, hollow-fiber ultrafiltration for clarification and for cell recycling) contribute about 28% of the total fixed capital costs and less than 5% of the operating cost. The two largest costs are whey transportation and yeast extract, contributing about 35% and 38% to the total product cost of US $ 0.98/kg 85% lactate. Without these two costs, unpurified lactate could be produced for $ 0.27/kg.

Introduction

Cheese whey is a by-product of the cheese manufacturing industry with an annual worldwide production of about $40 \cdot 7 \times 10^6$ tonnes (40×10^6 tons), half of which is produced in USA. A large portion of it is treated by ultrafiltration to produce whey protein concentrates. However, this process concomitantly produces large quantities of permeate, which contains mostly lactose and ash. The disposal of the permeate is still a problem. One solution is to convert the lactose by fermentation into value-added industrial chemicals, such as lactic acid. Lactates are widely used in the food industry as acidulants, preservatives, precursors for stearoyl-2-lactylates and for the production of industrial polymers such as polylactic acid and acrylic acid. About half of the world's production is made via fermentation. The remainder is produced via synthetic routes (Vick Roy 1985).

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Conventional methods of fermentation that use free cells in batch processes have several limitations, such as low productivity, product inhibition and batch-to-batch variation in the product, leading to high fermentation costs (Cheryan 1986; Cheryan and Mehaia 1984; Mehaia and Cheryan 1986, 1987). Continuous fermentation, on the other hand, although overcoming some of the problems associated with batch processes, is limited by cell wash-out. In order to increase the productivity significantly, it is necessary to use high cell concentrations and to remove the inhibitory product (lactic acid) from the fermentation mixture.

Membrane-based cell-recycling bioreactors can substantially improve productivity of fermentation processes, with few of the problems of immobilized cells or continuous fermentation systems that use free cells (Cheryan 1986, Mehaia and Cheryan 1986 1987). A membrane bioreactor allows us to integrate the fermentation and separation steps, thus simultaneously (a) maintaining high cell density, (b) recycling the cells for further use, and (c) continuously removing lactic acid from the fermenter.

The overall process for the conversion of whey to lactic acid is shown in Fig. 1. This paper focuses on two aspects: technical factors concerning the membrane recycle bioreactor (shown as the fermentation vessel/membrane module loop in Fig. 1) and cost estimates (from the reverse osmosis of the whey permeate to the evaporation step).

Materials and methods

Microorganism

A selected strain of *Lactobacillus bulgaricus* (a freeze-dried culture from Chr. Hansen's Laboratory, Milwaukee, Wis.) was used throughout the study. The bacteria were kept in a frozen form in a dormant state in 0.01 M Na_2HPO_4 buffer solution at –40° C. They were brought to an active state of growth by transferring the buffer solution to either of the liquid media described below.

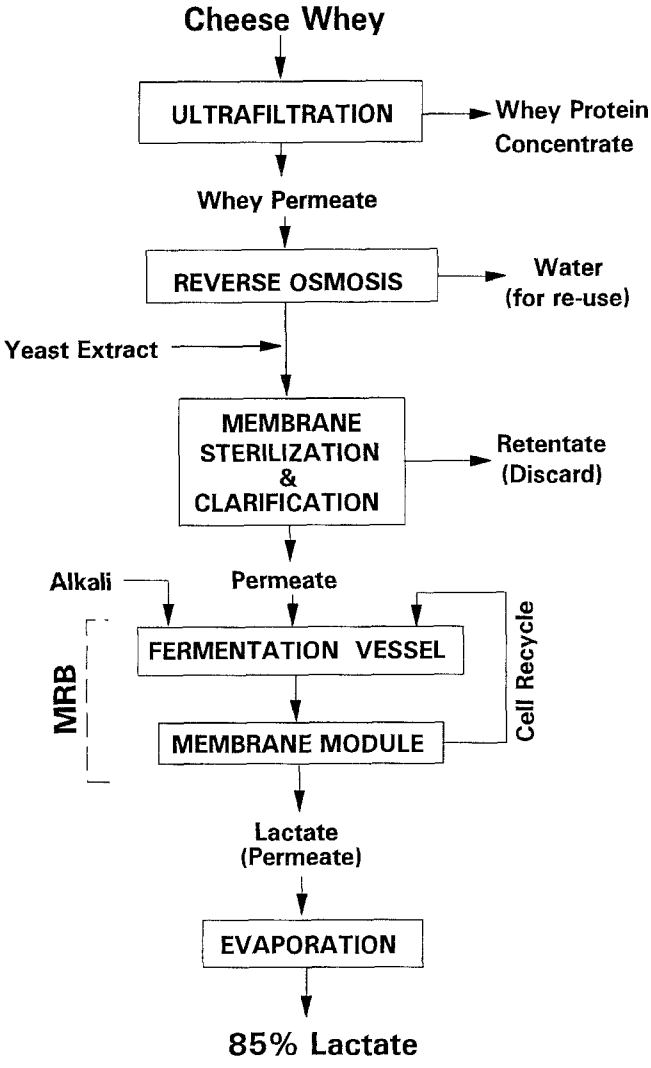

Fig. 1 Scheme of process for converting cheese whey into lactic acid. *MRB* is membrane recycle bioreactor

Media

The feedstock used for the continuous bioreactor studies was whey permeate with added yeast extract. Whey permeate powder was obtained from Express Foods Inc. (Lexington, Ky.). This was the dried mineral/lactose fraction resulting from the ultrafiltration of fluid sweet whey. Its composition was 98%–99% total solids, 79%–81% lactose, 8%–10% ash and 2.5%–4% protein (N×6.38). Yeast extract from Difco Laboratories, Detroit, Mich. was used for all experimental work reported in this study.

The whey permeate powder was suspended in distilled water to the desired solids concentration, mixed with yeast extract at the desired concentration and the pH was adjusted with HCl or NaOH as needed. The medium was cold-sterilized by passing it through either a 0.2-μm cross-flow microfilter (Acroflux capsule, Gelman Sciences, Ann Arbor, Mich.) or an ultrafilter with a 100kDa cut-off (obtained from Amicon, Danvers, Mass., or A/G Technology, Needham, Mass.). The sterile, supplemented whey permeate was stored in a sterile container at 4° C until needed.

Bioreactor

The batch fermenter was a 2-l glass vessel equipped with temperature control, pH controller and agitation. The membrane recycle bioreactor (MRB) has been described earlier (Mehaia and Cheryan 1986, 1987). The fermentation vessel was coupled in a semi-closed loop configuration to a membrane module by a pump. The product stream (containing the lactate and unutilized lactose and nutrients) was withdrawn from the system whereas the cells were recycled back to the bioreactor. When necessary, a small portion of the retentate was bled off from the MRB to keep the cell density constant. To obtain the higher cell concentrations, several cycles of fermentation/ultrafiltration/refermentation were conducted until the desired cell concentration was reached. All pumps were Masterflex peristaltic pumps obtained from Cole Palmer, Chicago, Ill.).

The membrane recycle bioreactor system was operated as a continuous stirred tank reactor (CSTR) as determined by a residence time distribution study (Tejayadi 1990). The total fermentation volume was kept constant by matching the feed flow rate to the permeate flux. During this phase of study, the independent variables were dilution rate (h^{-1}) 0–1.84, substrate concentration ($g\ l^{-1}$) 50, 75, 90, 100, and cell concentration ($g\ l^{-1}$) 5, 10, 12, 20, 40.

The following parameters were kept fixed: pH at 5.6±0.1 by the addition of 8M NH_4OH, temperature at 45° C and MRB volume at 300 ml. The volume of the MRB included the contents of the fermentation vessel, the tube side (retentate channel) of the hollow fiber and the connecting tubings and fittings.

The measured parameters were the concentrations of residual sugars (lactose, glucose and galactose) and product (lactic acid). Samples (5–10 ml) were collected from the permeate and subjected to analyses as described later. Samples from the retentate were collected to measure cell concentration.

Membrane module

Our earlier studies (Tejayadi and Cheryan 1988) with a hollow fiber module indicated that satisfactory flux could be maintained over a long term with lactic acid fermentation broths and that fouling of the membrane by the cells and media components could be controlled within acceptable limits. Reported here are studies with polysulfone hollow fiber modules, which were obtained from A/G Technology, Needham, Mass., Model UFP-30-E-4 with a molecular weight cut-off of 30,000. It contained 104 fibers 26.8 cm in length, 1 mm diameter and 0.035 m^2 surface area.

Calculated parameters

$$\text{Productivity } (g l^{-1}) = \frac{\text{amount of lactate produced}}{\text{fermentation volume} \times \text{time}}$$

$$\text{Yield } (Y_{P/S})(g g^{-1}) = \frac{\text{lactate produced}}{\text{substrate utilized}}$$

In a batch fermentation,

$$\text{Productivity} = \frac{\text{Lactate concentration } (g l^{-1})}{\text{fermentation time (h)}}$$

$$\text{Substrate utilization } (\%) = \left(\frac{S_{\text{initial}} - S_{\text{final}}}{S_{\text{initial}}}\right) \times 100$$

where S is the substrate concentration.

In a continuous fermentation, Productivity = lactate concentration ($g\ l^{-1}$) × dilution rate (h^{-1})

$$\text{Dilution rate } (D)(h^{-1}) = \frac{\text{product stream flow rate or flux } (l h^{-1})}{\text{volume of system (l)}}$$

$$\text{Substrate utilization } (\%) = \left(\frac{S_{\text{inlet}} - S_{\text{outlet}}}{S_{\text{inlet}}}\right) \times 100$$

Analytical methods

Lactose, glucose, galactose and lactic acid concentrations were measured by HPLC. An Aminex HPX-87H column (BioRad, Richmond, Calif.) was used with 0.005 M H_2SO_4 as the eluant at a flow rate of 0.8 ml/min at 65° C. A refractive index detector (Waters Associates) was used with a Fisher recorder.

Cell concentration was determined optically at 525 nm and then calibrated with dry weight. Cells were centrifuged, washed with distilled water twice and dried at 70° C until constant weight was obtained.

Results

Membrane recycle bioreactor

Figure 2 shows a typical long-term stability study of the membrane recycle bioreactor operated at a dilution rate of 0.5 h^{-1} and an average cell concentration of 40 g l^{-1}. A gradual approach to steady state and complete substrate utilization was observed. After 130 h of operation, the lactate concentration was 46 g l^{-1} with an initial lactose concentration of 50 g l^{-1}, resulting in a productivity of 23 g $l^{-1}h^{-1}$. Increasing the substrate concentration to 75 g l^{-1} resulted initially in a dramatic decrease in performance, but the MRB eventually stabilized at a lactic acid concentration of 70 g l^{-1} and a productivity of 35 g $l^{-1}h^{-1}$.

Figure 3 shows another long-term stability study conducted with different variables. The initial substrate concentration was 100 g l^{-1} and the dilution rate was first set at 0.5 h^{-1}. This combination of concentrations and dilution rate was too much for the MRB, resulting in fairly high levels of unutilized lactose and galactose in the broth even after 150 h of operation. Lowering the dilution rate to 0.25 h^{-1} resulted in 99% substrate utilization and correspondingly higher lactic acid concentration of 89 g l^{-1}, with a productivity of 22 g $l^{-1}h^{-1}$.

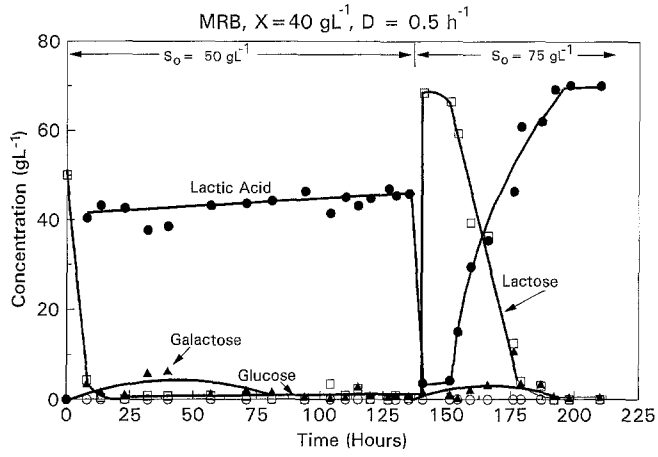

Fig. 2 Performance of MRB with whey permeate. Cell concentration (X) was 40 g l^{-1}, and dilution rate (D) was kept at 0.5 h^{-1}

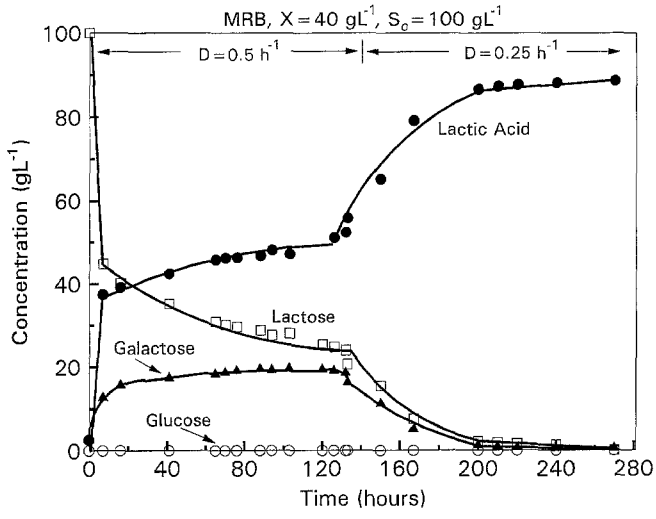

Fig. 3 Stability of MRB at a cell concentration of 40 g l^{-1} and inlet lactose concentration of 100 g l^{-1}. The dilution rate was varied as shown in the figure

Process design

The single most important factor controlling production or operating cost is the lactate concentration (P) from the MRB, because it determines several design parameters. The other parameters can be calculated as follows:

1. Substrate concentration to use in the feed to the reactor (S), where $S = P/Y_{P/S}$

This is also the substrate concentration from the reverse osmosis unit. The degree of concentration necessary in the reverse osmosis system is the concentration factor (X), where $X = S/S_0$, S_0 being the substrate concentration in the feed (in this case, the lactose concentration in the whey permeate, 45 g l^{-1}). Therefore, if the whey permeate to be processed on an hourly basis is W, then the flow rate of the reverse osmosis retentate (F_{RO}) is given by $F_{RO} = W/X$.

2. The membrane area for the RO system will increase with an increase in P: membrane area = $(W - F_{RO})/J_{RO}$

where J_{RO} is the flux of the reverse osmosis system. Flux was estimated from the data of Schlicher and Cheryan (1990), from which membrane permeability coefficients and osmotic pressure data for a thin-film composite membrane were obtained. This membrane is available in a spiral-wound configuration, which today costs about U.S. $ 300/m^2 for the complete plant, including the first set of membranes [personal communication (1993), Filtration Engineering, New Hope, Minn.; Niro Filtration Hudson, Wis.; Osmonics Inc., Minnetonka, Minn.].

3. The membrane area for the membrane clarifier/sterilizer system will decrease as P increases. Assuming a 5% loss of the reverse-osmosis-concentrated whey permeate in this system: membrane clarifier/sterilizer area = 0.95 $(F_{RO})/J_{STER}$.

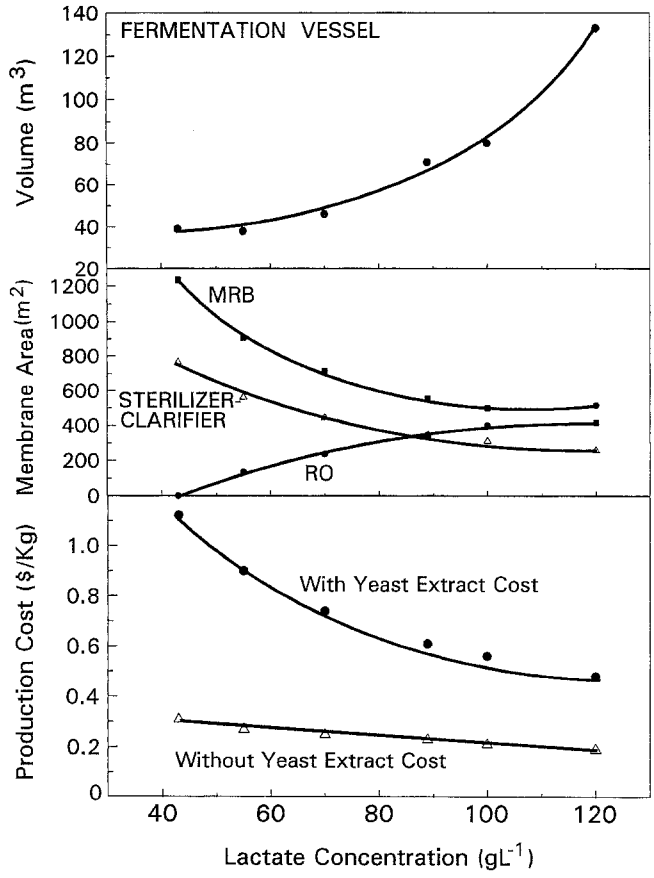

Fig. 4 Effect of lactate concentration on volume of the fermentation vessel (*top*); membrane area in the MRB, sterilizer/clarifier and reverse osmosis (*RO*) system (*middle*); and on production cost, assuming no whey transportation cost (*bottom*).

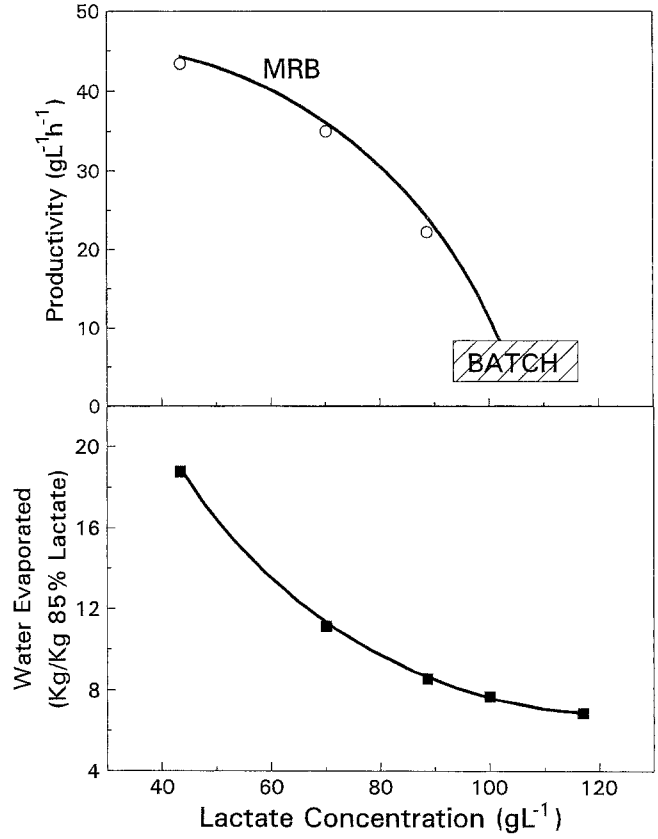

Fig. 5 Effect of lactate concentration in the MRB on productivity (at maximum yield of product and substrate utilization) and on the amount of water to be evaporated to produce 85% lactate

4. The membrane area for the ultrafiltration unit in the MRB will decrease as P increases. Assuming a loss of 1% of the flow into the MRB as cell bleed: MRB ultrafiltration area $=(0.99)\ (0.95)\ (F_{RO})/J_{MRB}$. The values of flux for the above two cases (J_{STER} and J_{MRB}) were obtained in our laboratory (Tejayadi and Cheryan 1988; Tejayadi 1990).

5. The dilution rate (D) will decrease with an increase in P. This can be obtained from Fig. 5, since $D=$ productivity$/P$.

6. The volume of the MRB will increase with P: volume $=(0.99)\ (0.95)\ F_{RO}/D$

These factors are shown in Fig. 4 as a function of product concentration (P). The net effect of the various factors on the production cost is shown in Fig. 5 (bottom). The amount of yeast extract needed is a function of the flow rate of the substrate into the MRB (i.e., a function of F_{RO}, which decreases as P increases). Thus, the amount of yeast extract needed will decrease as P increases. Yeast extract is the largest single operating cost in the analysis. Figure 4 also shows the operating cost minus the yeast extract cost as a function of product concentration. In this case, the production cost becomes much less dependent on the lactate concentration from the MRB.

Economic analysis

Cost estimates are based on the assumption that 300×10^6 l of whey permeate (containing 4.5% lactose) is available per year (300 operating days/year). The economic analysis has been done from the reverse osmosis step to the evaporation step shown in Fig. 1. Annual production would be about 12×10^6 kg (about 26×10^6 lb) 85% lactic acid, assuming a $Y_{P/S}$ of 0.89. Based on the process design outlined earlier, the daily production of 85% lactate is 44712 kg. Standard costs are taken from several sources indicated in the tables, as well as from Axtell and Robertson (1986), Garrett (1989), Reisman (1988) and Remer and Chai (1990). Costs given in those sources were averaged and adjusted by using the Consumer Price Index published in current issues of *Chemical Engineering* (McGraw-Hill, New York). The cost of converting to the acid form of lactic acid is not included in this analysis.

Table 1 shows the total fixed capital investment required for the cheese whey permeate-to-lactate plant. The membrane units (reverse osmosis and ultrafiltration) contribute about 28% of the total fixed capital costs.

Table 1 Fixed capital investment of whey permeate-to-lactate plant Basis: product (lactate) concentration from membrane recycle bioreactor (MRB) = 89 $g l^{-1}$

Item	Size	Unit cost ($/m^2$)	Cost ($)	Reference[a]
Direct costs				
1. Installed equipment				
Ultrafiltration				
Sterilization	350 m^2	900	315000	1
MRB	560 m^2	900	504000	1
Reverse osmosis	350 m^2	300	105000	2
Fermentation vessel (including agitator)	72000 l		100000	3, 4
Holding tank for whey permeate	1×10^6 l		150000	5
Holding tank for yeast extract	4700 l		11000	5
Mixing tank	25000 l		55000	5
Seed culture tank	400 l		3000	5
Six-stage multiple-effect evaporator			700000	6
Total installed equipment			1943000	
2. Piping, wiring, instrumentation (30% of 1)			582900	7
3. Buildings (10% of 1)			194300	7
4. Land improvements (5% of 1)			97200	7
5. Total direct costs			2817400	
Indirect costs				
Contingencies, engineering, contracting fees (27.5% of 5)			775600	7
Fixed capital investment			3593000	
Working capital (15% of fixed capital)			538950	
Total capital investment			4131950	

[a] *1* Personal communication (1993) A/G Technology, Needham, Mass.; Koch/Romicon, Wilmington, Mass.; CeraMem Corp., Waltham, Mass.; U.S. Filter, Warrendale, Pa. *2* Personal communication (1993) Filtration Engineering, New Hope, Minn.; Niro Filtration, Hudson, Wis.; Osmonics Inc., Minnetonka, Minn. *3* Reisman (1988). *4* Pace and Goldstein (1975). *5* Naser and Fournier (1988). *6* Personal communication (1993) Swenson Process Equipment Inc., Harvey, Ill. *7* Peters and Timmerhaus (1988)

Table 2 shows the operating costs and production cost, assuming (a) the whey permeate was obtained from a cheese factory situated 160 km (100 miles) from the lactic acid plant and was transported by road, and (b) there was no cost of the whey permeate. In this case, the two largest costs are whey transportation and yeast extract, contributing about 35% and 38% to the total product cost.

Discussion

The goals in any industrial fermentation process should be:

1. Maximum productivity, to minimize capital and operating costs
2. Maximum product concentration, to minimize downstream (concentration) costs
3. Maximum substrate utilization, to minimize feedstock costs
4. Maximum yield ($Y_{P/S}$), to minimize by-product formation and consequent downstream (separation) costs.

However, it is well-known in high-rate fermentation processes that, under conditions of highest substrate utilization and yield, high product concentration and high productivity are mutually exclusive. Figure 5 (top) shows such a relationship for our process. The literature indicates that productivities in batch fermentors range from 1 g $l^{-1}h^{-1}$ to 7.5 g $l^{-1}h^{-1}$ (this does not include the cycle time of the fermentors). In our laboratory, productivities ranged from 4.5 g $l^{-1}h^{-1}$ at 117 g l^{-1} lactate (Mehaia and Cheryan 1987) to 7.5 g $l^{-1}h^{-1}$ at 43 g l^{-1} (Tejaydi 1990). In contrast, the MRB could achieve much higher productivities, but at lower product concentration. As a result, higher amounts of water have to be removed at higher productivities. This is shown in the bottom portion of Fig. 5. There is a steep increase in the amount of water removal required below 80 g l^{-1} lactate, with not much change above 100 g l^{-1}.

The whey transportation cost could be reduced by installing the lactic acid plant in the same location as the cheese plant. It would eliminate this cost from Table 2 and reduce the cost from $ 0.98/kg to $ 0.64/kg. If it is not feasible to locate the lactic acid plant in the same vicinity as the whey plant, then at least the whey permeate should be concentrated 2.2 times before transportation, i.e., the reverse osmosis should be done at the whey plant. This would reduce the transportation cost in Table 2 to less than half the amount, reducing the production cost from $ 0.98/kg to $ 0.79/kg of 85% lactate.

Table 2 Operating costs for lactate plant. Same basis as Table 1. Whey permeate is available from a cheese or whey processing plant situated 160 km (100 miles) from the lactic acid plant (*UF* ultrafiltration, *RO* reverse osmosis)

Item	Unit cost	Quantity per day	Cost ($/day)	Percentage of total cost	Reference[a]
Operating costs					
1. Raw materials					
Whey permeate (transportation cost)	$ 1.50/100 l	10^6 l	15000	34.6	1
Yeast extract	$ 3.85/kg	4320 kg	16632	38.3	2
Ammonium hydroxide	$ 1.10/l	190 l	210	0.5	
Sulfuric acid	$ 0.88/kg	955 kg	840	1.9	
2. Utilities (excluding evaporation)					
Water	$ 0.26/1000 l	77000 l	20	0.1	3
Electricity	$ 0.06/kW h	1400 kW h	84	0.2	3
3. Energy costs for evaporation					
Steam (50 lb/in^2; 3.5 kg/cm^2)	$ 11/1000 kg	82000 kg	900	2.1	3–7
4. Labor	$ 15/h	50 h	750	1.7	
5. Membranes					
Replacement	$ 600/m^2 (UF)		1937	4.5	8, 9
Cleaning	$ 100/m^2 (RO)		50	0.1	8, 9
6. Maintenance and repairs (6% of fixed capital investment/year)			826	1.9	10
7. Operating supplies (2% of fixed capital investment/year)			275	0.6	10
8. Plant overhead (70% of 4, 6, 7)			876	2.0	10
Fixed charges					
1. Depreciation (20% per year of fixed capital investment)			2754	6.3	10
2. Insurance (1% per year of fixed capital investment)			138	0.4	10
General and administrative expenses (5% of manufacturing cost)			2090	4.8	10
Total production cost/day			43383	100	
Cost of 85% lactate = $ 43383/day for 44712 kg/day = $ 0.98/kg					

[a] *1* Lenz and Moreira (1980). *2* Personal communication (1992) Universal Foods Red Star Division, Milwaukee, Wis.; Sheffield Products (Quest International), Sarasota, Fla.; *3* Personal communication (1993) from local utility companies. *4* Axtell and Robertson (1986). *5* Garrett (1989). *6* Personal communication, Swenson Process Equipment Inc., Harvey, Ill. *7* Gienger and Ray (1988). *8* Personal communication (1993) A/G Technology, Needham, Mass.; Koch/Romicon, Wilmington, Mass.; CeraMem Corp., Waltham, Mass.; U.S. Filter, Warrendale, Pa. *9* Personal communication (1993) Filtration Engineering, New Hope, Minn.; Niro Filtration, Hudson, Wis.; Osmonics Inc., Minnetonka, Minn. *10* Peters and Timmerhaus (1988)

The current market price of lactic acid is $ 1.60–$ 2.20/kg, depending on the form of the lactate (acid or salt), the strength (60%–85%) and purity (personal communication 1993: ADM, Decatur, Ill.; Purac Inc., Arlington Heights, Ill.; Sterling Chemicals, Houston, Tex.; Eco-Chem, Adell, Wis.). If the lactic acid were to be used for the manufacture of biodegradable polymers, it would have to be considerably purer than that produced by a fermentation process. Thus, the production cost must be lowered even further, especially if the whey permeate is to be purchased (our analysis assumed the whey permeate is available at no cost).

The membrane-related costs account for less than 5% of the operating cost. Polymeric ultrafiltration hollow-fiber membranes were used for the process design. Continuing improvements in membrane technology could lower this cost further in the near future. For example, although ceramic/inorganic membranes are more expensive, they have much longer operating lifetimes and generally much higher flux for this application. Thus they may prove to be more economical than polymeric membranes in the long term. Electrodialysis could also be used to reduce the downstream costs (Yen and Cheryan 1991, 1993).

The remaining large cost is the nutrient cost. The nutrient (yeast extract) levels used in our experiments (10 g l^{-1}) were based on previous work in our laboratory that indicated this fermentation was critically dependent on certain nutrients available from the yeast extract. Finding a cheaper nutrient and adapting the selected lactic culture to that nutrient will be a key factor in the commercial success of this process.

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