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Dialysis Continuous Process for Ammonium-Lactate Fermentation: Improved Mathematical Model and Use of Deproteinized Whey†

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Separate terms for substrate limitation and product inhibition were incorporated into an equation describing the rate of cell growth for the steady-state fermentation of lactose to lactic acid with neutralization to a constant pH by ammonia. The equation was incorporated into a generalized mathematical model of a dialysis continuous process for the fermentation, developed previously, in which the substrate is fed into the fermentor and the fermentor contents are dialyzed through a membrane against water. The improved model was used to simulate the fermentation on a digital computer, and the results agreed with previous experimental tests using whole whey as the substrate. Further simulations were then made to guide experimental tests using deproteinized whey as the substrate. Dried cheese-whey ultrafiltrate was rehydrated with tap water to contain 242 mg of lactose per ml, supplemented with 8 mg of yeast extract per ml, charged into a 5-liter fermentor without sterilization, adjusted in pH (5.5) and temperature (44°C), and inoculated with an adapted culture of *Lactobacillus bulgaricus*. The fermentor and dialysate circuits were connected, and a series of steady-state conditions was managed nonaseptically for 71 days. The fermentation of deproteinized whey relative to whole whey, with both highly concentrated, resulted in similar extents of product accumulation but at a lesser rate.

Whey utilization continues to be a problem for the dairy industry. Whey can be processed by pressure filtration through semipermeable membranes to obtain protein concentrates (3). Forty to 70% of the original protein is recovered (10, 15). The protein has many commercial uses because of its high nutritional quality (20, 23, 24). However, the capital costs of production are high and large volumes of lactose-containing ultrafiltrate are left as residue, which is nearly as much an environmental burden and an economic and nutrient loss as the whole whey. Thus, feasibility of the process is dependent upon use of the deproteinized but lactose-rich ultrafiltrate.

A potential solution to this problem lies with the conversion of whey ultrafiltrate into feedstuff for ruminant animals, accomplished by the bacterial fermentation of the lactose into lactic acid and its neutralization to constant pH by ammonia (7). A background for this development exists in studies of the fermentation using whole whey as the substrate. The fermentation can be managed as a batch process (18), continuous process (11), or dialysis continuous process (4, 22). The latter process relative to the nondialysis processes enables the use of more concentrated substrate, is more efficient in the rate of substrate conversion, and additionally produces a dialysate effluent of less concentrated but purer ammonium lactate.

In the studies reported here, a rate expression for bacterial growth was developed containing separate terms for substrate limitation and product inhibition. The expression was incorporated into a mathematical model of the dialysis continuous process generalized with dimensionless parameters so that it could be widely applied (4). The resulting improved set of equations was employed to simulate the fermentation on a digital computer, and the results were verified using previous experimental results with whole whey as the substrate (22). Further simulations were then made to guide experimental tests in which deproteinized whey was used as the substrate. The simulated and experimental results were used to compare the relative value of the process applied to the two substrates.

MATHEMATICAL MODEL

Growth rate theory. An expression describing the rate of cell growth, previously used in a mathematical

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model of the dialysis continuous ammonium-lactate fermentation (see equation 11 in reference 4) is as follows:

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

The symbols in this and subsequent equations are described in Table 1.

In the studies of Stieber et al. (22), an exaggerated K_p value (1,000 mg/ml) was used to correlate the simulation with the experimental results. The high value for K_p eliminates the effect of the product inhibition term, and equation 1 is reduced to the following equation:

$$r_g = \mu_m\left(\frac{S_f}{K_s + S_f}\right)X_f \quad (2)$$

This equation is based on classical Michaelis-Menten kinetics and adequately describes the rate of cell growth for the fermentation. However, the separate effects of substrate limitation and product inhibition cannot be determined. Moreover, equation 2 is based on an enzyme-substrate-product relationship which implies that there is no product inhibition (21).

These restrictions were considered, and the following competitive relationship was proposed:

$$X_f + S_f \xrightarrow{k_1} X_fS_f \xrightarrow{k_2} X_fP_f \underset{k_4}{\overset{k_3}{\rightleftarrows}} X_f + P_f \quad (3)$$

where k_1, k_2, k_3, and k_4 are rate constants. Once the cells (X_f) obtain substrate (S_f), it is metabolized to product (P_f). P_f cannot be converted to S_f. Further, since $k_4 \neq 0$, the cells are inhibited by P_f (i.e., they cease obtaining substrate). In equation 3, assuming there is an adequate concentration of cells and substrate, the rates of substrate utilization and product formation depend mostly on the concentration of product in the environment of the cells.

An expression describing the rate of cell growth was obtained from equation 3 by a steady-state approach (21):

$$r_g = \mu_m\left(\frac{S_f}{K_s + S_f + K_pP_f}\right)X_f \quad (4)$$

where $\mu_m = k_2k_3/(k_2 + k_3)$, $K_s = k_2k_3/[k_1(k_2 + k_3)]$, and $K_p = k_2k_4/[k_1(k_2 + k_3)]$. The substrate-limitation con-

TABLE 1. *Glossary of mathematical symbols*

Symbol	Description	Units
A_m	Area of membrane available for dialysis	cm^2
F_d	Flow rate into and out of dialysate circuit	ml/h
F_f°	Flow rate into fermentor circuit	ml/h
F_f	Flow rate out of fermentor circuit	ml/h
K_p	Product-inhibition constant	mg/mg
K_s	Substrate-limitation constant	mg/ml
P_d	Product concentration in dialysate circuit	mg/ml
P_f°	Product concentration in fermentor feed	mg/ml
P_{mp}	Permeability of membrane to product	mg/cm^2-h
P_{ms}	Permeability of membrane to substrate	mg/cm^2-h
r_g	Rate of cell growth	mg/ml-h
r_p	Rate of product formation	mg/ml-h
$-r_s$	Rate of substrate utilization	mg/ml-h
S_d	Substrate concentration in dialysate circuit	mg/ml
S_f°	Substrate concentration in fermentor feed	mg/ml
S_f	Substrate concentration in fermentor circuit	mg/ml
t	Time	h
V_d	Volume of liquid in dialysate circuit	ml
V_f	Volume of liquid in fermentor circuit	ml
X_f	Cell-mass concentration in fermentor circuit	mg/ml
α	Substrate/cell ratio	mg/mg
β	Specific maintenance rate	h^{-1}
γ	Product/substrate ratio	mg/mg
E	Efficiency of lactose conversion	%
μ_m	Maximum specific growth rate of cells	h^{-1}
T_f	Cell-retention time in fermentor circuit	h

TABLE 2. *Glossary of dimensionless parameters*

Type	Symbol and definition	Description
Material parameters	$\bar{P}_d = P_d/(\gamma S_f^\circ)$	Product factor in dialysate circuit
	$\bar{P}_f = P_f/(\gamma S_f^\circ)$	Product factor in fermentor circuit
	$\bar{P}_f^\circ = P_f^\circ/(\gamma S_f^\circ)$	Product factor in fermentor feed
	$\bar{S}_d = S_d/S_f^\circ$	Substrate factor in dialysate circuit
	$\bar{S}_f = S_f/S_f^\circ$	Substrate factor in fermentor circuit
	$\bar{X}_f = \alpha X_f/S_f^\circ$	Cell factor in fermentor circuit
Operational parameters	$R = P_{mp}/P_{ms}$	Ratio of product/substrate membrane permeabilities
	$\Pi = P_{ms}A_m/F_f$	Membrane permeability factor
	$\phi = F_d/F_f$	Flow-rate ratio
Kinetic parameters	$\bar{K}_s = K_s/S_f^\circ$	Substrate-limitation factor
	$\bar{K}_p = \gamma K_p$	Product-inhibition factor
	$\theta = \mu_m T_f$	Time factor

stant (K_s) and the product-inhibition constant (K_p) express the relationships between the actual steady-state concentrations of the various cell, substrate, and product states.

Generalized model. The design of the fermentation system, the assumptions for purposes of modeling, the material balance equations, and the rate equations for substrate utilization and product formation were the same as those used previously (4). The rate equations and equation 4 were combined with the material balance equations, and the variables were defined in dimensionless parameters (Table 2) to obtain a generalized model for dialysis continuous fermentation.

The resulting equations for the fermentor circuit are as follows:

$$\frac{d\bar{S}_f}{dt} = \left[-(1 + \Pi)\bar{S}_f - \left[\theta\left(\frac{\bar{S}_f}{\bar{K}_s + \bar{S}_f + \bar{K}_p\bar{P}_f}\right) + \frac{\beta T_f}{\alpha}\right]\bar{X}_f + \Pi\bar{S}_d + 1\right]/T_f \quad (5)$$

$$\frac{d\bar{P}_f}{dt} = \left[-(1 + R\Pi)\bar{P}_f + \left[\theta\left(\frac{\bar{S}_f}{\bar{K}_s + \bar{S}_f + \bar{K}_p\bar{P}_f}\right) + \frac{\beta T_f}{\alpha}\right]\bar{X}_f + R\Pi\bar{P}_d + \bar{P}_f^o\right]/T_f \quad (6)$$

$$\frac{d\bar{X}_f}{dt} = \left[\theta\left(\frac{\bar{S}_f}{\bar{K}_s + \bar{S}_f + \bar{K}_p\bar{P}_f}\right) - 1\right]\bar{X}_f/T_f \quad (7)$$

where $T_f = V_f/F_f$ and is an operational parameter.

For the dialysate circuit, the corresponding equations are:

$$\frac{d\bar{S}_d}{dt} = \left[-(\Pi + \phi)\bar{S}_d + \Pi\bar{S}_f\right]F_f/V_d \quad (8)$$

$$\frac{d\bar{P}_d}{dt} = \left[-(R\Pi + \phi)\bar{P}_d + R\Pi\bar{P}_f\right]F_f/V_d \quad (9)$$

This generalized model improves upon that developed previously (see equations 12 to 16 in reference 4).

Generalized steady-state solution. The equations of the generalized model were rearranged and combined and the time derivatives were set at zero to obtain a generalized solution for the steady state, comparable to that developed previously (see equations 17 to 21 in reference 4).

Equations for the substrate, product, and cell mass in the fermentor circuit are as follows:

$$\bar{S}_f = \frac{\bar{K}_s\pi_p + \bar{K}_p + \bar{K}_p\bar{P}_f^o}{\pi_p(\theta - 1) - \pi_s\bar{K}_p} \quad (10)$$

$$\bar{P}_f = \frac{\bar{K}_s\pi_s + \theta - 1 + \bar{P}_f^o(\theta - 1)}{\pi_p(\theta - 1) - \pi_s\bar{K}_p} \quad (11)$$

$$\bar{X}_f = \frac{1 - \bar{S}_f + \Pi(\bar{S}_d - \bar{S}_f)}{1 + \beta T_f/\alpha} \quad (12)$$

where $\pi_s = \Pi[\Pi/(\phi + \Pi) - 1] - 1$, and $\pi_p = 1 + R\Pi[1 - R\Pi/(\phi + R\Pi)]$.

For the dialysate circuit, the corresponding equations are:

$$\bar{S}_d = \Pi\bar{S}_f/(\phi + \Pi) \quad (13)$$

$$\bar{P}_d = R\Pi\bar{P}_f/(\phi + R\Pi) \quad (14)$$

Equations 10 to 14 thus comprise a generalized steady-state solution for substrate, product, and cells in the fermentor and dialysate circuits of the system.

COMPUTER SIMULATIONS

Previous experimental results with whole whey (22) were used in digital-computer simulations to validate the improved mathematical model. The values used are listed in Table 3. The values for ϕ and Π were calculated directly from the experimental results. The values for μ_m, K_s, and K_p were determined by successive curve fitting of the simulated and experimental results.

A side effect of the dialysis process was a large osmotic influx of water from the dialysate into the fermentor, diluting its contents. The dilution was accounted for by assuming that the diluting water entered the fermentor with the feed stream rather than from the dialysate and by correcting S_f^0 accordingly.

Figure 1 shows the computer-simulated curves obtained by using the steady-state solution with the values in Table 3 to describe the correlation between one important operating parameter and the conversion efficiency, e.g., T_f versus S_f and S_d. The curves all fit closely with the superimposed points of the previous experimental results with whole whey (22).

TABLE 3. *Values used for computer simulations of previous fermentations with whole whey (22)*

Figure (days)	μ_m (h^{-1})	$\bar{K}_p$	$\bar{K}_s$	T_f (h)	ϕ	Π	R
1 (19–24)	0.145	0.6	0.0004		3.7	0.66	3.0
1 (50–75)	0.25	0.6	0.0004		3.4	0.62	3.0
2 (40–63)	0.25	0.6	0.0004	16		0.59	3.0

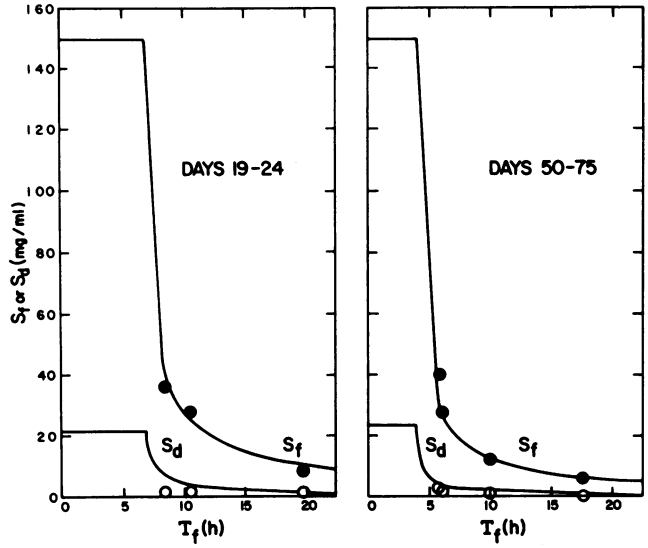

FIG. 1. *Computer-simulated effects of cell-retention time (T_f) on residual lactose in the fermentor circuit (S_f) and the dialysate circuit (S_d) during two periods of dialysis continuous fermentation. The curves were plotted by use of the steady-state solution with the values in Table 3. The points were replotted from Fig. 2 in reference 22 to demonstrate the close fit between the experimental results and the computer simulations.*

A similar comparison between the simulated and the experimental results was also made for a second operating parameter, e.g., F_d versus S_f and S_d (Fig. 2). Although not fitting as closely to the experimental points, the simulation curves described the trends well. The discrepancy mostly was caused by decreased permeability of the membranes.

Altogether, the results demonstrated the validity of the mathematical model, which then was used to guide experimental tests for the ammonium-lactate fermentation of deproteinized whey. The improved model predicted the same effects of changes in the various parameters on the process and the same regions for experimental tests as did the previous model (4).

MATERIALS AND METHODS

Inoculum. The inoculum culture was obtained from the fermentor effluent (day 94) of a previous dialysis continuous fermentation (22) originally started with *Lactobacillus bulgaricus* 2217 (Chris Hanson's Laboratory, Milwaukee, Wis.).

Substrate. Dried deproteinized cheese whey (prepared by ultrafiltration of whole sweet-cheese wheys by Stauffer Chemical Co., Rochester, Minn.) was rehydrated to contain 242 mg of lactose per ml and was supplemented with 8 mg of yeast extract per ml. The reconstituted whey was made up in 7-liter batches without sterilization, stored at 4°C, and held in a stirred, heated (60°C) reservoir to keep the lactose in solution.

Dialysis continuous fermentation system. The experimental dialysis fermentation system was conducted continuously at a temperature of 44°C and a pH of 5.5 with essentially the same equipment as used previously (22). However, plunger-type reciprocating pumps (type " P", Bran and Lubbe, Inc., Evanston, Ill.) were used to meter the whey and water, and the pH was regulated with a different automatic device (model pH-40, New Brunswick Scientific Co., New Brunswick, N. J.). The circulation rates through the dialyzer, for both the fermentor and dialysate circuits, were 2 liters per min. Operation of the system was interrupted once, for 3 weeks, during which the fermentor-circuit contents were stored at 4°C.

Analytical procedures. Samples from the dialysate and fermentor circuits were taken at 12-h intervals from the dialysate effluent and from a glass "T" inserted in the tubing between the fermentor and dialyzer. Steady-state data were determined from samples taken at five times the cell retention time or 48 h after changing a parameter. Lactose in the samples was determined by the colorimetric method of Morris (14). Lactic acid was determined by use of a gas chromatograph (series 1420, Varian Associates, Palo Alto, Calif.) with an integrator (model CDS 111, Varian Associates), using a stainless-steel column (6 feet by ⅛ inch [ca. 1.83 m by 0.32 cm] outside diameter) packed with 10% SP-1000/1% H_3PO_4 on 100/120 Chromosorb WAW (Supelco, Inc., Bellefonte, Pa.). Samples were prepared by the procedure of Holdeman and Moore (9). Lactic acid also was determined by the colorimetric method of Pryce (17). Specific conductance was measured with a conductivity bridge (model RC-16B2, Beckman Instruments, Inc., Cedar Grove, N. J.).

RESULTS

Cell-retention time versus conversion efficiency. The results of experimental variation in the cell-retention time (T_f) affecting the resid-

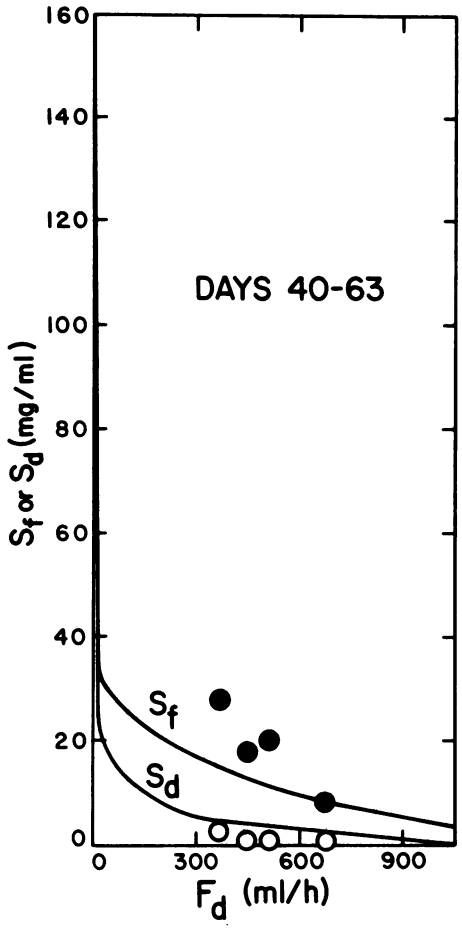

FIG. 2. *Computer-simulated effects of dialysate flow rate (F_d) on residual lactose in the fermentor circuit (S_f) and the dialysate circuit (S_d) with cell-retention time held constant (T_f = 16 h). The curves were plotted by use of the steady-state solution and the values in Table 3. The points were replotted from Fig. 4 in reference 22 to demonstrate the fit between the experimental results and the computer simulations.*

ual lactose in the fermentor circuit (S_f) and the dialysate circuit (S_d) showed that the lactose concentrations in both circuits decreased with increased T_f (Fig. 3). All of the curves reached a point where an increase in T_f did little to decrease the lactose concentration.

Dialysis rate versus conversion efficiency. The flow-rate ratio (ϕ) is an operational parameter used to manipulate the rate of dialysis. Results of experimental variation in ϕ affecting the residual lactose in the fermentor circuit (S_f) and the dialysate circuit (S_d) are also shown in Fig. 3. The lactose concentrations were less with increased dialysis (ϕ = 2.5) than with decreased dialysis (ϕ = 1.0). The effect was mostly on S_d.

Cell-retention time versus lactate concentration. Figure 4 shows the results of variation in T_f affecting the lactate concentrations in the fermentor circuit (P_f) and in the dialysate circuit (P_d). The lactate concentrations increased to a maximum with increasing T_f.

Dialysis rate versus lactate concentration. Figure 4 also shows the results of the flow-rate ratio affecting P_f and P_d. ϕ had little effect on P_f. However, P_d was much greater with decreased dialysis (ϕ = 1.0) than with increased dialysis (ϕ = 2.5). Values for the dialysate product yield ($\bar{P}_d\phi$, which represents the fraction of substrate leaving as lactate in the dialysate effluent) were similar at either value of ϕ.

Lactose conversion and lactate productivity. Figure 5 shows that the lactate productivity decreased with increased T_f, whereas the percentage of lactose converted to product increased with increased T_f. High lactate productivity and high lactose conversion along with high lactate concentrations are all desirable.

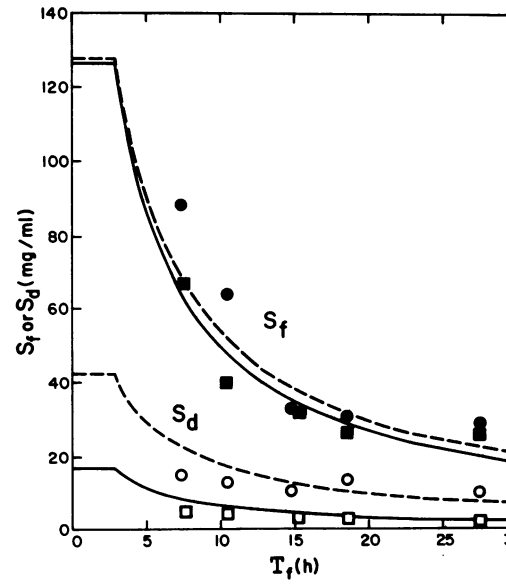

FIG. 3. *Computer-simulated effects of cell-retention time (T_f) on residual lactose in the fermentor circuit (S_f) and the dialysate circuit (S_d) at two flow-rate ratios (ϕ) and during two time periods of dialysis continuous fermentation. The curves were plotted by use of the steady-state solution and the values in Table 5. The points are experimental data and demonstrate the fit between the experimental results and the computer simulations. The dashed curves and circle points were obtained at ϕ = 1.0 and during days 26 to 36. The smooth curves and square points were obtained at ϕ = 2.5 and during days 40 to 49.*

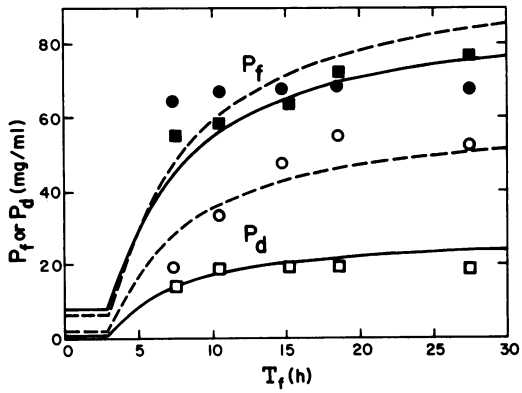

FIG. 4. *Computer-simulated effects of cell-retention time (T_f) on accumulated lactate in the fermentor circuit (P_f) and the dialysate circuit (P_d) at two flow-rate ratios (ϕ) and during two time periods of dialysis continuous fermentation. The curves were plotted by use of the steady-state solution and the values in Table 5. The points are experimental data and demonstrate the fit between the experimental results and the computer simulations. The dashed curves and circle points were obtained at ϕ = 1.0 and during days 26 to 36. The smooth curves and square points were obtained at ϕ = 2.5 and during days 40 to 49.*

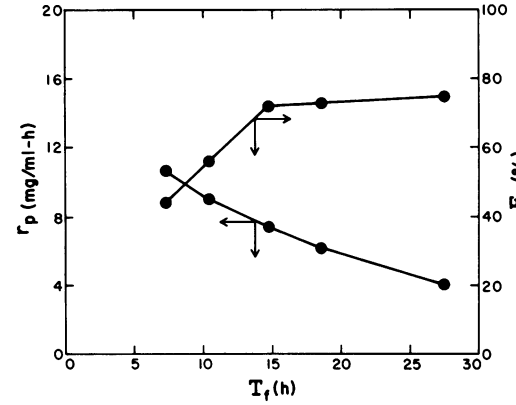

FIG. 5. *Experimental effect of changes in cell-retention time (T_f) on lactose conversion (E) and lactate productivity (r_p) at a flow-rate ratio (ϕ) of 1.0. Percent lactose conversion = $[1 - (F_fS_f + F_dS_d)/(F_f^0S_f^0)] \times 100$. Lactate productivity = $(P_fF_f + P_dF_d - P_f^0F_f^0)/(T_fF_f)$.*

Consequently, trade-offs in the regulation of T_f are required in designing the system for practical use.

Product/substrate ratio. Results of experimental variation in T_f and ϕ showed that these operational parameters had little effect on the product/substrate ratio (γ, results not shown). Consequently, for purposes of modeling the ammonium-lactate fermentation, a fixed value was assigned to γ (0.96). The ratio was calculated from r_p/r_s, where values for r_p were obtained from Fig. 5 and where $r_s = (S_f^0F_f^0 - S_fF_f - S_dF_d)/(T_fF_f)$.

Dialyzer dependability. The preceding experimental results all were obtained during uninterrupted operation of the system for 56 days. After a hiatus of three weeks, operation was resumed for an additional 15 days. The fermentor and dialysate compartments of the dialyzer stayed clean throughout the total 71 days of operation. However, the membrane surfaces in the fermentor compartments were fouled with a film of debris after the 56 days, and fresh membranes were used for the final 15 days.

Product quality. Samples from the fermentor circuit were regularly analyzed by gas chromatography, not only to determine the concentration of lactic acid but also to monitor the possible presence of atypical metabolic products. The results indicated that the fermentation remained homofermentative, i.e., only negligible amounts of products other than lactic acid were found.

Prolonged steady-state operation. The dialysis continuous fermentation was operated at set steady-state conditions (T_f = 27.2, ϕ = 2.5) during the 15-day period to determine other characteristics of the process (Table 4). The mean rate of ammonium-lactate production was 4.7 mg/ml-h and the lactose conversion was 81%.

By day 58 of the fermentation, the residual lactose levels were very low, but then the levels increased with time. The lactose increase was possibly caused by a buildup of inhibitory substance in the fermentor circuit resulting from membrane fouling, as seen by the greater differences between S_f and S_d and between P_f and P_d as time progressed.

During the fermentation, a mean of 36.5% of the fermentor effluent represented water which osmosed from the dialysate circuit, i.e., a mean of 56.4 ml of water per h ($F_f - F_f^0$ in Table 4) entered the fermentor from the dialysate circuit.

From material-balance data (Table 4), the equivalent substrate concentration in the fermentor-feed stream (S_f^0) was calculated by use of a conservation-of-mass equation. The mean calculated value (247 mg/ml) agreed well with the mean analytical value (242 mg/ml) for S_f^0. Thus, the results confirmed that no significant portion of the substrate was lost to products other than lactic acid.

Validation of the mathematical model. The values shown in Table 5 were used to correlate the experimental and simulated results. The values for ϕ and γ were calculated directly from the experimental data. The values for μ_m, K_s, and K_p were obtained by successive curve fitting of simulated with experimental results of

TABLE 4. *Material-balance data for operation of the dialysis continuous fermentation system at steady-state conditions*

Day	T_f (h)	ϕ	S_f (mg/ml)	S_d (mg/ml)	P_f (mg/ml)	P_d (mg/ml)	F_f° (ml/h)	F_f (ml/h)	F_d (ml/h)	S_f° (mg/ml)[a]
	25.5	2.29	77.0	16.3	22.5	9.4	104.4	164.4	376.8	245.1
57	24.2	2.26	33.0	8.8	55.0	23.6	88.4	172.8	390.0	314.5
	25.7	2.36	12.6	2.6	61.5	31.2	85.2	163.2	385.8	293.7
58	28.2	2.69	6.7	0.5	65.0	29.6	84.7	148.8	400.2	265.6
	27.4	2.62	7.5	0.4	68.0	28.0	94.2	153.0	400.8	239.8
59	26.4	2.46	10.0	1.1	61.0	29.4	93.2	158.4	389.4	245.1
	27.4	2.60	15.0	1.2	67.0	25.2	97.0	153.0	398.4	233.4
60	27.9	2.66	15.8	1.4	65.0	27.0	101.3	150.0	399.0	227.9
	27.5	2.62	17.5	2.1	62.5	26.2	96.5	152.4	399.6	238.8
61	27.0	2.53	17.5	2.7	73.0	25.6	101.4	155.4	393.0	243.8
	27.0	2.53	21.7	2.8	68.0	22.0	102.9	155.4	393.6	224.3
62	26.9	2.53	25.9	2.6	65.0	22.0	100.3	156.0	394.2	233.0
	26.3	2.46	22.2	2.8	66.5	25.0	102.2	159.0	391.8	240.1
63	27.1	2.55	21.2	2.5	67.0	24.6	94.7	154.8	395.4	253.2
	27.7	2.61	18.4	2.2	67.5	22.8	99.4	151.2	394.8	224.9
64	27.6	2.61	19.7	2.2	69.0	22.6	97.1	151.8	396.0	235.2
	27.6	2.58	21.3	2.1	74.5	24.6	100.0	151.8	391.2	244.4
65	27.2	2.53	22.9	2.1	69.0	26.6	98.0	154.2	389.4	256.5
	27.4	2.53	23.6	2.0	80.0	23.6	100.0	153.0	387.6	253.1
66	27.7	2.58	22.9	2.0	74.0	23.4	95.0	151.2	389.4	254.5
	27.8	2.59	24.9	1.9	80.0	22.0	97.2	150.6	390.0	254.4
67	28.2	2.61	24.3	1.8	83.5	19.0	96.9	148.8	388.8	244.5
	27.9	2.60	24.2	1.6	75.5	21.2	104.0	150.0	390.6	225.1
68	27.8	2.58	23.1	1.5	75.5	20.2	99.5	150.6	388.2	229.8
	27.6	2.53	27.7	2.1	69.5	25.4	↓	151.8	384.6	251.0
69	27.5	2.54	26.6	2.0	72.5	22.6	99.4	152.4	386.4	243.8
	27.2	2.49	27.5	2.2	79.0	21.0	↓	154.2	384.6	251.4
70	27.0	2.47	27.5	2.3	77.5	23.6	107.6	155.4	384.6	240.1
	27.5	2.53	27.7	1.8	83.5	21.4	98.4	152.4	385.8	259.6
71	27.3	2.52	28.6	2.2	77.5	23.0	99.0	153.6	387.6	259.9
Mean	27.2	2.54	23.2	2.7	69.2	23.7	97.9	154.3	390.9	247.0

[a] $S_f^\circ = (F_fS_f + F_fP_f + F_fX_f + F_dS_d + F_dP_d)/F_f^\circ - P_f^\circ$, where $P_f^\circ = 9.1$ mg/ml.

TABLE 5. *Values used for computer simulations of experimental fermentations with deproteinized whey*

Figure (days)	μ_m (h^{-1})	$\bar{K}_p$	$\bar{K}_s$	ϕ	Π	R	γ
3 (26–36)	0.35	2.2	0.0004	1.0	0.50	3.0	0.96
3 (40–49)	0.35	2.2	0.0004	2.5	0.40	3.0	0.96
4 (26–36)	0.35	2.2	0.0004	1.0	0.50	3.0	0.96
4 (40–49)	0.35	2.2	0.0004	2.5	0.40	3.0	0.96

a nondialysis continuous fermentation (unpublished results). The value for Π was obtained similarly with the experimental results of the dialysis continuous fermentation. S_f^0 was taken as 160.3 mg/ml instead of 237.5 mg/ml (which was obtained from material balances of the experimental data) because a mean 32.5% of the fermentor contents represented water which osmosed from the dialysate circuit.

Figure 3 shows the computer-simulated curves obtained by use of the steady-state solution with the values in Table 5 to describe the relation between the two principle operating parameters (T_f and ϕ) and the conversion efficiency (S_f and S_d). Figure 4 shows the relation between the same operating parameters and the accumulation of product (P_f and P_d). The curves in both figures fitted well with the superimposed points of experimental results and thereby further demonstrated the validity of the mathematical model. Moreover, the kinetic constants (μ_m, K_s, and K_p) used for the simulations in Fig. 3 and 4 were obtained from the results of nondialysis continuous fermentations, adding to the validation of the model.

Process evaluation by use of the mathematical model. The experimental and simulated results were correlated to evaluate the fermentation process. The values for μ_m (0.35 h^{-1}), K_s (0.0004), and K_p (2.2) were the same in both time periods, indicating no change in the bacterial culture from days 26 to 49. The values

for $\bar{K}_s$ and $\bar{K}_p$ also showed that the fermentation was not affected by substrate limitation but was greatly limited by increasing concentrations of product. The values for Π (0.5 at days 26 to 36; 0.4 at days 40 to 49) showed that the permeability of the membrane decreased as the fermentation progressed. Calculations of Π using a P_{ms} of 0.06 mg/cm^2-h showed that Π should have been about 0.9. Thus, by 4 weeks into the fermentation, the permeability factor decreased by 50%. Coulman et al. (4) showed that a Π of 2.0 should be used for the process to obtain suitable relief from product inhibition.

Process monitoring. On-line sensors are becoming increasingly important in the operation of fermentations (16). In the present process, the dialysate effluent is a relatively pure solution of ammonium lactate and should be measurable by its electrical conductivity. Figure 6 illustrates the positive correlation between the concentration of ammonium lactate and the conductance in the dialysate. Thus, a conductivity bridge could be readily used for the on-line monitoring of product concentration. Moreover, if coupled with the model, this parameter could indicate other parameters of the fermentation, e.g., cell concentration in the fermentor and membrane permeability in the dialyzer.

DISCUSSION

Various models have been used to describe the growth of lactic acid bacteria (4–6, 8, 11–13, 19). Although these models correlate with experimental results, they have only been used for fermentations of very low substrate and product concentrations, they contain equations or terms which have no biological basis, or they lack a strong product-inhibition effect. Recently, Aborhey and Williamson (1) developed a model which appeared useful, but the model was complicated for our purposes because it incorporated the concept of intracellular substrate concentration. Since acid production cannot continue once all of the substrate has been utilized, Rogers et al. (19) suggested improving Luedeking and Piret's (13) rate equation for acid production by incorporating a substrate-limitation term into the maintenance term. Their simulation results agreed with experimental results, but the K_s (0.371 mg/ml) used for the simulations was high and probably represents a product-inhibition effect (2, 11, 22). A better way to improve the equation would be to incorporate a product-inhibition term as well as a substrate-limitation term.

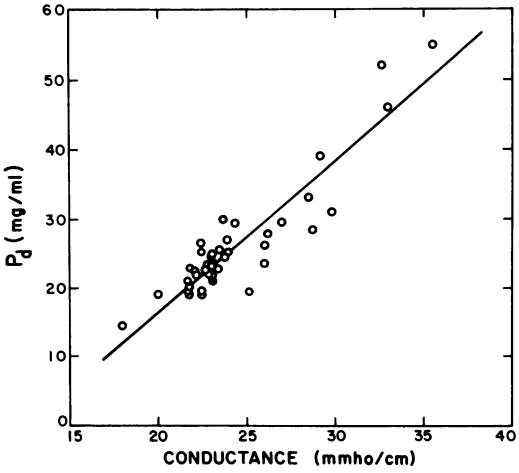

FIG. 6. *Regression correlation between concentration of ammonium-lactate (P_d) and electrical conductance in the dialysate circuit.*

The model developed in the present study is simple and correlated well with experimental tests of dialysis and nondialysis (unpublished) continuous processes for the ammonium-lactate fermentation over a wide range of operating parameters with high substrate and product concentrations. Specifically, the model agreed with experimental results of substrate utilization (Fig. 1 to 3), product formation (Fig. 4), and cell-mass accumulation (results not shown). The model also contains substrate-limitation and product-inhibition terms which have a biological basis. Moreover, the values used for these terms are realistic. To date, the model has only been correlated with steady-state data. The major shortcoming of the model is that the product-inhibition effect is not strong enough at lactate concentrations greater than 70 mg/ml. As suggested above, the incorporation of a product-inhibition term into the maintenance term may provide a solution. Another cause of the problem is that, since a dialysis culture is able to tolerate greater lactate concentrations than is a nondialysis culture (22), there may be an unknown dialyzable factor which also has an inhibition effect on the lactic acid fermentation.

The mean product/substrate ratio of the ammonium-lactate fermentation was determined as 0.96 mg/mg. Thus, 96% of the lactose utilized was converted to ammonium-lactate and 4% was incorporated into bacterial cells. On a substrate efficiency basis, the fermentation thus would be more useful for production of ammonium-lactate than for bacterial cells.

The dialysis continuous process has been used to ferment whole whey (22) and deproteinized whey. Both fermentations are most efficient when using a T_f of about 20 h, i.e., further increases in T_f do little to decrease S_f and S_d (compare Fig. 1 and 3). A comparison of the use of these substrates at similar conditions is shown

TABLE 6. *Comparison of wheys used for production of ammonium-lactate by dialysis continuous fermentation*

Substrate	S_f° (mg/ml)	T_f (h)	ϕ	E (%)	S_f (mg/ml)	P_f (mg/ml)	S_d (mg/ml)	P_d (mg/ml)
Whole whey (22)	230	19	2.5	97	2.6	80	0.4	24
Deproteinized whey	242	27	2.5	81	23.2	69	2.7	24

in Table 6. The fermentation of whole whey relative to deproteinized whey occurred at a greater fermentation rate and resulted in more complete substrate conversion. The fermentor contents of both processes were similarly diluted by a net osmosis of water from the dialysate circuit. Both fermentations were similarly efficient in the percent conversion of lactose into ammonium-lactate only and in the high concentration of accumulated ammonium-lactate in the fermentor. The present study also showed that a high concentration of cell-free ammonium lactate (50 mg/ml) could be maintained in the dialysate effluent (Fig. 4).

The mathematical model showed the permeability factor to be as low as 0.4. Consequently, the fermentation could be improved considerably (e.g., more efficient conversion of substrate and greater lactate concentration in the dialysate effluent) with use of a more permeable membrane. Greater conversion of substrate could also be had by increasing the cell-mass concentration in the dialysis fermentor. This might be accomplished by recycling the cells or by employing a nondialysis prefermentor which is optimized for cell production and the effluent from which flows into the dialysis fermentor, which is optimized for conversion efficiency and product accumulation. Such fermentation systems can be easily modeled before conducting experimental tests.

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