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

R. W. STIEBER and PHILIPP GERHARDT*

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824

Received for publication 5 December 1978

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 feed-stuff 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

† Journal article no. 8608 from the Michigan Agricultural Experiment Station.
model of the dialysis continuous ammonium-lactate fermentation (see equation 11 in reference 4) is as follows:

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

(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 inhi-
bition term, and equation 1 is reduced to the following equation:

$$r_g = \mu_m \frac{S_f}{(K_S + S_f)} X_f$$

(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:

$$X_f + S_f \rightarrow X_f S_f \rightarrow X_f P_f \rightarrow X_f + P_f$$

(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 $S_{f0}$. 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):

$$r_g = \mu_m \frac{S_f}{(K_S + S_f + K_p P_f)} X_f$$

(4)

where $\mu_m = k_3 k_4 / (k_2 + k_3)$, $K_p = k_3 k_4 / [k_2 (k_2 + k_3)]$, and $K_p = k_3 k_4 / [k_2 (k_2 + k_3)]$. The substrate-limitation con-

### Table 1. Glossary of mathematical symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_m$</td>
<td>Area of membrane available for dialysis</td>
<td>cm$^2$</td>
</tr>
<tr>
<td>$F_d$</td>
<td>Flow rate into and out of dialysate circuit</td>
<td>ml/h</td>
</tr>
<tr>
<td>$F_r'$</td>
<td>Flow rate into fermentor circuit</td>
<td>ml/h</td>
</tr>
<tr>
<td>$F_r$</td>
<td>Flow rate out of fermentor circuit</td>
<td>ml/h</td>
</tr>
<tr>
<td>$K_p$</td>
<td>Product-inhibition constant</td>
<td>mg/mg</td>
</tr>
<tr>
<td>$K_a$</td>
<td>Substrate-limitation constant</td>
<td>mg/ml</td>
</tr>
<tr>
<td>$P_d$</td>
<td>Product concentration in dialysate circuit</td>
<td>mg/ml</td>
</tr>
<tr>
<td>$P_{mp}$</td>
<td>Permeability of membrane to product</td>
<td>mg/cm$^2$-h</td>
</tr>
<tr>
<td>$P_{ms}$</td>
<td>Permeability of membrane to substrate</td>
<td>mg/cm$^2$-h</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
<td>h</td>
</tr>
<tr>
<td>$V_{d}$</td>
<td>Volume of liquid in dialysate circuit</td>
<td>ml</td>
</tr>
<tr>
<td>$V_r$</td>
<td>Volume of liquid in fermentor circuit</td>
<td>ml</td>
</tr>
<tr>
<td>$X_r$</td>
<td>Cell-mass concentration in fermentor circuit</td>
<td>mg/ml</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Substrate/cell ratio</td>
<td>mg/mg</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Specific maintenance rate</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Product/substrate ratio</td>
<td>%</td>
</tr>
<tr>
<td>$E$</td>
<td>Efficiency of lactose conversion</td>
<td></td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>Maximum specific growth rate of cells</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$T_r$</td>
<td>Cell-retention time in fermentor circuit</td>
<td>h</td>
</tr>
</tbody>
</table>

### Table 2. Glossary of dimensionless parameters

<table>
<thead>
<tr>
<th>Type</th>
<th>Symbol and definition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material parameters</td>
<td>$P_d = P_d/(\gamma S_f)$</td>
<td>Product factor in dialysate circuit</td>
</tr>
<tr>
<td></td>
<td>$P_f = P_f/(\gamma S_f)$</td>
<td>Product factor in fermentor circuit</td>
</tr>
<tr>
<td></td>
<td>$P_r = P_r/(\gamma S_f)$</td>
<td>Product factor in fermentor feed</td>
</tr>
<tr>
<td></td>
<td>$S_d = S_d/S_f$</td>
<td>Substrate factor in dialysate circuit</td>
</tr>
<tr>
<td></td>
<td>$S_f = S_f/S_f$</td>
<td>Substrate factor in fermentor circuit</td>
</tr>
<tr>
<td></td>
<td>$X_r = \alpha X_r/S_f$</td>
<td>Cell factor in fermentor circuit</td>
</tr>
<tr>
<td>Operational parameters</td>
<td>$R = P_{mp}/P_{ms}$</td>
<td>Ratio of product/substrate membrane permeabilities</td>
</tr>
<tr>
<td></td>
<td>$\Pi = P_{mp} A_m$</td>
<td>Membrane permeability factor</td>
</tr>
<tr>
<td></td>
<td>$F_r$</td>
<td>Flow-rate ratio</td>
</tr>
<tr>
<td>Kinetic parameters</td>
<td>$k_i = K_i/S_f$</td>
<td>Substrate-limitation factor</td>
</tr>
<tr>
<td></td>
<td>$k_i = \gamma K_i$</td>
<td>Product-inhibition factor</td>
</tr>
<tr>
<td></td>
<td>$\theta = \mu_m T_r$</td>
<td>Time factor</td>
</tr>
</tbody>
</table>
stant (K_v) 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:

\[
\frac{d\mathbf{S}_f}{dt} = \frac{-K_s \pi + K_p + K_p \mathbf{P}_f}{\pi_p (\theta - 1) - \pi_{sp}} \mathbf{S}_f + \pi_{sp} \mathbf{P}_f
\]  

(10)

\[
\frac{d\mathbf{P}_f}{dt} = \frac{-K_s \pi_s + \theta - 1 + \mathbf{P}_f (\theta - 1)}{\pi_p (\theta - 1) - \pi_{sp}} \mathbf{S}_f
\]  

(11)

\[
\mathbf{X}_f = \frac{1 - \mathbf{S}_f + \Pi (\mathbf{S}_d - \mathbf{S}_f)}{1 + \beta T_f/\alpha}
\]  

(12)

where \(\pi_\eta = \Pi [\Pi (\phi + \Pi) - 1] - 1, \) and \(\tau_p = 1 + RII[1 - RII/(\phi + RII)].\)

For the dialysate circuit, the corresponding equations are:

\[
\mathbf{S}_d = \Pi \mathbf{S}_f/\phi + \Pi
\]  

(13)

\[
\mathbf{P}_d = RII \mathbf{P}_f/\phi + RII
\]  

(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 \(\phi \) and \(\Pi\) were calculated directly from the experimental results. The values for \(\mu_m, K_v, \) 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 \(\mathbf{S}_f^0\) 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)

<table>
<thead>
<tr>
<th>Figure (days)</th>
<th>(\mu_m ) (h(^{-1}))</th>
<th>(K_v)</th>
<th>(K_p)</th>
<th>(T_f) (h)</th>
<th>(\phi)</th>
<th>(\Pi)</th>
<th>(R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (19–24)</td>
<td>0.145</td>
<td>0.6</td>
<td>0.0004</td>
<td>3.7</td>
<td>0.66</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>1 (50–75)</td>
<td>0.25</td>
<td>0.6</td>
<td>0.0004</td>
<td>3.4</td>
<td>0.62</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>2 (40–63)</td>
<td>0.25</td>
<td>0.6</td>
<td>0.0004</td>
<td>16</td>
<td>0.59</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

Downloaded from http://aem.asm.org on September 20, 2017 by guest
A similar comparison between the simulated and the experimental results was also made for a second operating parameter, e.g., \( F_d \) versus \( S_t \) 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 ¼ inch [ca. 1.83 m by 0.32 cm] outside diameter) packed with 10% SP-1000/1% H₃PO₄ 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-
DIALYSIS CONTINUOUS FERMENTATION

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 simula-
tions.

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 ($\phi$) 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 demon-
strate the fit between the experimental results and the
computer simulations. The dashed curves and
circle points were obtained at $\phi = 1.0$ and during
days 26 to 36. The smooth curves and square points
were obtained at $\phi = 2.5$ and during days 40 to 49.

usual 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 de-
crease the lactose concentration.

Dialysis rate versus conversion efficiency. The flow-rate ratio ($\phi$) is an operational parameter used to manipulate the rate of di-
alysis. Results of experimental variation in $\phi$
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 ($\phi = 2.5$) than with
decreased dialysis ($\phi = 1.0$). The effect was
mostly on $S_d$.

Cell-retention time versus lactate concentra-
tion. Figure 4 shows the results of vari-
ation in $T_f$ affecting the lactate concentrations
in the fermentor circuit ($P_d$) and in the dialysate
circuit ($P_d$). The lactate concentrations in-
creased to a maximum with increasing $T_f$.

Dialysis rate versus lactate concentra-
tion. Figure 4 also shows the results of the flow-
rate ratio affecting $P_d$ and $P_d$. $\phi$ had little effect
on $P_d$. However, $P_d$ was much greater with de-
creased dialysis ($\phi = 1.0$) than with increased
dialysis ($\phi = 2.5$). Values for the dialysate prod-
uct yield ($P_{d\phi}$, which represents the fraction of
substrate leaving as lactate in the dialysate eff-
luent) were similar at either value of $\phi$.

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 in-
creased with increased $T_f$. High lactate productivity and high lactose conversion along with
high lactate concentrations are all desirable.
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 \( \phi \) showed that these operational parameters had little effect on the product/substrate ratio (\( \gamma \), results not shown). Consequently, for purposes of modeling the ammonium-lactate fermentation, a fixed value was assigned to \( \gamma \) (0.96). The ratio was calculated from \( r_p/r_w \) where values for \( r_p \) were obtained from Fig. 5 and where \( r_p = (S_i^0F_f^0 - S_iF_f - S_dF_d)/(T_fF_f) \).

**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 (\( T_f = 27.2, \phi = 2.5 \)) 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 \( S_i \) 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 \( \phi \) and \( \gamma \) were calculated directly from the experimental data. The values for \( \mu_m \) \( K_s \), 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 | $T_r$ (h) | $\phi$ | $S_i$ (mg/ml) | $S_o$ (mg/ml) | $P_i$ (mg/ml) | $P_o$ (mg/ml) | $F'_i$ (ml/h) | $F_r$ (ml/h) | $F_a$ (ml/h) | $S^*$ (mg/ml)
|-----|----------|--------|---------------|---------------|--------------|--------------|--------------|-------------|-------------|--------------
| 25.5 | 2.29 | 77.0 | 16.3 | 22.5 | 9.4 | 104.4 | 164.4 | 376.8 | 245.1
| 57 | 24.2 | 2.25 | 33.0 | 8.8 | 55.0 | 23.6 | 88.4 | 172.8 | 390.0 | 314.5
| 58 | 25.7 | 2.36 | 12.6 | 2.6 | 6.5 | 3.12 | 85.2 | 163.2 | 385.8 | 293.7
| 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
| 28.4 | 2.46 | 10.0 | 1.1 | 61.0 | 29.4 | 93.2 | 158.4 | 388.4 | 245.1
| 27.4 | 2.60 | 15.0 | 1.2 | 67.0 | 26.2 | 97.0 | 153.0 | 389.4 | 233.4
| 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
| 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 | 242.3
| 62 | 26.9 | 2.53 | 25.9 | 2.6 | 65.0 | 22.0 | 100.3 | 156.0 | 394.2 | 233.0
| 63 | 26.3 | 2.46 | 22.2 | 2.8 | 66.5 | 25.0 | 102.2 | 159.0 | 391.8 | 240.1
| 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
| 65 | 27.6 | 2.58 | 21.3 | 2.1 | 74.5 | 24.6 | 100.0 | 151.8 | 391.2 | 244.4
| 66 | 27.2 | 2.53 | 22.9 | 2.1 | 69.0 | 26.6 | 98.0 | 154.2 | 389.4 | 254.5
| 27.4 | 2.53 | 23.6 | 2.0 | 80.0 | 23.6 | 100.0 | 153.0 | 387.6 | 253.1
| 27.7 | 2.58 | 22.9 | 2.0 | 74.0 | 23.4 | 95.0 | 151.2 | 389.4 | 254.5
| 27.8 | 2.62 | 23.6 | 1.8 | 80.0 | 22.2 | 97.2 | 150.6 | 390.0 | 254.4
| 67 | 27.2 | 2.60 | 24.3 | 1.6 | 83.5 | 19.0 | 95.9 | 148.8 | 388.8 | 244.5
| 27.8 | 2.58 | 23.1 | 1.5 | 75.5 | 20.2 | 99.5 | 150.6 | 388.2 | 229.8
| 69 | 27.6 | 2.53 | 27.7 | 2.1 | 69.5 | 25.4 | 151.8 | 384.6 | 251.0
| 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 | 23.7 | 2.2 | 69.2 | 23.7 | 97.9 | 154.3 | 390.9 | 247.0

$S^*$ = $(F'_S + P_i P_r + F'_D + F'_S D + F'_D D)/F'_r - P'_r$, where $P'_r = 9.1$ mg/ml.

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

| Figure (days) | $\mu_m$ (h$^{-1}$) | $K_s$ | $K_p$ | $\phi$ | $\Pi$ | $R$ | $\gamma$
|---------------|------------------|-------|-------|-------|------|-----|-------
| 3 (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| 1.0   | 0.50 | 3.0 | 0.96
| 5 (26-36)     | 0.35             | 2.2   | 0.0004| 2.5   | 0.40 | 3.0 | 0.96
| 6 (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 $\Pi$ was obtained similarly with the experimental results of the dialysis continuous fermentation. $S^*_S$ 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 ($T_r$, and $\phi$) and the conversion efficiency ($S_o$ and $S_d$). Figure 4 shows the relation between the same operating parameters and the accumulation of product ($P_r$ 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 ($\mu_m$, $K_s$, 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 $\mu_m$ (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 $K_\alpha$ and $K_\beta$ also showed that the fermentation was not affected by substrate limitation but was greatly limited by increasing concentrations of product. The values for $\Pi$ (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 $\Pi$ using a $P_{\text{ma}}$ of 0.06 mg/cm$^2$-h showed that $\Pi$ 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 $\Pi$ 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 Pi- rets' (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 $K_\alpha$ (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.

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 $T_t$ of about 20 h, i.e., further increases in $T_t$ do little to decrease $S_t$ and $S_\alpha$ (compare Fig. 1 and 3). A comparison of the use of these substrates at similar conditions is shown.
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.

ACKNOWLEDGMENT

This work was supported by grant ENG 76-17260 from the National Science Foundation.

LITERATURE CITED