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Lactic acid production by batch fermentation of whey permeate: a mathematical model

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SUMMARY

The batch fermentation of whey permeate to lactic acid was improved by supplementing the broth with enzyme-hydrolyzed whey protein. A mathematical model based on laboratory results predicts to a 99% confidence limit the kinetics of this fermentation. Cell growth, acid production and protein and sugar use rates are defined in quantifiable terms related to the state of cell metabolism. The model shows that the constants of the Leudeking-Piret model are not true constants, but must vary with the medium composition, and especially the peptide average molecular weight. The kinetic mechanism on which the model is based also is presented.

INTRODUCTION

Lactic acid may be produced by batch fermentation of whey permeate; however, acid production rates of existing processes are low. Fermentation rates have been improved markedly by supplementing the broth with enzyme-hydrolyzed whey protein [4,5]. It was the purpose of this work to develop a mathematical model to correlate the effects of whey protein hydrolyzate average molecular weight and concentration on the fermentation kinetics.

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The semi-empirical model describing the fermentation kinetics was developed in much the same way as are enzyme rate equations. Similar rough structural approaches have been used successfully to develop 'structured' models for other fermentations [1,2,9,10].

MODEL DEVELOPMENT

Cell growth

The proposed, simplified mechanism assumes that substrate (lactose) and protein combine independently with free cells, X, to form equilibrium

complexes X·S and X·PR. Substrate then combines independently with X·PR and protein combines independently with X·S to form the 'active equilibrium complex', X·S·PR, which yields cells and lactic acid:

$$S + X \overset{K_s}{\rightleftharpoons} X \cdot S$$

$$PR + X \overset{K_{pr}}{\rightleftharpoons} X \cdot PR$$

$$X \cdot S + PR \overset{K_{pr}}{\rightleftharpoons} X \cdot S \cdot PR$$

$$X \cdot PR + S \overset{K_s}{=} X \cdot S \cdot PR \overset{k}{\rightarrow} X + LA$$

where PR is the amount of *usable* protein. Note that not all the protein in the broth is usable (see Ref. 4 for experimental support). Obviously, each of the steps represents a consolidation of many individual steps in various metabolic pathways.

The equilibrium expressions are:

$$K_s = \frac{[S]\,[X]}{[X \cdot S]} \tag{1}$$

$$K_{pr} = \frac{[PR]\,[X]}{[X \cdot PR]} \tag{2}$$

The material balance for cell mass is:

$$X_T = [X] + [X \cdot S] + [X \cdot PR] + [X \cdot S \cdot PR] \tag{3}$$

where X_T is the total cell mass.

The rate of cell production, r_x, is:

$$r_x = (dX_T/dt) = k\,[X \cdot S \cdot PR] \tag{4}$$

Combining equations (1) through (4);

$$r_x = \frac{\mu_{max}\,([PR]/K_{pr})}{1 + ([PR]/K_{pr}) + ([PR]/K_{pr})\,(K_s/[S]) + (K_s/[S])} X_T \tag{5}$$

During cell growth $[S] \gg K_s$ [4] so equation (5) simplifies to:

$$r_x = \frac{\mu_{max}\,[PR]}{[PR] + K_{pr}} X_T \tag{6}$$

At this point the model does not account for lactic acid (LA) inhibition which is known to occur. One could expand the model to include the inhibition effect by adding additional simple mechanisms such as

$$X + LA \rightleftharpoons X \cdot LA$$
$$X \cdot LA + S \rightleftharpoons X \cdot LA \cdot S$$
$$X \cdot LA + PR \rightleftharpoons X \cdot LA \cdot PR$$
$$X \cdot LA + LA \rightleftharpoons LA \cdot X \cdot LA$$

etc., but these do not lead to models which fit published data [3]. Therefore, it was decided that for now the inhibition effect would be incorporated empirically as:

$$r_x = \frac{\mu_{max}[PR]}{[PR] + K_{pr}{}^{*} f(LA)} X_T \tag{7}$$

therefore,

$$\mu = \frac{\mu_{max}[PR]}{[PR] + K_{pr}{}^{*} f(LA)} \tag{8}$$

The f(LA) can be determined by plotting the experimental data in accord with a rearranged version of equation (8):

$$\frac{[PR]}{\mu} - \frac{[PR]}{\mu_{max}} = \frac{f(I)}{\mu_{max}} \tag{9}$$

An example plot is presented in Fig. 1. The parabolic shape of the curve indicates that [9]:

$$f(I) = K_{pr}([LA]/K_i + 1)^2 \tag{10}$$

where K_i is the inhibition constant. Note that the corresponding plot for $f(I) = [LA]/K_i$, as proposed by Steiber and Gerhardt [11], is linear. Substituting equation (10) into equation (7):

$$\left(\frac{dX_T}{dt}\right) = \mu\, X_T = \frac{\mu_{max}([PR]/K_{pr})\, X_T}{[PR]/K_{pr} + ([LA]/K_i + 1)^2} \tag{11}$$

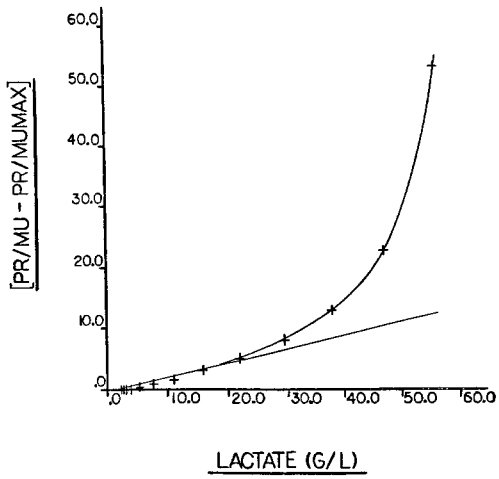

Fig. 1. $([PR]/\mu) - ([PR]/\mu_{max})$ vs. lactate concentration: for the fermentation supplemented with 50% hydrolyzate (+); Steiber and Gerhard model (———).

Substrate consumption: growth and maintenance

Substrate is used and acid is produced by growth and maintenance metabolisms. As a first approximation, it is assumed that during growth the rate of substrate use for acid production via maintenance is small in comparison to that used for growth. However, as the cell approaches the resting state ($\mu \rightarrow 0$), the use pattern reverses. This shift in metabolism can be estimated by defining the fraction of cell metabolism dedicated to growth as [4]:

$$F_g = \mu/\mu_{max} \tag{12}$$

and the fraction dedicated to maintenance metabolism as:

$$F_m = 1 - F_g \tag{13}$$

The general equation for lactose consumption is:

$$\left(\frac{d[S]}{dt}\right)_{tot} = \left(\frac{d[S]}{dT}\right)_g + \left(\frac{d[S]}{dT}\right)_m \tag{14}$$

From equation (11):

$$\left(\frac{dX_T}{dt}\right) = \frac{\mu_{max}([PR]/K_{pr})\ X_T}{[PR]/K_{pr} + ([LA]/K_i + 1)^2}$$

Then the substrate use rate for growth is:

$$\left(\frac{d[S]}{dt}\right)_g = (X_T F_g) \frac{\upsilon([PR]/K_{pr})}{[PR]/K_{pr} + ([LA]/K_i + 1)^2} \tag{15}$$

since F_g is the fraction of cell metabolism dedicated to growth.

Lactose and nitrogen (PR) are required for cell maintenance. Evidence of the maintenance requirement is provided by Ohleyer et al. [7] who observed that in a steady state recycle reactor a low concentration of nitrogen was required to maintain a constant biomass concentration (although the use rate is too low to measure). Therefore, the maintenance mechanism is proposed to be:

$$X + S \overset{K'_s}{\rightleftharpoons} X \cdot S$$

$$X + PR \overset{K'_{pr}}{\rightleftharpoons} X \cdot PR$$

$$X \cdot S + PR \overset{K'_{pr}}{\rightleftharpoons} X \cdot S \cdot PR$$

since new cell mass is not generated. Note that K_s and K_{pr} may not equal K'_s and K'_{pr} as the cellular machinery for maintenance may not be the same as that for cell growth. Thus, using similar techniques as previously, the lactose use during maintenance is:

$$\left(\frac{d[S]}{dt}\right)_m = (X_T F_m) \times \frac{\xi([PR]/K'_{pr})\ ([S]/K_s)}{([PR]/K'_{pr} + [S]/K'_s + ([PR]/K'_{pr})\ ([S]/K'_s) + I)} \tag{16}$$

where

$$I = ([LA]/K_i + 1)^2 \tag{17}$$

and ξ is the specific sugar use rate for maintenance.

Acid production

Because the acid yield during growth may not be the same as the yield for maintenance, the overall rate of acid production is defined as:

$$\left(\frac{d[LA]}{dt}\right)_{tot} = -Y_{g,LA/S}\left(\frac{d[S]}{dt}\right)_g - Y_{m,LA/S}\left(\frac{d[S]}{dt}\right)_m \tag{18}$$

where $Y_{g,LA/S}$ and $Y_{m,LA/S}$ are the yield coefficients for the growth and maintenance stages, respectively.

Protein use

In general, the rate of protein consumption may be defined as:

$$\left(\frac{d[PR]}{dt}\right)_{tot} = \left(\frac{d[PR]}{dt}\right)_{g} + \left(\frac{d[PR]}{dt}\right)_{m} \tag{19}$$

indicating that nitrogen is required for growth and cell maintenance [7]. Equation (19) is of the same form as (14) and can be derived from the proposed mechanisms as were equations (15) and (16):

$$\frac{d[PR]}{dt} = X_T F_g \frac{V_1([PR]/K_{pr})}{([PR]/K_{pr} + I)} + X_T F_m \frac{V_2([PR]/K'_{pr})([S]/K'_s)}{[PR]/K'_{pr} + ([PR]/K'_{pr})([S]/K'_s) + I} \tag{20}$$

where V_1 and V_2 are the specific use rates. The experimental results show that the rate of protein use during maintenance is negligible in comparison to the use during growth, and cannot be measured accurately [4]. In addition, the protein used for growth and the protein used for maintenance cannot be distinguished readily; thus, both effects are lumped into Y_0:

$$\frac{d[PR]}{dt} = Y_0 X_T \frac{V([PR]/K_{pr})}{([PR]/K_{pr} + I)} \tag{21}$$

and the equation is simplified by substituting (11) into (21):

$$\frac{d[PR]}{dt} = Y^{-1}_{x/pr} \frac{d[X]}{dt} \tag{22}$$

Comparison with experimental data

An example fit of the model to smoothed laboratory data for the fermentation supplemented to 50% with enzyme-hydrolyzed whey protein (average molecular weight (AMW) = 700) is presented in Fig. 2. (Plots for all other cases are in Ref. 4.) The kinetic constants evaluated from the laboratory data for the fermentations supplemented to 0–75% with enzyme-hydrolyzed whey protein are given in Table 1. The model predicts the laboratory results at a 99% confidence interval (based on the χ^2 distribution) [4].

Comparison with the Leudeking-Piret model

The model may be compared to Leudeking and Piret's mixed growth model [6]:

$$\frac{d[LA]}{dt} = \alpha \frac{d[X]}{dt} + \beta[X] = (\alpha \mu + \beta)[X] \tag{23}$$

or

$$\frac{1}{[X]} \frac{d[LA]}{dt} = \alpha \mu + \beta \tag{24}$$

to show that

$$\alpha = \frac{Y_{g,LA/S}}{\mu_{max}} \frac{([PR]/K_{pr})\, v}{([PR]K_{pr} + I)} \tag{25}$$

and

$$\beta = (F_m) \frac{Y_{m,LA/S}\, \xi([PR]/K'_{pr})([S]/K'_s)}{([PR]/K'_{pr} + [S]/K'_s + ([PR]/K'_{pr})([S]/K'_s) + I)} \tag{26}$$

These relationships predict that α and β should vary during a batch fermentation, which is what was observed at high lactic acid concentration. For example, a plot of $(1/[X]) \cdot (d[LA]/dt)$ versus μ (Fig. 3) shows that α (the slope) and β (the intercept) vary at high acid concentrations. They also predict that α and β should be constant for a continuous, steady-state fermentor but should vary with changes in the steady-state medium composition. This has been observed by several investigators [3,7,8]. All this implies that the kinetic constants of the model should change with hydrolyzate AMW. The comparison shown in Table 2 for AMW 700 and 1000 hydrolyzates shows this to be true.

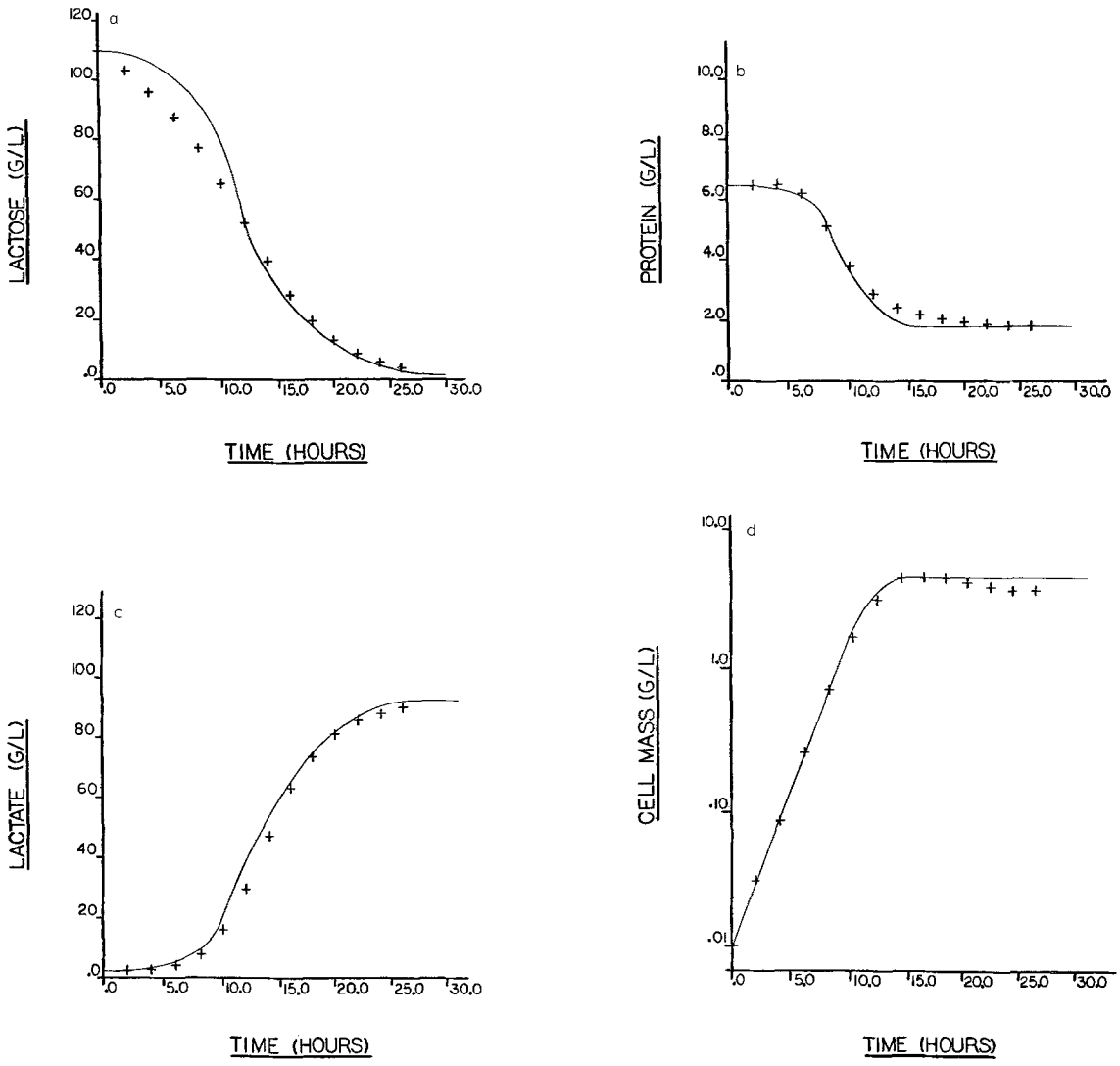

Fig. 2. (a) Model fit to lactose concentration data (AMW 700, 50% fermentation). (b) Model fit to protein concentration data (AMW 700, 50% fermentation). (c) Model fit to lactate concentration data (AMW 700, 50% fermentation). (d) Model fit to cell mass concentration data (AMW 700, 50% fermentation).

CONCLUSIONS

(1) A mathematical model which simulates the fermentation kinetics was developed. (2) A goodness of fit test between the laboratory and simulated values for the fermentation kinetics, based on the χ^2 distribution, shows that the model fits at a 99% confidence limit [4]. (3) The model is most sensitive to variation in μ, $Y_{g,LA/S}$ and $Y_{m,LA/S}$ while variations in K_s', K_i, K_{pr} have little effect [4]. (4) The fermentation kinetics appear to be a function of hydrolyzate AMW. (5) The 'constants' of the Leudeking-Piret model are not true constants; they vary during the course of a batch fermentation.

Table 1

Fitted kinetic constants

μ_{max}	0.56/h
$Y_{g,\ LA/S}$	0.75 g LA/g S
$Y_{m,\ LA/S}$	0.99 g LA/g S
v	8.0 g S/h·g cell
ξ	2.0 g S/h·g cell
$Y_{x/pr}$	1.0 g X/g PR
K_i	20.0 g LA/l
K_s'	0.5 g S/l
K_{pr}	0.12 g PR/l
K_{pr}'	0.01 g PR/l

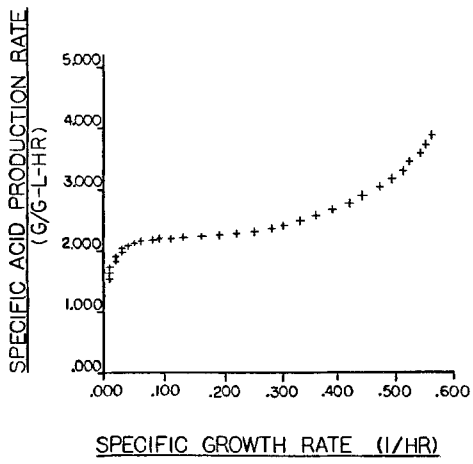

Fig. 3. Specific acid production rate vs. specific growth rate (AMW 700, 50% fermentation).

Table 2

Kinetic constants as a function of AMW

Constant	Value at AMW 700	Value at AMW 1000
μ_{max}	0.56	0.64
v	8.0	9.0
ξ	2.0	3.5
K_i	20	20
$Y_{g,\ LA/S}$	0.75	0.95
$Y_{m,\ LA/S}$	0.99	0.99
$Y_{x/pr}$	1.0	1.15
K_s'	0.5	0.5
K_{pr}	0.12	0.12
K_{pr}'	0.01	0.01

NOMENCLATURE

K_i = lactic acid inhibition constant (g/l); K_{pr} = protein saturation constant during cell growth (g/l); K_{pr}' = protein saturation constant during maintenance (g/l); K_s' = lactose saturation constant (g/l); [LA] = lactic acid concentration (g/l); [PR] = protein concentration (g/l); [S] = lactose concentration (g/l); t = time (h); [X] = cell mass concentration (g/l); α, β = fermentation constants of Leudeking and Piret; μ = specific growth rate (1/h); $Y_{g,LA/S}$ = acid yield during cell growth (g acid/g sugar); $Y_{m,LA/S}$ = acid yield during maintenance (g acid/g sugar); $Y_{x/pr}$ = yield (g cells/g protein); υ = specific sugar use rate during cell growth (g sugar/h · g cell); ξ = specific sugar use rate during maintenance (g sugar/h · cell).

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