0273-2289/97/63–65—0495$11.75

# A Mathematical Model of Ethanol Fermentation from Cheese Whey

## I: Model Development and Parameter Estimation

CHEN-JEN WANG AND RAKESH K. BAJPAI*

*Department of Chemical Engineering, University of Missouri-Columbia*
*Columbia, MO 65211*

## ABSTRACT

The cybernetic approach to modeling of microbial kinetics in a mixed-substrate environment has been used in this work for the fermentative production of ethanol from cheese whey. In this system, the cells grow on multiple substrates and generate metabolic energy during product formation. This article deals with the development of a mathematical model in which the concept of cell maintenance was modified in light of the specific nature of product formation. Continuous culture data for anaerobic production of ethanol by *Kluyveromyces marxianus* CBS 397 on glucose and lactose were used to estimate the kinetic parameters for subsequent use in predicting the behavior of microbial growth and product formation in new situations.

**Index Entries:** Cheese whey; cybernetic model; *Kluyveromyces marxianus*; kinetics; chemostat.

## INTRODUCTION

Glycosides are utilized in many anaerobic fermentations as a carbon source for ethanol production. Commonly encountered examples are those involving sucrose in molasses, sucrose, maltose, and maltotriose in brewer's wort, and lactose in cheese whey. Oligo- and polysaccharides in cellulose and starch are often used, too. Although fermentations start with glycosides, they may result in emergence of monosaccharides in broth to hydrolysis of glycosides. Cofermentations with other sugars to enhance ethanol production are also common. For example, fermentations from reconstituted cheese whey powder (CWP) may result in extracellular accu-

*Author to whom all correspondence and reprint requests should be addressed.

mulation of glucose and galactose in addition to the lactose, depending on the source of whey, the initial concentration of CWP, and the operating conditions *(1,2)*. Cheese whey is often either spiked with additional sugars *(3)* or cofermented with agricultural products *(4–6)*. As a result, fermentation from whey may involve the presence of multiple substrates in broth.

When multiple sugars are present in a pure culture system, sugars may be taken up by the cells either sequentially and/or simultaneously, depending on environmental conditions, microorganism, and sugars. In our previous study *(2)* on fermentations of multiple sugars in cheese whey by yeast *Kluyveromyces marxianus* CBS 397, it was shown that glucose and lactose are consumed simultaneously in batch fermentations under conditions of growth limitation by the nitrogen source. Under nitrogen-sufficient situations, however, a diauxic growth on both sugars was observed.

Although multiple-substrate systems are very common, only sporadic attempts have been made to study quantitatively the interactions between mono- and disaccharides and their impact on the overall kinetics observed in product-forming fermenters. A systematic study dealing with the effect of monosaccharides formed by the hydrolysis of sucrose in ethanol fermentations from molasses has been conducted by Jayanata *(7)*. In this case, it was found that fast invertase-mediated hydrolysis of sucrose causes rapid increase in concentrations of glucose and fructose in the broth immediately after the start of the fermentation. The rates of cell growth and product formation from each of these monosaccharides were inhibited by the presence of the other sugar. Since the hydrolysis rate was fast and the yeast exhibited very similar uptake behavior of these monosugars, it was possible to consider the system as one consisting of a single sugar with its concentration equal to the sum total of those of glucose and fructose.

In another system involving five sugars in brewer's wort *(8)*, a complex interaction between the sugars as a result of both simultaneous and sequential sugar uptake behaviors has been shown. Although glucose, fructose, and sucrose were simultaneously taken up by the cells, maltose and maltotriose were consumed later sequentially. This was because of the glucose-mediated repression of maltose uptake; utilization of maltotriose was shut off by both glucose and maltose. Given the critical levels of glucose and maltose in broth, the model ably described sugar consumption behavior and cell growth profile; kinetics of ethanol formation was, however, not presented.

The scarcity of a robust mathematical model for the product-forming systems is primarily because most microbial genomic as well as physiological characteristics, such as intracellular regulatory processes, are usually not well understood. This difficulty may be resolved by cybernetic modeling. The cybernetic approach introduced by Ramkrishna and coworkers *(9–13)* has found great success in modeling the diverse behavior of bacterial growth on multiple substrates in batch, fed-batch, and chemostat operations. The underlying assumption is that the complex

cellular metabolism is driven by an optimal policy that attempts to maximize either cell growth or the efficiency of resource utilization under given environmental conditions. These models are based on a general observation that in an environment containing more than one nonessential substrate, the order of utilization of the substrates follows the decreasing pattern of growth rates. Formulation of the cybernetic models does not require *a priori* knowledge of actual mechanisms of intracellular regulatory processes, such as the order of preferential uptake of the substrate. As a result, the same cybernetic approach may find a wide range of applicability in various microbial systems with different regulatory mechanisms under varied environmental conditions.

A straightforward application of the cybernetic model to the product-forming system, however, requires consideration of product formation, which represents an important and major metabolic flux for carbon substrates. In light of the energetic coupling and net energy production involved in the formation of ethanol, it also requires a reconsideration of maintenance terms. In this article, a cybernetic model has been developed with special attention to anaerobic growth of yeasts on multiple substrates. Kinetic parameters have been estimated using continuous-culture data. Applications of this model have been considered in a companion article (Part II).

## MATHEMATICAL MODEL

The cybernetic models *(9,10)* consider that cellular metabolism of a given substrate is controlled by a single key enzyme whose synthesis and activity are modulated by appropriate cybernetic variables. Maintenance consumption of substrate is interpreted as an outcome of uncoupling between catabolism and anabolism and, hence, as a linear function of specific growth rates of cells *(11)*. Since the control of substrate utilization in yeasts is also achieved via induction/repression of enzyme synthesis coupled with inhibition/activation of those already synthesized, fermentations involving yeasts can be modeled in a similar way.

Unlike the aerobic growth of bacteria, anaerobic growth of yeasts involves both cell growth and ethanol production. In a cybernetic framework where synthesis and activity of key enzyme are considered to be governed by appropriate allocations of critical resources, a question arises regarding whether product formation and cellular growth involve a single common enzyme or two separate key enzymes. In order to resolve this question, it is necessary to understand the interactions between growth and ethanol production in yeasts.

Under unsteered conditions (i.e., in the absence of bisulfite or salt stress) of anaerobic growth of yeasts, the nicotinamide adenine dinucleotide (NAD) reduced by metabolism of sugar is regenerated during reduction of pyruvate to ethanol *(14)*. Thus, ethanol is the major product

formed under these conditions. The adenosine 5'-triphosphate (ATP) produced during the metabolism is utilized to support growth of cells, the extent of which depends on the coupling between catabolic and anabolic processes. This is also manifested in the observation that ethanol production rate is often growth-related in batch cultures *(15)*. Therefore, it should be possible to use a single common key enzyme for growth as well as the ethanol production process. Under this circumstance, each substrate will be associated with a single set of cybernetic variables ($\varepsilon_i$, $\delta_i$) representing the control of the synthesis and inhibition of the key enzyme, respectively. Another cybernetic variable, $\delta_M$, is associated with maintenance-related activities as proposed by Turner and Ramkrishna *(11)*. It indicates the uncoupling of anabolic and catabolic processes, and is representative of the state of cells rather than of specific physiological state of broth. In other words, it has the same value for all carbon substrates in the system.

As a result, the model equations, also considering substrate and product inhibitions, for fermentation of multiple substrates by yeasts can be expressed as:

Specific cell growth rate:

$$r_X = \sum_{i=1}^{n} r_{x,i}\delta_i = \sum_{i=1}^{n} [\mu_i^e e_i S_i / (K_{SX,i} + S_i + S_i^2 / K_{IX,i})] \\ [1/(1 + P/K_{P1,i} + P^2/K_{P2,i})]\delta_i \quad (1)$$

Specific product formation rate:

$$r_P = \sum_{i=1}^{n} r_{P,i}\delta_1 = \sum_{i=1}^{n} [\nu_i^e e_i S_i / (K_{SP,i} + S_i + S_i^2 / K_{IP,i})][K_{P,i}/(K_{P,i} + P)]\delta_i \quad (2)$$

Specific formation rate of enzyme $e_i$:

$$r_{e,i} = [\alpha_i S_i / (K_{SX,i} + S_i + S_i^2 / K_{IX,i})][1/(1 + P_i/K_{P1,i} + P_i^2/K_{P2,i})]\varepsilon_i \\ - e_i[\beta_i + (1/X)(dX/dt)] + \alpha_i \quad (3)$$

Specific rate of consumption of substrate $i$ for maintenance:

$$r_{M,i} = \phi_{M,i} e_i S_i / (K_{SM,i} + S_i) \quad (4)$$

Specific substrate consumption rate:

$$r_{S,i} = (r_{X,i}\delta_i / Y_{X/S_i}) + (r_{P,i}\delta_i / Y_{P/S_i}) + r_{M,i}\delta_M + \phi_{MO,i} \quad (5)$$

Here, the specific rate of substrate consumption for maintenance purposes is split into a growth-independent value ($\phi_{MO,i}$) and a growth-dependent value ($r_{M,i}$). This is in accordance with the observation of Pirt *(16)*. The

maintenance process is characterized by uncoupling of catabolism from anabolism, which becomes more pronounced as growth rate decreases. Under conditions of fast growth, the efficiency of coupling appears to be at a maximum. One major cause of lowered cell yields at low specific growth rates has been suggested to be the energy uncoupling under these conditions *(17)*. The parameters $\mu_i^e$, $\nu_i^e$, and $\phi_{M,i}^e$ are related to $\mu_i^{max}$, $\nu_i^{max}$, and $\phi_{M,i}^{max}$, respectively, as *(9)*:

$$\mu_i^e = \mu_i^{max}[(\mu_i^{max} + \beta_i)/(\alpha_i + a_i)] \tag{6a}$$

$$\nu_i^e = \nu_i^{max}[(\mu_i^{max} + \beta_i)/(\alpha_i + a_i)] \tag{6b}$$

$$\phi_{M,i}^e = \phi_{M,i}^{max}[(\mu_i^{max} + \beta_i)/(\alpha_i + a_i)] \tag{6c}$$

The cybernetic variable $\delta_i$ *(10)* represents the regulation of enzyme activity by inhibition/activation and is given by:

$$\delta_i = [r_{X,i}/\max(r_{X,i})] \tag{7}$$

The cybernetic variable $\varepsilon_i$ *(10)* in Eq. (3) represents induction/repression of synthesis of key enzyme $e_i$, and is given by:

$$\varepsilon_i = \left( r_{X,i} \Big/ \sum_{i=1}^{n} r_{X,i} \right) \tag{8}$$

In cybernetic models, $\delta_M$ is considered as a measure of cellular control of activity of a key enzyme for maintenance functions. Turner et al. *(13)* have suggested a linear functional dependence of $\delta_M$ on specific growth rate in their model. Similar dependence has been suggested by the experimental data of Neijssel and Tempest *(18)* and further modeled by Pirt *(16)*. However, all of these studies have involved growth-only processes. For a product formation system, especially one that involves ethanol production, which results in net ATP generation, product formation rate should also affect the maintenance metabolism. This is in agreement with the cybernetic perspective according to which the cells aspire to achieve maximum return (growth rate) by efficiently allocating the resources to the metabolic function. Under anaerobic conditions, the cells cannot achieve such a cybernetic goal without a continuous regeneration of NAD via ethanol production. As a result, cell growth and ethanol production are interrelated in the sense of cybernetics. This dependence of $\delta_M$ on specific growth and product formation rates has been derived as follows.

For a single-substrate environment with adapted cells, the cybernetic variables $\delta$ and $\varepsilon$ can be taken to be equal to unity. At any specific rate of growth and product formation, Eq. (5) may be written as

$$r_S = (1/Y_{X/S})r_X + (1/Y_{P/S})r_P + r_M\delta_M + \phi_{MO} \tag{9}$$

For this problem, $r_S$ may reach its maximum value when $r_X$ and $r_P$ reach their maxima. These maxima of $r_X$ and $r_P$ appear together for ethanol fermentations *(15)*. Under these conditions, cells are very active, and therefore, the maintenance consumption of substrate is minimal; hence, $\delta_M$ may be equated to zero, and Eq. (9) becomes:

$$r_S^{max} = (1/Y_{X/S})\mu^{max} + (1/Y_{P/S})\nu^{max} + \phi_{MO} \quad (10)$$

Since $r_S \leq r_S^{max}$:

$$(r_X/Y_{X/S}) + (r_P/Y_{P/S}) + r_M\delta_M + \phi_{MO} \leq (\mu^{max}/Y_{X/S}) + (\nu^{max}/Y_{P/S}) + \phi_{MO} \quad (11)$$

Therefore:

$$\delta_M \leq (1/r_M)[(1/Y_{X/S})(\mu^{max} - r_X) + (1/Y_{P/S})(\nu^{max} - r_P)] \quad \text{for } r_M > 0 \quad (12)$$

Based on the above arguments, this equation is subject to the following equality constraints:

$$\delta_M = 0 \text{ at } r_X = \mu^{max} \quad (13a)$$

$$\delta_M = 0 \text{ at } r_P = \nu^{max} \quad (13b)$$

$$\delta_M = 1 \text{ at } r_X = 0 \text{ and } r_P = 0 \quad (13c)$$

Since this is true for all conditions of fermentations (even when $r_M = \phi_M^{max}$, its maximum value):

$$\delta_M \leq (1/\phi_M^{max})[(1/Y_{X/S})(\mu^{max} - r_X) + (1/Y_{P/S})(\nu^{max} - r_P)] \quad (14)$$

Therefore:
$$\delta_M \leq [c_1(\mu^{max} - r_X) + c_2(\nu^{max} - r_P)] \quad (15)$$

where $c_1 = (1/\phi_M^{max}) \cdot (1/Y_{X/S})$ and $c_2 = (1/\phi_M^{max}) \cdot (1/Y_{P/S})$, and $c_1 \neq 0$ and $c_2 \neq 0$.

Equation (15) implies that $\delta_M$ is a function of both $r_X$ and $r_P$. The inequality in Eq. (15) can be rewritten in a form of equality as:

$$\delta_M = -w + c_1(\mu^{max} - r_X) + c_2(\nu^{max} - r_P) \quad (16)$$

where $w \geq 0$.

The term w in Eq. (15) may be a function of $r_X$, or $r_P$, or both. Its form may be chosen to satisfy the equality constraints (Eqs. [13a–c]). Assuming a function of the following form for w: $w = w_1 + w_2r_X + w_3r_P + w_4r_Xr_P$, the following equation can be derived with equality constraints (Eqs. [13a–c]):

$$\delta_M = [1 - (r_X/\mu^{max})][1 - (r_P/\nu^{max})] \quad (17)$$

Table 1
Composition of Semisynthetic Medium

| Nutrient | Composition g/L |
|---|---|
| Sugar | 5.20 |
| Yeast Extract | 4.0 |
| $(NH_4)_2SO_4$ | 2.038 |
| $KH_2PO_4$ | 0.334 |
| $MgSO_4 \cdot 7H_2O$ | 0.122 |
| $FeSO_4 \cdot 7H_2O$ | 0.012 |
| $CaCl_2 \cdot 2H_2O$ | 0.007 |

According to Eq. (17), $\delta_M$ is a joint function of deviations from maximum growth and product formation rates of the cells. For multiple substrate systems, an analogous function for $\delta_M$ can be suggested as a joint function of these deviations

$$\delta_M = \left[1 - \left(\sum_{j=1}^{n} r_{X,j}\delta_j \Big/ \sum_{j=1}^{n} \mu_j^{\max}\varepsilon_j\right)\right]\left[1 - \left(\sum_{j=1}^{n} r_{P,j}\delta_j \Big/ \sum_{j=1}^{n} \nu_j^{\max}\varepsilon_j\right)\right] \quad (18)$$

Equations (18) and (1)–(5) constitute the cybernetic model for a multiple-substrate, product-forming system.

## MATERIALS AND METHODS

Experimental conditions for fermentations of semisynthetic media were kept the same as those reported before *(2)*, except for the composition of supplemental nutrients as shown in Table 1. This composition was made to ensure the growth conditions without limitation of nitrogen source.

Analytical methods were also the same as previously described *(2)*. Samples containing <0.3 g/L glucose or lactose were analyzed by the Nelson-Somagyi method *(19)*. In a mixture of glucose and lactose at low concentrations, glucose content was analyzed by a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH), and lactose concentration was determined by subtracting glucose concentration from the total sugar concentration obtained from the Nelson-Somagyi method.

For determination of cell concentration, 5-mL samples were vacuum-filtered on preweighed 0.2-μm cellulose-acetate membrane filter (Gelman Sciences, Ann Arbor, Michigan). The solids were washed once with 5 mL distilled water and dried along with the filter in a microwave oven for 5 min at full power (700 W). The solids were cooled in a desiccator for 10 min before weighing.

## ESTIMATION OF KINETIC PARAMETERS

The distinctive advantage of the cybernetic approach over the other models describing microbial metabolism in the presence of multiple substrates is its sole use of single-substrate kinetic parameters as the model inputs to simulate the multiple-substrate system. Single-substrate kinetic parameters can be estimated from either batch or continuous-culture data. With batch data, the dynamic equations for cell mass (X), product (P), and sugar (S) need to be solved together. These solutions depend on initial conditions, including the initial concentration of "key enzyme," which is unknown. Therefore, the solutions always involve an unknown initial condition, which may change from experiment to experiment. Use of steady-state continuous-culture data presents no such problem and was made in estimation of the kinetic parameters for sugars in semisynthetic media.

For continuous cultures of *Kluyveromyces marxianus* on two substrates, the governing equations for the rates of cell growth, ethanol production, substrate utilization, and enzyme production can be written as follows

$$(dX/dt) = \sum_{j=1}^{2} r_{X,j}\delta_j X - DX \tag{19}$$

$$(dP/dt) = \sum_{j=1}^{2} r_{P,j}\delta_j X - DP \tag{20}$$

$$(dS_i/dt) = D(S_{i,o} - S_i) - X\{[(r_{x,i}/Y_{X/S_i}) + (r_{P,i}/Y_{P/S_i})]\delta_i + r_{M,i}\delta_M\} \quad i = 1,2 \tag{21}$$

$$(de_i/dt) = [\alpha_i S_i/(K_{SX,i} + S_i + S_i^2/K_{IX,i})]\,[1/(1 + S_i/K_{P_1,i} + S_i^2/K_{P2,i})]\varepsilon_i - e_i\left(\beta_i + \sum_{j=1}^{2} r_{X,i}\delta_i\right) + a_i - De_i \qquad i = 1,2 \tag{22}$$

$\delta_i$, $\varepsilon_i$ ($i$ = 1,2), and $\delta_M$ are the cybernetic variables defined in Eq. (7), (8), and (18), respectively. $S_{i,0}$ is the concentration of substrate $i$ ($i$ = 1,2) in the feed medium.

For a single substrate, the cybernetic parameters ($\varepsilon$ and $\delta$) are equal to 1. The constant specific maintenance rate, $\phi_{M0}$, is small compared to the rest of maintenance contribution and has been neglected. Under steady-state conditions, Eqs. *(19)–(22)* yield:

$$D = r_X = [\mu^e eS/(K_{SX} + S + S^2/K_{IX})]\,[1/(1 + P^2/K_{P1} + P^2/K_{P2})] \tag{23}$$

$$D = r_P[X/P] = [(v^e eS)/(K_{SP} + S + S^2/K_{1P})]\left[\left(K_P + P\right)\right](X/P) \tag{24}$$

$$D = X/(S_0 - S)[(r_X / Y_{X/S}) + (r_P / Y_{P/S}) + (\phi_M^e eS)/(K_{SM} + S)\delta_M]$$
$$= [X/(S_0 - S)]\,[(r_X / Y_{X/S}) + (r_X / Y_{X/S}) + \phi_M^e e\delta_M] \quad \text{for S} >> K_{SM} \tag{25}$$

$$e = \{[\alpha S/(K_{SX} + S + S^2/K_{IX})]\,[1/(1 + S/K_{P1} + S^2/K_{P2})] + a\}/(\beta + D) \tag{26}$$

$$\delta_M = [1 - (r_X / \mu^{max})]\,[1 - (r_P / \nu^{max})] \tag{27}$$

Substitution of e from Eq. (26) into Eq. (23) yields:

$$Z = \beta D + D^2$$
$$= \{[\alpha S/(K_{SX} + S + S^2/K_{IX})][1/(1 + P/(K_{P1} + P^2/K_{P2})] + a\}\cdot \tag{28}$$
$$[\mu^e S/(K_{SX} + S + S^2/K_{IX})]\,[1/(1 + P/K_{P1} + P^2/K_{P2})]$$

The values of $\alpha$, $\beta$, and a were assumed to be the same as those suggested by Kompala et al. *(10)* and Turner and Ramkrishna *(11)*. These values are 0.001, 0.05, and $10^{-5}$, respectively. The parameters $\mu^e$, $K_{SX}$, $K_{IX}$, $K_{P1}$ and $K_{P2}$ were estimated using Eq. (28) with the steady-state measurements of sugar and ethanol concentrations at different dilution rates.

The estimation procedure involves minimization of an objective function, which is usually formulated as the sum of the squares of the weighted differences between experimental data ($A_{i,j}$) and model predictions ($\hat{A}_{i,j}$). The subscript *i* refers to the measurements (1, . . ., *n*) and *j* refers to the component (1, . . ., *m*). A minimization with equal weights may be unsatisfactory where magnitudes of data for various components differ significantly. For example, in most fermentation experiments, the magnitudes of the concentrations of cell mass are considerably smaller than those of substrate and product. At the same time, the choice of weighting factors affects the calculated values of kinetic parameters and leads to different explanations of experimental observations.

Himmelblau *et al. (20)* have suggested the following weighting factors for different components.

$$W_j = \left\{\sum_{k=1}^{n}\left[A_{k,j} - (1/n)\sum_{i=1}^{n} A_{i,j}\right]^2\right\}^{1/2} \tag{29}$$

These weighting factors were used in this study. The objective function to be minimized is then:

$$\sum_{i}^{n}\sum_{j}^{m}\{(A_{i,j} - \hat{A}_{i,j})\cdot W_j\}^2 \tag{30}$$

In estimation of the parameters $\mu_e$, $K_{SX}$, $K_{IX}$, $K_{P1}$, and $K_{P2}$, for example, $A_{i,j}$ has been substituted by the corresponding measured variable in Eq. (28).

Table 2
Kinetic Parameters

| Parameter | | Glucose[a] | Lactose[b] |
|---|---|---|---|
| $\mu^e$ | | 425 | 293 |
| $\mu^{max}$ | ($hr^{-1}$) | 0.628 | 0.517 |
| $K_{SX}$ | (g/L) | 0.055 | 0.094 |
| $K_{IX}$ | (g/L) | 10,000 | 370 |
| $K_{P1}$ | (g/L) | 7.8 | 12.1 |
| $K_{P2}$ | ($g^2/L^2$) | 330 | 330 |
| $\nu^e$ | | 1037 | 867 |
| $\nu^{max}$ | ($hr^{-1}$) | 1.531 | 1.531 |
| $K_{SP}$ | (g/L) | 0.050 | 0.088 |
| $K_{IP}$ | (g/L) | 10,000 | 250 |
| $K_P$ | (g/L) | 24.9 | 33.0 |
| $\phi_M^e$ | | 1.7 | 4.0 |
| $\phi_M^{max}$ | ($hr^{-1}$) | 0.0025 | 0.007 |
| $K_{SM}$ | (g/L) | $10^{-6}$*(13)* | $10^{-6}$*(13)* |
| $Y_{X/S}$ | (g/g) | 0.957 | 0.839 |
| $Y_{P/S}$ | (g/g) | 0.510 (theor.) | 0.538(theor.) |

$\mu^e$, $\nu^e$, and $\phi_M^e$ are in unit of (g dry wt/[h of unit enzyme activity]).

$\mu^{max}$, $\nu^{max}$, and $\phi_M^{max}$ were calculated from the estimated values of $\mu^e$, $\nu^e$, and $\phi_M^e$, respectively.

$K_{SM}$ was fixed at the value of $10^{-6}$ as reported by Turner et al. *(13)*.

[a] Estimated from continuous-culture data using glucose feed (5 and 20 g/L).

[b] Estimated from continuous-culture data using lactose feed (6.4 and 20 g/L).

Minimization of the objective function was conducted using a simplex algorithm proposed by Nelder and Mead *(21)*. Computations were conducted on an IBM mainframe computer, and the estimated parameters are listed in Table 2.

The same procedures were also applied to Eqs. (24) and (25) to compute the rest of the kinetic parameters ($K_{SP}$, $K_{IP}$, $\nu^e$, $K_P$, and $\phi_M^e$). The yield coefficients, $Y_{P/S}$, were set at their theoretical values, i.e., 0.510 for glucose and 0.538 for lactose. The value of $K_{SM}$ has also been fixed at $10^{-6}$ g/L, as suggested by Turner and Ramkrishna *(11)*. All of the kinetic parameters estimated from pure culture data of glucose and lactose are presented in Table 2. The table shows, additionally, the values of $\mu^{max}$, $\nu^{max}$, and $\phi_M^{max}$, calculated by rearrangement of Eqs. (6a)–(6c). The parameters listed in Table 2 were used to simulate batch and continuous-culture experiments involving fermentation of pure and mixed sugars. Results of these simulations and their comparisons with experimental data are presented in the accompanying article (Part II).

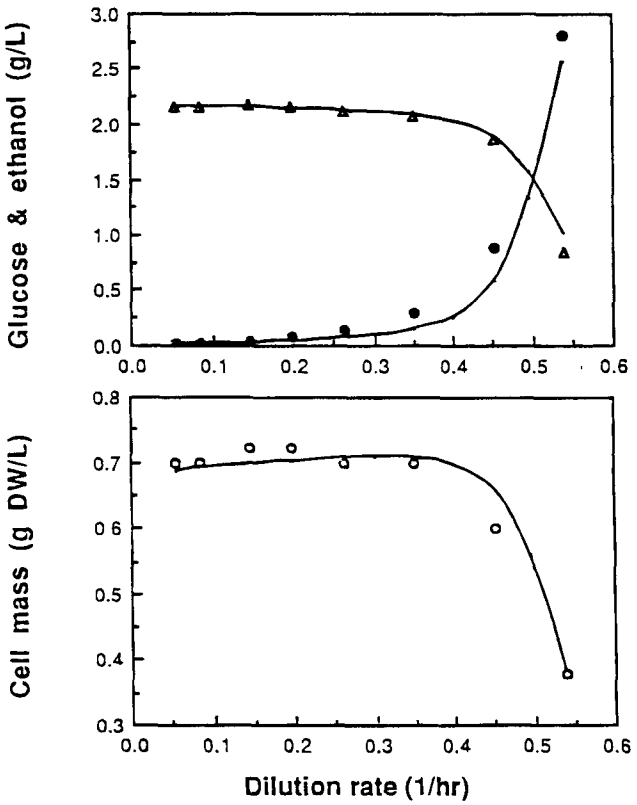


Fig. 1. Chemostat fermentation of 5 g/L glucose. Symbols: ●, glucose; ■, cell mass; Δ, ethanol. Solid lines represent simulation results.

The values of $\mu^{max}$ and $\nu^{max}$ are in the same range as those in ethanol fermentations *(22–25)*, whereas $K_{SX}$ and $K_{SP}$ are relatively low. Lactose has been observed to be inhibitory to cell growth as well as product formation processes, as suggested by finite low values of constants $K_{IX}$ and $K_{IP}$. This too is in accordance with the experimental observations of several researchers *(25–28)*. For the concentration range used in this work, glucose was not inhibitory, and $K_{IX}$ and $K_{IP}$ were given relatively large values (10,000). Ethanol is an inhibitory product in fermentations of glucose as well as lactose *(2,25–28)* as shown by the low values of $K_{PI}$ and $K_P$. The values of cell yield parameter $Y_{X/S}$ is rather high, but not surprising in the presence of the complex nature of medium.

## RESULTS

In order to show the goodness of fit between the experimental data and simulation results using estimated parameters, Eqs. (19–22) were solved in a dynamic manner for single-substrate situations ($i = j = 1$) until steady state was achieved. These simulation results have been presented in Figs. 1–4 for fermentations of glucose and lactose at two different feed

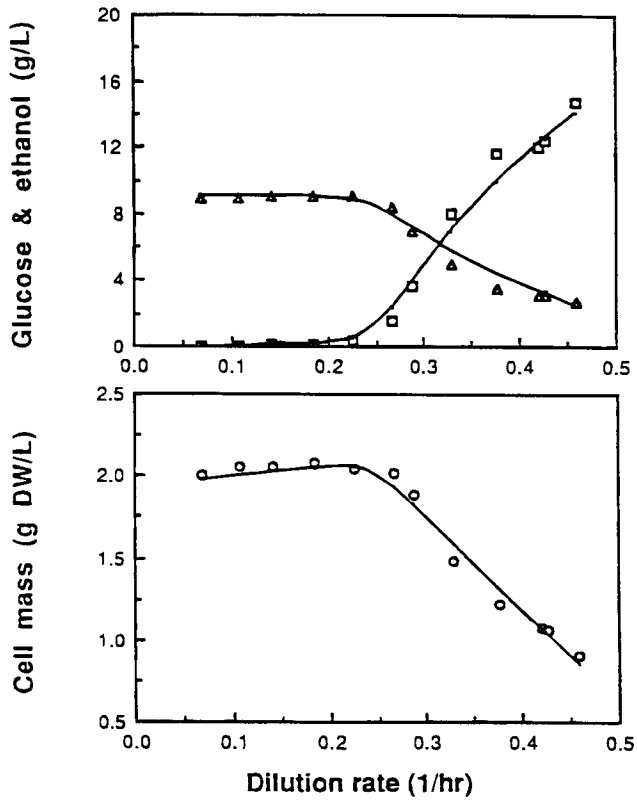


Fig. 2. Chemostat fermentation of 20 g/L glucose. Symbols: ●, glucose; ■, cell mass; Δ, ethanol. Solid lines represent simulation results.

concentrations levels. The feed concentrations were chosen to be the same as the values used in experimental work. The steady-state experimental data points for the concentrations of sugar (glucose or lactose), ethanol, and cell mass have been shown in these figures as discrete points. Simulation results have been presented as solid lines. Although the experimental measurements were used to calculate the parameters, it is reassuring to see that the predictions match the experimental observations quite closely in all the cases.

The simulation results with other conditions involving mixtures of sugars in batch and continuous cultures have been presented in detail in the accompanying article (Part II).

## CONCLUSIONS

Product formation has been interpreted as the outcome of the cybernetic goal for yeasts to grow in ethanol fermentation and incorporated into the cybernetic framework. As a result, the cybernetic variable for cell maintenance was modified to consider the effect of product formation activity.

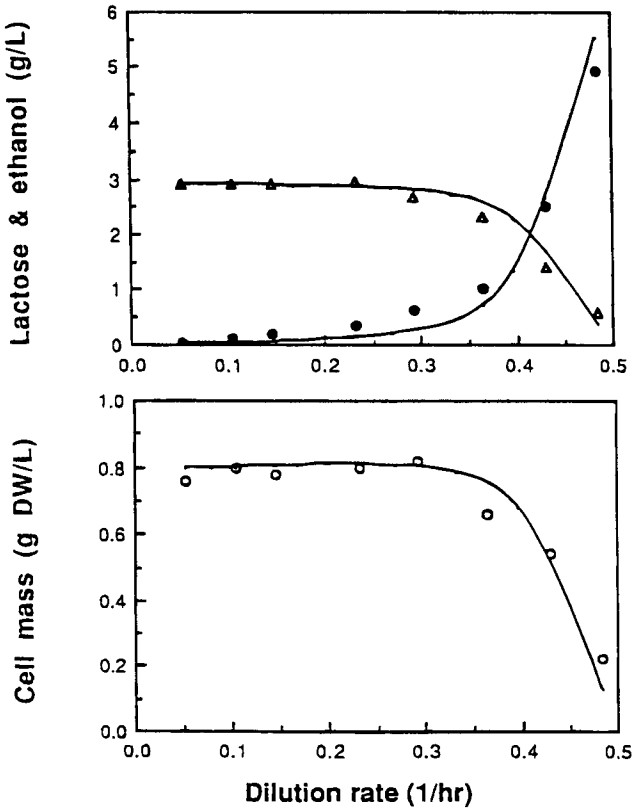


Fig. 3. Chemostat fermentation of 6.4 g/L lactose. Symbols:, ● lactose; ■, cell mass; Δ, ethanol. Solid lines represent simulation results.

The kinetic parameters of the proposed model were estimated using the continuous data. These parameters have been used with the model to simulate fermentations under various conditions as described in the accompanying article (Part II).

## NOMENCLATURE

| | |
|---|---|
| a | basal enzyme production, unit of enzyme activity/g |
| D | dilution rate, 1/h |
| e | key enzyme level, unit of enzyme activity/g |
| $K_{IX,i}$ | substrate inhibition constant for cell growth, g/l |
| $K_{IP,i}$ | substrate inhibition constant for product formation, g/l |
| $K_P$ | product inhibition constant for product formation, g/l |
| $K_{P1,j}$ | product inhibition constants for cell growth, g/l |
| $K_{P2,j}$ | product inhibition constants for cell growth, $g^2/l^2$ |
| $K_{SX,i}$ | substrate saturation constant for cell growth, g/l |
| $K_{SP,i}$ | substrate saturation constant for product formation, g/l |
| $K_{SM,i}$ | substrate saturation constant for maintenance, g/l |

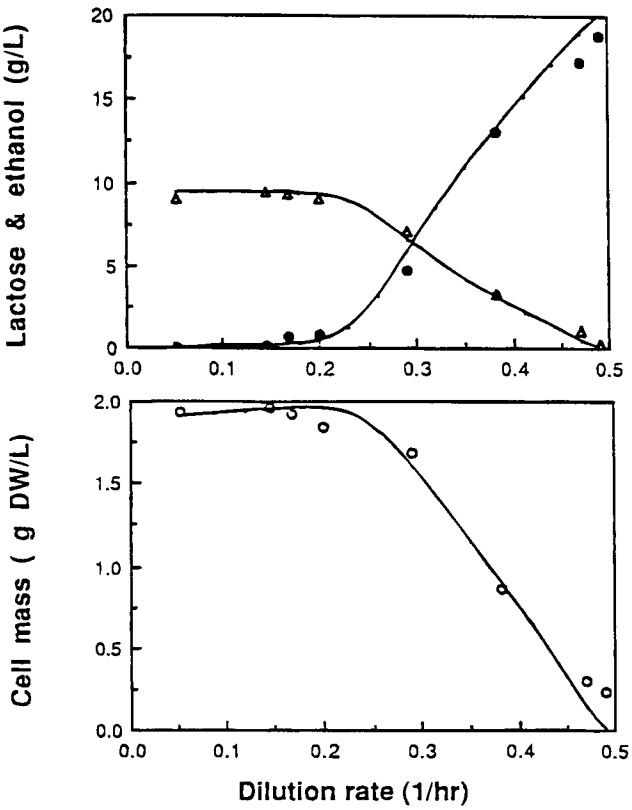


Fig. 4. Chemostat fermentation of 20 g/L lactose. Symbols:, ● lactose; ○ cell mass; Δ, ethanol. Solid lines represent simulation results.

| | |
|---|---|
| P | ethanol concentration in broth, g/l |
| S | sugar concentration in broth, g/l |
| t | time, h |
| X | cell mass concentration in broth, g/l |
| $\alpha$ | enzyme synthesis constant |
| $\beta$ | enzyme degradation constant |
| $\delta$ | cybernetic variable for inhibition/activation of enzyme activity |
| $\delta_M$ | cybernetic variable for uncoupling between catabolism and anabolism |
| $\varepsilon$ | cybernetic variable for induction/repression of enzyme synthesis |
| $\mu^{max}$ | maximum specific rate of cell growth, 1/h |
| $\nu^{max}$ | maximum specific rate of product formation, 1/h |
| $\phi_M^{max}$ | maximum specific substrate consumption rate for maintenance, 1/h |

## REFERENCES

1. Tu, C. W., Jayanata, Y., and Bajpai, R. K. (1985), *Biotechnol. Bioeng. Symp.* **15,** 295–305.
2. Wang, C. J., Jayanata, Y., and Bajpai, R. K. (1987), *J. Ferment. Technol.* **65,** 249–253.
3. Yoo, B. W. and Mattick, J. F. (1969), *J. Dairy Sci.* **52,** 900.

4. Whalen, P. J., Shahani, K. M., and Lowry, S. R. (1985), *Biotechnol. Bioeng. Symp.* **15,** 117–128.
5. Friend, B. A. and Shahani, K. M. (1981), *Fuels from biomass and wastes*, Klass, D. L. and Emert, G. H., eds., Ann Arbor Science Publishers, Michigan, pp. 343–355.
6. Friend, B. A., Cunningham, M. L., and Shahani, K. M. (1982), *Agricultural Wastes* **4,** 55–63.
7. Jayanata, Y. (1983), Einsatz Reaktionstechnischer Modelle zur Kinnzeichung und Optimierung der Kontinuierlichen Äthanolproduktion mit Biomasserückführung, Ph.D. thesis, TU Berlin, D83/FB 13 Nr. p. 148.
8. Fidgett, M. and Smith, E. L. (1975), *Appl. Chem. Biotechnol.* **25,** 355–366.
9. Kompala, D. S., Ramkrishna, D., and Tsao, G. T. (1984), *Biotechnol. Bioeng.* **26,** 1272–1281.
10. Kompala, D. S., Ramkrishna, D., Jansen, N. B., and Tsao, G. T. (1986), *Biotechnol. Bioeng.* **28,** 1044–1055.
11. Turner, B. G. and Ramkrishna, D. (1988), *Biotechnol. Bioeng.* **31,** 41–43.
12. Turner, B. G., Ramkrishna, D., and Jansen, N. B. (1989), *Biotechnol. Bioeng.* **34,** 252–261.
13. Turner, B. G., Ramkrishna, D., and Jansen, N. B. (1988), *Biotechnol. Bioeng.* **32,** 46–54.
14. Rose, A. H. (1976), *Chemical Microbiology*, 3rd ed., Plenum, New York, p. 209.
15. Gaden, E. L., Jr. (1959), *J. Biochem. Microbiol. Technol. Eng.* **1,** 413–420.
16. Pirt, S. J. (1982), *Arch. Microbiol.* **133,** 300–302.
17. Senez, J. C. (1962), *Bacteriol. Rev.* **26,** 95–107.
18. Neijssel, O. M. and Tempest, D. W. (1976), *Arch. Microbiol.* **107,** 215–221.
19. Somagyi, M. (1952), *J. Biol. Chem.* **195,** 19–23.
20. Himmelblau, D. M., Jones, C. R., and Bischoff, K. B. (1967), *Ind. Eng. Chem. Fundam.* **6,** 539–543.
21. Nelder, J. A. and Mead, R. (1965), *Comp. J.* **7,** 308–313.
22. Bazua, C. D. and Wilke, C. R. (1977), *Biotechnol. Bioeng. Symp.* **7,** 105–118.
23. Ghose, T. K. and Tyagi, R. D. (1979), *Biotechnol. Bioeng.* **21,** 1401–1420.
24. Hoppe, G. K. and Hansford, G. S. (1982), *Biotechnol. Lett.* **4,** 39–44.
25. Ruggeri, R., Specchia, V., and Gianetto, A. (1988), *Chem. Eng. J.* **37,** B23–B30.
26. Moulin, G., Boze, H., and Galzy, P. (1980), *Biotechnol. Bioeng.* **22,** 2375–2381.
27. Harbison, A. M., Kempton, A. G., and Stewart, G. G. (1984), *Dev. Ind. Microbiol.* **25,** 467–473.
28. Vienne, P. and von Stockar, U. (1985), *Enzyme Microbiol. Technol.* **7,** 287–294.