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Use of Novel Immobilized β-Galactosidase Reactor to Hydrolyze the Lactose Constituent of Skim Milk

II. Multiresponse Nonlinear Regression Analysis of Kinetic Data

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ABSTRACT

A novel chemical reactor, consisting of β-galactosidase from *Aspergillus oryzae* immobilized on a ribbed membrane made from polyvinylchloride and silica, was used to hydrolyze the lactose constituent of skim milk. Multiresponse nonlinear regression methods were employed to determine the kinetic parameters of rate expressions based on a proposed enzymatic mechanism that includes the formation of oligosaccharides. HPLC methods were employed to monitor the concentrations of all species present in the effluent stream. For the experimental conditions used in this research, a rate expression that includes the inhibition effect of α-galactose is sufficient to model the reaction network.

Index Entries: Immobilized-enzyme reactor; lactose hydrolysis; skim milk; multiresponse regression.

NOMENCLATURE

A	concentration of lactose	*M*
B	concentration of glucose	*M*

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C_α	concentration of α-galactose	M
C_β	concentration of β-galactose	M
C'	concentration of galactosyl group	M
CC	concentration of galactose–galactose disaccharide	M
[E]	concentration of free enzyme	M
$[E]_t$	total concentration of enzyme	M
(EQ)	enzyme complex with arbitrary species Q	
F	statistical F-ratio	
f	dimensionless fractional concentration	
$k_1, k_2, \ldots$	reaction rate constants	
k_{mut}	galactose mutarotation rate constant	s^{-1}
K_{ij}	competitive inhibition constant for species j	M^{-1}
K_{mut}	galactose mutarotation equilibrium constant	
N	number of experiments used in the nonlinear regression	
p	statistical probability	
Q	arbitrary sugar species (A, C′, C_α, C_β, CC, or T)	
r_j	rate of formation of species j per unit volume	M/min
R	gas constant	
T	concentration of trissacharides	M
V_j	grouped reaction rate constant	min^{-1}
X	composite concentration of species in rapid equilibrium	
Y_j	dimensionless concentration of species j	
Z	dimensionless distance from the reactor inlet	
Z	vector of residuals from the fit of the data via nonlinear regression	

Greek Symbols

α	α-galactose	
β	β-galactose	
γ_j	preexponential factor for species j in Arrhenius temperature model	
θ	kinetic parameter	
ν_j	energy-related term for species j in Arrhenius temperature model	
τ	space-time	min

Subscripts

C	total galactose
A	lactose
B	glucose
T	trisaccharide
0	initial
e	equilibrium

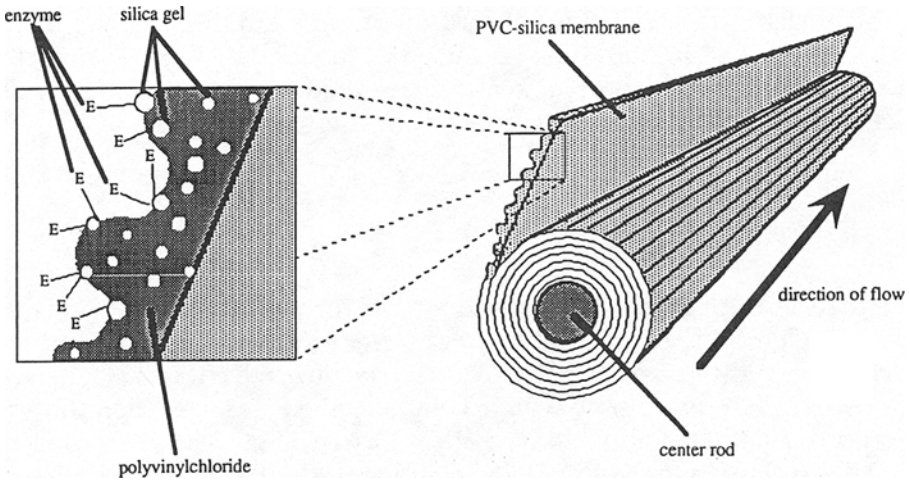


Fig. 1. Schematic diagram of axial-annular flow reactor.

INTRODUCTION

Many researchers have studied the enzymatic hydrolysis of lactose, and several review articles are available (*1–3*). In recent years, the application of β-galactosidase (β-galactoside galactohydrolase EC 3.2.1.23) to the hydrolysis of the lactose component in such dairy products as milk and cheese whey has received much attention (*4–6*). In particular, clinical studies have shown that people who suffer from lactose intolerance can consume such hydrolyzed dairy products with a concommitant decrease in symptoms (*7–10*). Hence it would be desirable to produce low-lactose milk in an economic fashion, so dairy products derived from them can be made available to such individuals. Several microbial sources of β-galactosidase have been used for this purpose. The lactases from *Escherichia coli* (bacteria) (*11–13*), *Kluyveromyces lactis* (yeast) (*14–16*), and *Aspergillus oryzae* (fungus) (*17–20*) have been the primary subjects of such studies. In order to use these enzymes efficiently, it is best to immobilize them on an insoluble support. Several critical reviews of the advantages and problems associated with the use of immobilized enzymes are available in the literature (*21–23*).

In this study, a novel immobilized-enzyme reactor was tested. This reactor consists of β-galactosidase from *Aspergillus oryzae* immobilized on a ribbed membrane. The ribbed membrane is rolled so that the flow of the reactant fluid is directed along the axis of a cylindrical reactor, through the annular openings formed by the ribs (*see* Fig. 1). This reactor is considered to be novel because the ribs on the membrane preclude the use of spacing material required by other rolled-membrane reactors. By eliminat-

ing this spacing material, the ratio of membrane surface area to reactor volume is increased. Skim milk was used as the feedstock for the reactor, and the effluent from the reactor was analyzed via HPLC to determine the concentration of each sugar present in the reacting fluid.

METHODS

The reactor membrane, a ribbed material formed from a 50:50 mixture of polyvinylchloride (PVC) and silica gel, was supplied by FMC Corporation Biosupport Materials (Pinebrook, NJ). The enzyme (30,000 FCC lactose U/g powder, 12% protein by weight) was supplied by Miles Inc., Biotechnology Products Division (Elkhart, IN). Pasteurized skim milk was obtained from the University of Wisconsin Dairy (Madison, WI). Polyethyleneimine (PEI, 50% w/v in water) was purchased from the Eastman Kodak Company (Rochester, NY). All other reagents were purchased from Sigma Chemicals (St. Louis, MO), and all were reagent grade or better.

Equipment

The ribbed PVC–silica sheet on which the enzyme is to be immobilized is rolled around a Plexiglas™ rod and inserted into a polycarbonate tube (Fig. 1). The skim milk flows along the axis of the tube in the annular channels formed by the ribs. This reactor is referred to as an axial-annular flow reactor (Axan) because of the flow configuration.

The HPLC system used to analyze the reacting fluid was obtained from Waters (Millipore Co., Millford, MA). It consisted of a Model 600E system controller, a Model 700 WISP autosampler, a Model 410 differential refractometer, and Model 825 computer analysis software.

Experimental Methods

Immobilization of β-Galactosidase

The procedure for immobilization of β-galactosidase using polyethyleneimine (PEI) and glutaraldehyde is similar to that employed by Goldberg (*24*). The axial-annular reactor was first flushed with deionized (DI) water. An aqueous solution of PEI (5% w/v) was then recirculated through the reactor for 1 h at 22°C. The reactor was subsequently flushed with 200 mL of DI water and 200 mL of aqueous NaCl (2*M*) to remove any PEI not chemically adsorbed on the surface of the membrane. After thorough flushing, glutaraldehyde (5% w/v in DI water) was recirculated through the reactor for 40 min. The reactor was then flushed with 100 mL of DI water and 100 mL of acetate buffer (pH 4.5, 0.1*M*).

The enzyme solution was prepared by dissolving 7 g of Miles lactase into 90 mL of acetate buffer (pH 4.5, 0.1*M*). This solution was centrifuged

for 20 min at 2000 rpm, and the supernatant was decanted. The enzyme solution (supernatant) was recirculated in the reactor for 1 h at 22°C. The system was then washed with buffer until no protein or enzyme activity could be detected in the wash buffer. The concentration of protein in the initial solution of enzyme, in the final solution of enzyme, and in the effluent obtained from the washing of the reactor were determined by spectrophotometric assay, following the procedure developed by Bradford (25). By this method it was calculated that 1.3 g of protein were immobilized on the surface of the membrane. This is equivalent to 1.9 mg of protein/cm^2 of the membrane. The relative activity of the immobilized enzyme, expressed in μmol of lactose hydrolyzed/mg of protein, is approx 90% of the relative activity of the free enzyme.

Procedure for the Hydrolysis of Lactose

Data relating sugar concentrations to the reactor space-time (ratio of the volume of reactor occupied by the process fluid to the volumetric flow rate) were obtained by first establishing steady-state conditions within the reactor at a given flow rate. Effluent concentrations (as determined from HPLC analysis) were monitored to determine when steady-state conditions had been achieved. The effective volume occupied by the process fluid was 21.2 mL.

A Sugar-Pak™ chromatography column (Waters) was used in the HPLC analysis. The Sugar-Pak™ column is a calcium-based ion-exchange column. The column was operated at 90°C; deionized water (degassed and filtered with a 0.45 μm filter) was the mobile phase. The flow rate was 0.5 mL/min. The various sugars were detected by changes in the refractive index (RI) of the effluent solution. The RI detector was operated at 35°C.

Milk samples (0.5-mL aliquots) were diluted to 10 mL with 75% acetone and 25% water (v/v) and then centrifuged for 30 min at 2000 rpm to precipitate the milk proteins. The supernatant was then filtered through a 10,000 NMWL ultrafilter (Millipore). These samples were then ready for injection into the HPLC column.

MATHEMATICAL MODELING

In order to characterize the axial-annular flow reactor, it is first necessary to model the reactor mathematically, and then to use these mathematical equations to determine the parameters of the reaction system. To accurately characterize these chemical reactors, the following mathematical concepts need to be addressed:

1. Steady-state material balances for each of the major chemical species
2. Reaction-rate equations based on a proposed kinetic mechanism

Material Balances Under Steady-State Conditions

Experimental residence-time distribution studies (*20,26*) have shown that, under the conditions employed in our investigations, the Peclet number is ≈50. Previous research (*20,26*) has shown that this value is large enough to assume that plug-flow conditions prevail in the axial-annular flow reactor. The plug-flow reactor model equation can be written as

$$\frac{dY_i}{dZ} = \frac{\tau r_i}{A_0} \tag{1}$$

where A_0 is the initial concentration of lactose, Y_i=concentration of species i/A_0, Z is the dimensionless distance from the reactor inlet (length coordinate/reactor length), r_i is the rate of production of species i per unit volume, and τ is the space-time of the reactor.

Proposed Enzymatic Mechanism

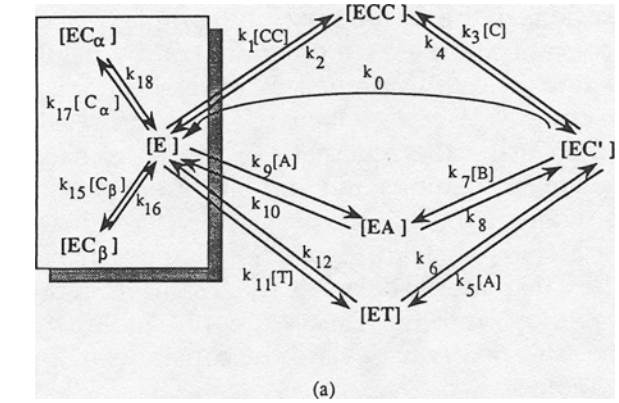
The first step in developing a mathematical model for a chemical reaction is to propose a reaction mechanism that is consistent with the available data. A proposed mechanism should include the primary reaction as well as any side reactions. Huber et al. (*27*) have postulated that the glucose molecule vacates the active site of the enzyme first, leaving a galactosyl group on the enzyme surface. This galactosyl group can then react with any molecule in the reacting fluid that contains a hydroxyl group (e.g., water, glucose, galactose, or lactose). A mechanism that includes such reactions is shown in Figure 2(a).

The algebra involved in deriving a rate expression based on this proposed mechanism is long and tedious. One can simplify the reaction mechanism to that shown in Fig. 2(b) by using the assumptions implicit in the use of the method developed by Segel (*28*). The rates of the reactions in the shaded box in Fig. 2(a) are assumed to be much faster than the overall rate of reaction. For this reason, the chemical species that are in this box can be subtotaled and indicated by the symbol X. The symbol f represents the relative proportion of the rapid equilibrium segment X that is actually involved in the indicated reaction. That is to say, of the species constituting X, it is E that reacts with A (with a rate constant k_1) to yield EA. The symbol f denotes the portion of X that is E.

$$f = \frac{[E]}{[E] + [EC_\beta] + [EC_\alpha]} \tag{2}$$

The rate of formation of lactose (r_A) is then

$$r_A = \frac{dA}{dt} = -fk_9X[A] + k_{10}[EA] - k_5[A][EC'] + k_6[ET] \tag{3}$$



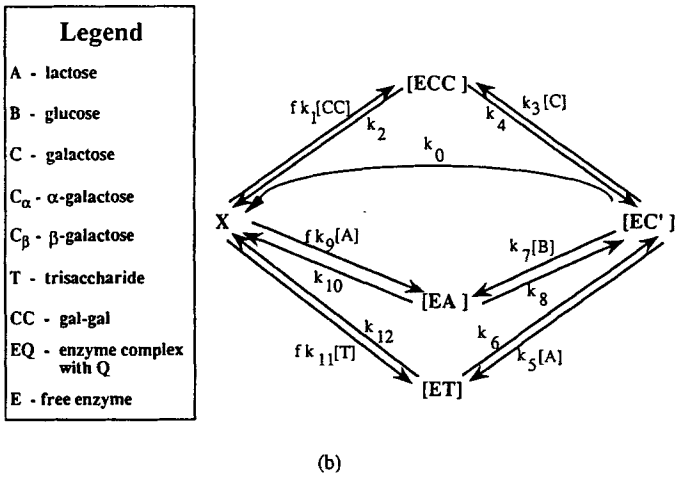


Fig. 2. Diagram of the reaction mechanism: (a) proposed mechanism; b) simplified mechanism.

The rates of formation of the other species can be derived in a similar manner:

$$r_B = \frac{dB}{dt} = k_8[EA] - k_7[EC'][B] \quad (4)$$

$$r_C = \frac{dC}{dt} = k_0[EC'] + k_4[ECC] - k_3[EC'][C] \quad (5)$$

$$r_T = \frac{dT}{dt} = -fk_{11}X[T] + k_{12}[ET] \quad (6)$$

$$r_{CC} = \frac{dCC}{dt} = -fk_1X[CC] + k_2[ECC] \quad (7)$$

The concentrations of the various enzyme complexes can all be written in terms of the concentration of free enzyme, [E], by using the assumption that steady-state conditions prevail. For this mechanism, the algebra required to perform steady-state balances on all the reacting species is greatly simplified by using the schematic approach of King and Altman (*29*). The algebraic manipulations and simplifications are similar to those presented in a previous paper (*26*).

The rate expressions that are produced based on this method are shown in Table 1a. The material balances that result from the substitution of these rate expressions into Eq. 1 are shown in Table 1b. The relationships between the rate constants (k_i) and the constants in Eqs. T1.7–T1.12 are presented in Table 2.

Mutarotation of Galactose

The proposed mechanism for the hydrolysis of lactose contains separate inhibition reactions for both the α and β anomers of galactose. These reactions are included because it has been shown by several researchers (*19,20,26,30,31*) that the two anomers inhibit the enzymatic hydrolysis of lactose to different degrees.

The kinetics of the mutarotation of galactose can be effectively modeled as a reversible reaction, first order in both the forward and the reverse directions (*32*). Consequently, the rate of the mutarotation reaction can be written in terms of the difference between the equilibrium concentration of α-galactose and the actual concentration of α-galactose, viz,

$$r_\alpha = \frac{dC_\alpha}{dt} = k_{mut}([C_{\alpha e}] - [C_\alpha]) \tag{8}$$

where $C_{\alpha e}$ is the equilibrium concentration of α-galactose and k_{mut} is the rate constant for the mutarotation reaction. If one introduces the terms $Y_\alpha = C_\alpha/A_0$ for the dimensionless concentration of α-galactose and $Y_{\alpha e} = C_{\alpha e}/A_0$ for the dimensionless equilibrium concentration of α-galactose, Eq. 8 becomes

$$r_\alpha = \frac{dC_\alpha}{dt} = k_{mut} A_0 (Y_{\alpha e} - Y_\alpha) \tag{9}$$

Thermodynamic and material balance considerations indicate that the equilibrium concentration of α-galactose is given by

$$Y_{\alpha e} = Y_C K_{mut} / (1+K_{mut}) \tag{10}$$

where Y_C is the dimensionless concentration of galactose ($\alpha+\beta$) and K_{mut} is the equilibrium constant for the mutarotation reaction. Both k_{mut} and K_{mut} were determined experimentally by measuring the optical rotation of

Table 1. Mathematical Expressions for the Non-linear Regression Analysis

Table 1a Rate Expressions

$$r_A = \frac{-V_A[A] - 2V_{AA}[A]^2 + V_T[T] - V_{AC}[A][C]}{(1 + K_{i\alpha}[C_\alpha] + K_{i\beta}[C_\beta])} \quad \text{(T1.1)}$$

$$r_B = \frac{V_A[A] + V_{AA}[A]^2 + V_{AC}[A][C]}{(1 + K_{i\alpha}[C_\alpha] + K_{i\beta}[C_\beta])} \quad \text{(T1.2)}$$

$$r_C = \frac{V_A[A] + 2V_{CC}[CC] + V_T[T] - V_{AC}[A][C]}{(1 + K_{i\alpha}[C_\alpha] + K_{i\beta}[C_\beta])} \quad \text{(T1.3)}$$

$$r_T = \frac{V_{AA}[A]^2 - V_T[T]}{(1 + K_{i\alpha}[C_\alpha] + K_{i\beta}[C_\beta])} \quad \text{(T1.4)}$$

$$r_{CC} = \frac{V_{AC}[A][C] - V_{CC}[CC]}{(1 + K_{i\alpha}[C_\alpha] + K_{i\beta}[C_\beta])} \quad \text{(T1.5)}$$

$$r_\alpha = k_{mut}\{[C]K_{mut} / (1 + K_{mut}) - [C_\alpha]\} \quad \text{(T1.6)}$$

Table 1b Mass Balances

$$\frac{dY_A}{dZ} = \tau\left\{\frac{-V_A Y_A - 2V_{AA}Y_A^2 A_0 + V_T Y_T - V_{AC}Y_A Y_C A_0}{(1 + K_{i\alpha}Y_\alpha A_0 + K_{i\beta}Y_\beta A_0)}\right\} \quad \text{(T1.7)}$$

$$\frac{dY_B}{dZ} = \tau\left\{\frac{V_A Y_A + V_{AA}Y_A^2 A_0 + V_{AC}Y_A Y_C A_0}{(1 + K_{i\alpha}Y_\alpha A_0 + K_{i\beta}Y_\beta A_0)}\right\} \quad \text{(T1.8)}$$

$$\frac{dY_C}{dZ} = \tau\left\{\frac{V_A Y_A + 2V_{CC}Y_{CC} + V_T Y_T - V_{AC}Y_A Y_C A_0}{(1 + K_{i\alpha}Y_\alpha A_0 + K_{i\beta}Y_\beta A_0)}\right\} \quad \text{(T1.9)}$$

$$\frac{dY_T}{dZ} = \tau\left\{\frac{V_{AA}Y_A^2 A_0 - V_T Y_T}{(1 + K_{i\alpha}Y_\alpha A_0 + K_{i\beta}Y_\beta A_0)}\right\} \quad \text{(T1.10)}$$

$$\frac{dY_{CC}}{dZ} = \tau\left\{\frac{V_{AC}Y_A Y_C A_0 - V_{CC}Y_{CC}}{(1 + K_{i\alpha}Y_\alpha A_0 + K_{i\beta}Y_\beta A_0)}\right\} \quad \text{(T1.11)}$$

$$\frac{dY_\alpha}{dZ} = \tau\, k_{mut}\{Y_C K_{mut} / (1 + K_{mut}) - Y_\alpha\} \quad \text{(T1.12)}$$

Table 2.
Relationships Between Rate Constants and Parameters

$$K_{i\beta} = k_{15}/k_{16} \quad \text{(T2.1)}$$

$$K_{i\alpha} = k_{17}/k_{18} \quad \text{(T2.2)}$$

$$V_A = (k_0k_4k_6k_8k_9 + k_0k_2k_8k_9k_{12} + k_0k_2k_6k_8k_9 + k_0k_4k_8k_9k_{12})[E]_t \quad \text{(T2.3)}$$

$$V_{AA} = (k_4k_5k_8k_9k_{12} + k_2k_5k_8k_9k_{12})[E]_t \quad \text{(T2.4)}$$

$$V_T = (k_0k_2k_6k_{10}k_{11} + k_0k_4k_6k_{10}k_{11} + k_0k_2k_6k_8k_{11} + k_0k_4k_6k_8k_{11})[E]_t \quad \text{(T2.5)}$$

$$V_{AC} = (k_2k_3k_6k_8k_9 + k_2k_3k_8k_9k_{12})[E]_t \quad \text{(T2.6)}$$

$$V_{ACC} = (k_1k_4k_5k_8k_{12} + k_1k_4k_5k_{10}k_{12})[E]_t \quad \text{(T2.7)}$$

$$V_{CT} = k_2k_3k_6k_{10}k_{11} + k_2k_3k_6k_8k_{11})[E]_t \quad \text{(T2.8)}$$

$$V_{CC} = (k_0k_1k_4k_6k_{10} + k_0k_1k_4k_{10}k_{12} + k_0k_1k_4k_6k_8 + k_0k_1k_4k_8k_{12})[E]_t \quad \text{(T2.9)}$$

α-galactose in a buffer solution (*19*). From a knowledge of these parameters and the total concentration of galactose, one can calculate the concentration of each of the anomers of galactose present in the reaction mixture at any time.

Effect of Temperature on the Kinetic Parameters

The temperature dependence of chemical reactions is most frequently modeled in terms of the Arrhenius equation:

$$\theta = \gamma \exp(\nu/RT) \quad (11)$$

where θ is a generalized kinetic parameter (either a rate constant, or a inhibition constant), γ is the preexponential factor, ν is a term related to the activation energy or standard enthalpy change associated with the molecular process of interest, and R is the gas constant. When these new parameters are fitted via nonlinear regression, they are often highly correlated (*33*). This undesirable correlation can be reduced by centering the temperature term around a mean temperature (T_0).

$$\theta = \gamma' \exp\{\nu/R[(1/T) - (1/T_0)]\} \quad (12)$$

where $\gamma' = \gamma \exp(\nu/RT_0)$. The value for T_0 is arbitrary.

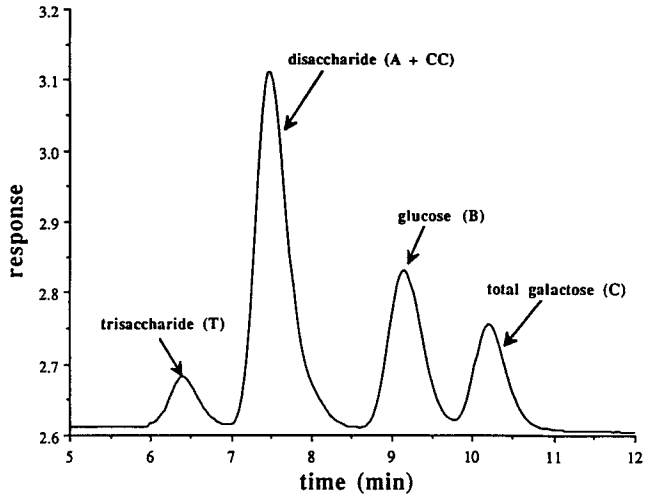


Fig. 3. HPLC chromatogram of a typical product stream: T = 30°C, τ = 2.54 min (the conditions for the HPLC analysis are presented in the Experimental Methods section).

The most common method of determining the temperature dependence of kinetic parameters is to fit data for each temperature independently and then fit these kinetic parameters to the above Arrhenius relationship. However, in doing this, one underestimates the variances of γ' and ν (33). The appropriate method is to employ a global model to simultaneously fit data taken at all the temperatures investigated. This global model is formed by substituting Eq. 12 directly into the mass balance equations (Eqs. T1.7–T1.12). This method allows one to estimate the values and the variances of the parameters solely on the basis of measured data.

RESULTS

Experiments similar to those detailed in the experimental section were performed to determine the values of the kinetic parameters for the rate expressions. Experiments were performed at 20, 30, 40, and 50°C. The mean temperature (T_0) employed in Eq. 12 was chosen to be 35°C. When temperature effects are included, the model based on Eqs. T1.7–T1.12 has 18 parameters (a preexponential parameter and an energy-related parameter for each of the nine kinetic parameters).

A typical chromatogram is shown in Fig. 3. The column was unable to clearly separate the lactose (A) peak from the galactose-galactose (CC) peak. Since the HPLC analysis could provide concentration data for only one disaccharide, it was assumed that this concentration was the sum of the concentrations of lactose and galactose-galactose. This assumption is included in the mathematical model.

Table 3
Parameter Estimates for *Aspergillus oryzae*
14 parameter and 12 parameter model for skim milk as a feedstock

			parameter value ± 95% HPD[a]	
		Units	14 parameters	12 parameters
V_A				
	γ_1	min^{-1}	0.22±0.01	0.19±0.01
	ν_2	kcal/mol	-4.4±0.08	-9.0±0.8
V_{AA}				
	γ_3	$(M \cdot min)^{-1}$	0.33±0.04	0.32±0.04
	ν_4	kcal/mol	-4.0±1.8	-8.4±1.6
V_T				
	γ_5	min^{-1}	0.50±0.07	0.49±0.05
	ν_6	kcal/mol	-12.0±2.4	-15.8±2.6
V_{AC}				
	γ_7	$(M \cdot min)^{-1}$	1.2±0.2	0.88±0.25
	ν_8	kcal/mol	-24.0±2.6	-24.0±2.6
$K_{i\alpha}$				
	γ_9	M^{-1}	170±30	180±30
	ν_{10}	kcal/mol	-10.0±3.2	-9.4±3.6
V_{CC}				
	γ_{11}	min^{-1}	1.3±0.3	1.0±0.3
	ν_{12}	kcal/mol	-36.0±4.0	-36.0±4.0
$K_{i\beta}$				
	γ_{13}	M^{-1}	4.2±2.2	
	ν_{14}	kcal/mol	-20.0±6.0	
obj.			-1286.3	-1228.2

[a]HPD = Highest Posterior Density

In order to fit these rate expressions, one needs to solve a system of six nonlinear differential equations (Eqs. T1.7–T1.12). These differential equations were solved using a Gear-method integrator from the NAG Library (*34*). The multiresponse nonlinear regression analysis was performed with the GREG program (*35*), Level 20. The preexponential parameters for V_{CT} and V_{ACC} were determined to be several orders of magnitide smaller than the other preexponential parameters, so the terms associated with V_{CT} and V_{ACC} were dropped from the model. The results of the nonlinear regression analysis leading to values for the remaining 14 parameters are presented in Table 3 and Fig. 4.

In order to determine if it is necessary to include the effects of inhibition by β-galactose, the data were also fit to the 12-parameter model, which does not include the β-galactose inhibition parameters. The results of the nonlinear regression analysis with 12 parameters also are presented in Table 3. Since the 12-parameter model is nested within the 14-parameter model, one can use an extra determinant analysis to determine whether the additional parameters significantly improve the fit (*33*). The objective function of the regression program can be related to the residual determinant $|\mathbf{Z}^T\mathbf{Z}|$ as follows:

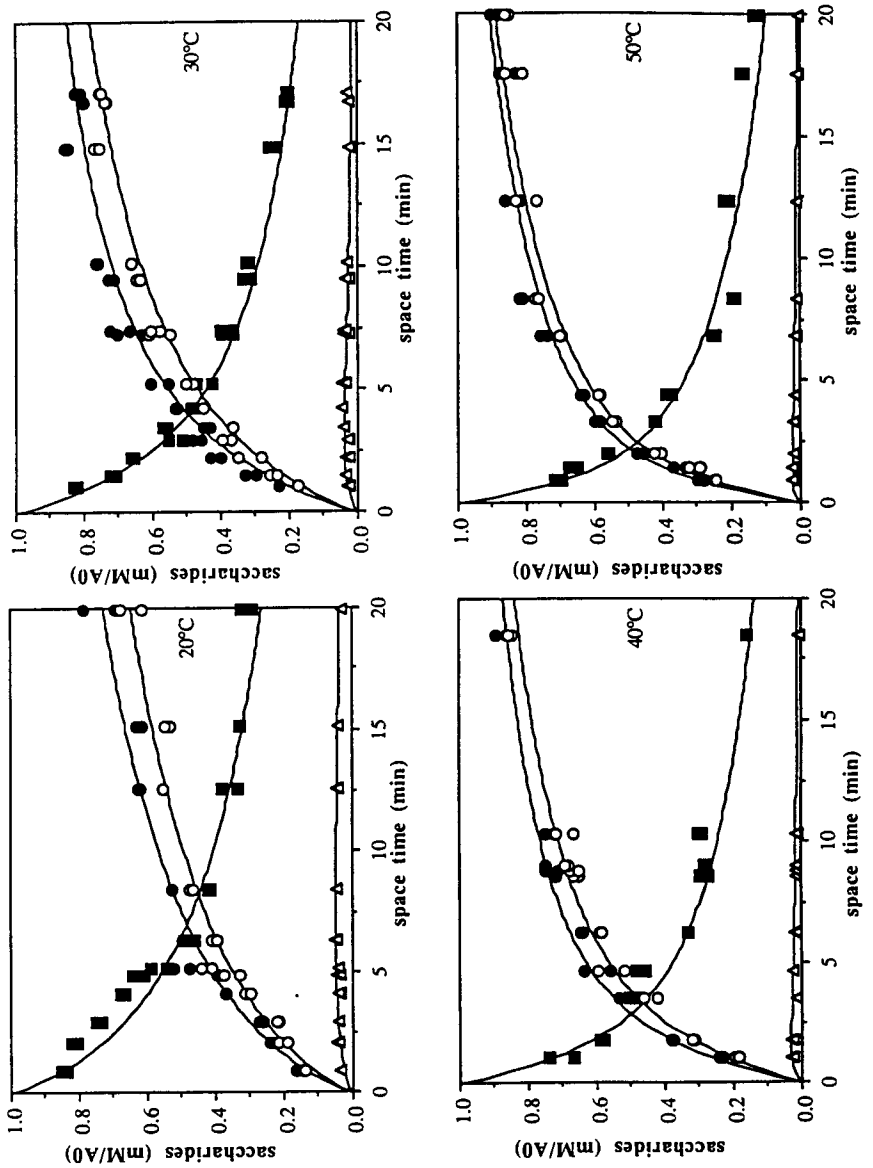


Fig. 4. Concentrations of sugars as a function of space-time for reactions at 20, 30, 40, and 50°C: (■), disaccharide; (●), glucose; (○), galactose; (△), trisaccharide. $T_0 = 35$°C.

Table 4
Extra Determinant Analysis
Comparison of 14 Parameter and 12 Parameter Models

Source	Determinant	Degrees of Freedom[b]	Mean Determinant	F Ratio	p Value[c]
Extra	5.59×10^{-7}	2	2.80×10^{-7}	34.3	$<<0.001$
Full model	6.21×10^{-7}	76	8.17×10^{-9}		
partial model	1.18×10^{-6}	78			

[b] degrees of freedom for the models = N - # of parameters. N = 90
[c] $F(2, 76; 0.001) << 34.3$

$$\text{objective} = N \ln \{ |Z^T Z| \} \quad (13)$$

where N is the number of experiments. The determinant $|Z^T Z|$ is analogous to the sum of squares of the residuals for a uniresponse model. The extra determinant analysis (Table 4) shows that the additional parameters in the 14-parameter model are statistically significant ($p << 0.001$), compared to the 12-parameter model.

DISCUSSION

The ultimate objective of our research is to design and test a reactor that will produce a low-lactose skim milk that can be consumed without discomfort by persons suffering from lactose intolerance. The axial-annular flow reactor tested will achieve the necessary level of conversion in a reasonable space-time, and the enzyme appears to be stable to moderately high temperatures. Therefore this reactor has much promise as an industrial reactor, provided that the process economics are favorable. The modeling and parameter estimates presented in this paper are necessary to design a reactor for use in an industrial setting. To this end, it is advantageous to have available a rate expression that provides a good fit to the data over the entire range of operating conditions anticipated (extent of reaction, temperature, and the like).

From a mathematical standpoint, the immediate objective of the component of the research reported in this paper is to determine the kinetic mechanism that provides the best fit to the data. To this end the following conclusions can be drawn:

1. The proposed mechanism provides a good fit to the data. The concentrations of glucose, galactose, lumped disaccharide, and trisaccharide can all be fitted with this multiresponse model.
2. Fourteen parameters are sufficient to provide the best fit of the data. This conclusion is based on an extra determinant analysis, which shows that the additional parameters in this model are statistically significant when compared to the 12-parameter model. A residual plot for this model shows no hidden trends.

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