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Simultaneous Estimation of V_{max}, K_m, and the Rate of Endogenous Substrate Production (R) from Substrate Depletion Data

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Abstract. The nonlinear and 3 linearized forms of the integrated Michaelis-Menten equation were evaluated for their ability to provide reliable estimates of uptake kinetic parameters, when the initial substrate concentration (S_0) is not error-free. Of the 3 linearized forms, the one where $t/(S_0 - S)$ is regressed against $\ln(S_0/S)/(S_0 - S)$ gave estimates of V_{max} and K_m closest to the true population means of these parameters. Further, this linearization was the least sensitive of the 3 to errors ($\pm 1\%$) in S_0. Our results illustrate the danger of relying on r^2 values for choosing among the 3 linearized forms of the integrated Michaelis-Menten equation. Nonlinear regression analysis of progress curve data, when S_0 is not free of error, was superior to even the best of the 3 linearized forms. The integrated Michaelis-Menten equation should not be used to estimate V_{max} and K_m when substrate production occurs concomitant with consumption of added substrate. We propose the use of a new equation for estimation of these parameters along with a parameter describing endogenous substrate production (R) for kinetic studies done with samples from natural habitats, in which the substrate of interest is an intermediate. The application of this new equation was illustrated for both simulated data and previously obtained H_2 depletion data. The only means by which V_{max}, K_m, and R may be evaluated from progress curve data using this new equation is via nonlinear regression, since a linearized form of this equation could not be derived. Mathematical components of computer programs written for fitting data to either of the above nonlinear models using nonlinear least squares analysis are presented.

Introduction

The Michaelis-Menten kinetic model has been used by microbial physiologists and ecologists to describe substrate consumption (and product formation) by (1) nongrowing bacterial suspensions and (2) samples obtained from both natural habitats and enrichment cultures in which the activity is approximately at steady-state. In several of these studies [4, 7, 12, 15, 16, 18, 19], the integral form of the Michaelis-Menten equation was used since V_{max} and K_m may be estimated from a single substrate depletion (or product formation) curve, in contrast to the differential form of the Michaelis-Menten kinetic model which requires several experiments run at different initial substrate concentrations for estimation of V_{max} and K_m [14].

In some of the above studies [12, 16, 18, 19], a linearized form of the integrated Michaelis-Menten kinetic model was used to permit estimation of V_{max} and K_m via linear least squares analysis. This practice, although useful because of its simplicity, does not lead to the calculation of the best estimates of V_{max} and K_m from the data. Further, use of any linearized form of the integrated Michaelis-Menten equation violates an important assumption of least squares analysis: namely, that the independent variable is free of error. In most cases, it is better to fit nonlinear data directly to the nonlinear form of a model (e.g., the integrated Michaelis-Menten equation) rather than fitting transformed data to a linearized version of the same model.

In addition to the above limitations, linearized forms of the integrated Michaelis-Menten equation require that the initial substrate concentration (S_0) be known without error, a condition not often met in practice. In contrast, directly fitting progress curve data to the integrated Michaelis-Menten model allows S_0 to be treated as another parameter (like K_m and V_{max}) to be estimated. One objective of the present study was to develop a procedure for estimating V_{max}, K_m, and S_0 from Michaelis-Menten substrate depletion data.

If substrate production occurs concomitant with depletion of added substrate during a progress curve experiment, significant errors in estimates of V_{max} and K_m may arise when the resultant data are fitted to the integrated Michaelis-Menten equation [15]. Although this problem is typically not encountered when pure bacterial cultures are studied, it may obfuscate kinetic investigations conducted with samples from natural habitats or enrichments in which the added substrate is endogenously produced. A second objective of our work was to develop a nonlinear regression routine capable of estimating V_{max}, K_m, and S_0 along with a parameter describing zero-order endogenous substrate production (R) from progress curve data.

The superiority of nonlinear regression methods for estimating V_{max} and K_m when S_0 is unknown, and simultaneous estimation of these parameters along with R, were shown using both experimental and theoretical data. The latter were generated using stochastic procedures that introduced either simple (constant standard deviation) or relative (constant coefficient of variation) errors into numerical solutions (i.e., substrate concentration versus time curves) of the integrated Michaelis-Menten expressions. Lastly, we used sensitivity analysis to determine the range of endogenous substrate production rates that can in practice be simultaneously estimated with V_{max}, K_m, and S_0 from substrate depletion data.

Theory and Methods

Theory

The rate of substrate consumption by a nongrowing bacterial suspension, in the absence of endogenously produced substrate, may be described by

$$dS/dt = -V_{max}S/(K_m + S) \quad (1)$$

where V_{max} = maximum rate of substrate consumption, K_m = half-saturation constant, S = substrate concentration and t = time. Equation 1 is the differential form of the Michaelis-Menten equation, and it may be integrated to give

$$V_{max}t = S_0 - S + K_m \ln(S_0/S) \quad (2)$$

In Eq. 2, S_0 = initial substrate concentration. This equation, along with the integral form of Eq. 1 solved for product appearance, have appeared in many textbooks on enzyme kinetics (e.g., Cornish-Bowden [6] and Roberts [14].

If substrate is produced (e.g., by another organism) during consumption of added substrate, then Eq. 2 no longer applies and it should not be used to estimate V_{max} and K_m from substrate depletion data. Allowing for a zero-order (i.e., constant) rate of endogenous substrate production (R), Eq. 1 becomes

$$dS/dt = -V_{max}S/(K_m + S) + R \quad (3)$$

After integration, Eq. 3 becomes

$$(C_2/C_1^2)\ln(C_3/C_4) + (S_0 - S)/C_1 - t = 0 \quad (4)$$

where

$$C_1 = V_{max} - R,\ C_2 = 2RK_m - V_{max}K_m,\ C_3 = C_1S - RK_m, \text{ and } C_4 = C_1S_0 - RK_m.$$

Equation 4, like Eq. 2, is implicit in S (i.e., the substrate concentration cannot be explicitly solved for time) and solution curves must be numerically approximated. This can be accomplished by numerically integrating the differential forms (viz., Eqs. 1 and 3) of these 2 expressions. Equations 2 and 4 describe similar curves when R is less than $V_{max}S_0/(K_m + S_0)$, but while S asymptotically decreases to zero for Eq. 2, Eq. 4 predicts S will approach a steady-state value of $RK_m/(V_{max} - R)$ (Fig. 1). When the endogenous rate of substrate production is greater than $V_{max}S_0/(K_m + S_0)$, Eq. 4 predicts S will increase to its steady-state value (Fig. 1).

In order to fit data to Eqs. 2 and 4 (or any nonlinear model) it is necessary to have a way of detecting how sensitive solutions to the model equation—S vs. t, in this case—are to slight changes in the parameters. This requirement can be met by evaluating the sensitivity equations for each of the parameters in the model. A sensitivity equation is defined as the first derivative of the dependent variable with respect to a particular parameter of a nonlinear model. Sensitivity equations can be readily derived for Eqs. 2 and 4 using implicit differentiation [20], and for Eq. 2 they are

$$dS/dV_{max} = t/(1 + K_m/S) \quad (5)$$

$$dS/dK_m = -\ln(S_0/S)/(1 + K_m/S) \quad (6)$$

and

$$dS/dS_0 = (1 + K_m/S_0)/(1 + K_m/S) \quad (7)$$

The sensitivity equations for the parameters of Eq. 4 are

$$dS/dV_{max} = \{\ln(C_4/C_3)[(V_{max}K_m - 3RK_m)/C_1] + (C_2S_0/C_4 - C_2S/C_3) + S_0 - S\}/C_5 \quad (8)$$

$$dS/dK_m = [(2R - V_{max})\ln(C_4/C_3) + C_2R(1/C_3 - 1/C_4)]/C_5 \quad (9)$$

$$dS/dR = \{\ln(C_4/C_3)(2K_m - C_2/C_1^2) + C_2[(S + K_m)/C_3 - (S_0 + K_m)/C_4] - (S_0 - S)\}/C_5 \quad (10)$$

and

$$dS/dS_0 = (C_1C_2/C_4 - C_1)/C_5 \quad (11)$$

In Eqs. 8–11, $C_5 = C_1C_2/C_3 - C_1$.

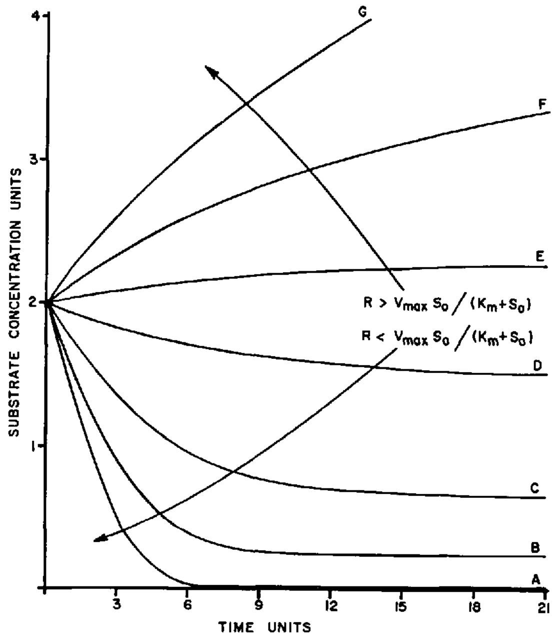

Fig. 1. Solutions to the integrated Michaelis-Menten equation that incorporates zero-order endogenous substrate production (Eq. 4). The solution curves were obtained by numerically integrating Eq. 3 using a fourth-order Runge-Kutta technique (Burden et al. [5]), given V_{max} and K_m values of 1 and 1, respectively. Beginning with the bottommost curve the R values were 1, 20, 40, 60, 70, 80, and 90% of V_{max} (=1). Note that as R decreases, the solution to Eq. 4 approaches that given by Eq. 2.

The fitting of data directly to Eq. 2 or 4 consists of essentially 3 steps. First, theoretical S values (S_T) are calculated using the model equations—either Eq. 2 or 4—at the times for which S was measured, given initial estimates or guesses of the parameters. The second step involves evaluating the sensitivity equations (Eqs. 5–7 for model Eq. 2 or Eqs. 8–11 for model Eq. 4) at the same times for which theoretical S values were calculated. Third, the above information is used in the following expression to calculate "correction terms" for the initial parameter estimates:

$$S - S_T = C_V dS/dV_{max} + C_K dS/dK_m + C_R dS/dR + C_{S0} dS/dS_0 \quad (12)$$

When data are fitted to Eq. 2, then the third term on the right-hand side of Eq. 12 is absent. In Eq. 12, the left-hand side equals the residual errors: observed S minus predicted S (S_T). The correction terms (C_V, C_K, C_R, and C_{S0}) are determined via multiple linear regression, and added—they may be positive or negative—to the initial parameter estimates forming the next set of parameters. The above 3 steps are repeated until the correction terms are less than the convergence criterion (e.g., 0.0001). At this point, the parameter estimates that yield the smallest residual sum of squares have been determined. Nonlinear regression is thus an iterative or recursive process in which a set of initial parameter estimates are sequentially refined until the best values are calculated.

In addition to their use for nonlinear regression, the sensivity equations predict (1) whether unique estimates of the parameters in a given model may be determined, and (2) optimal values of the independent variable (time, in this case) at which the dependent variable should be measured in order to obtain most accurate estimates of the parameters [3]. For Eqs. 2 and 4 the sensitivity coefficients predict that when S_0 is less (10-fold say) than K_m, unique estimates of the parameters in these 2 nonlinear models cannot be determined. This is because the sensitivity coefficients for the parameters of Eqs. 2 and 4—excluding S_0—are approximately proportional (i.e., multiples of one another), in the first-order region (Figs. 2–3). Conversely, when the initial substrate concentration is saturating, the sensitivity coefficients predict that changes in individual parameters produce relatively small changes in the dependent variable over a significant portion of the progress curve described by either Eq. 2 or 4 (Figs. 2–3). Optimal estimation of V_{max} and K_m, and R if data are to be fitted to Eq. 4, require that S_0 be in the mixed-order region (Figs. 2–3).

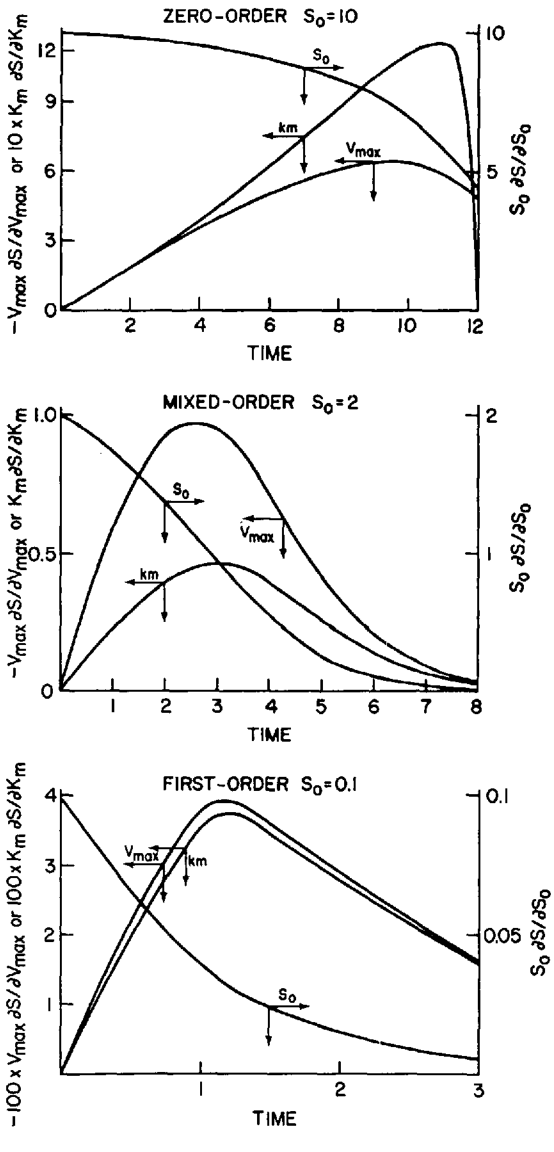

Fig. 2. The relative sensitivity equations for the parameters (V_{max}, K_m, S_0) of the integrated Michaelis-Menten equation (Eq. 2) for the zero-, mixed-, and first-order regions. Note, the sensitivity equations plotted in this way (viz., parameter value × sensitivity equation) all have the same units, making them directly comparable.

Simulated Data

In order to evaluate least squares analysis for Eqs. 2 and 4, theoretical data were generated by solving these 2 equations for chosen values of the parameters and the initial substrate concentration, S_0. The solution curves were obtained by numerically integrating Eqs. 1 and 3 using a fourth-order Runge-Kutta [5] method. Errors of either the relative (constant coefficient of variation) or simple (constant standard deviation) were added to these solution curves resulting in data containing known errors. The errors were introduced into the solution curves using Monte Carlo simulation techniques [9].

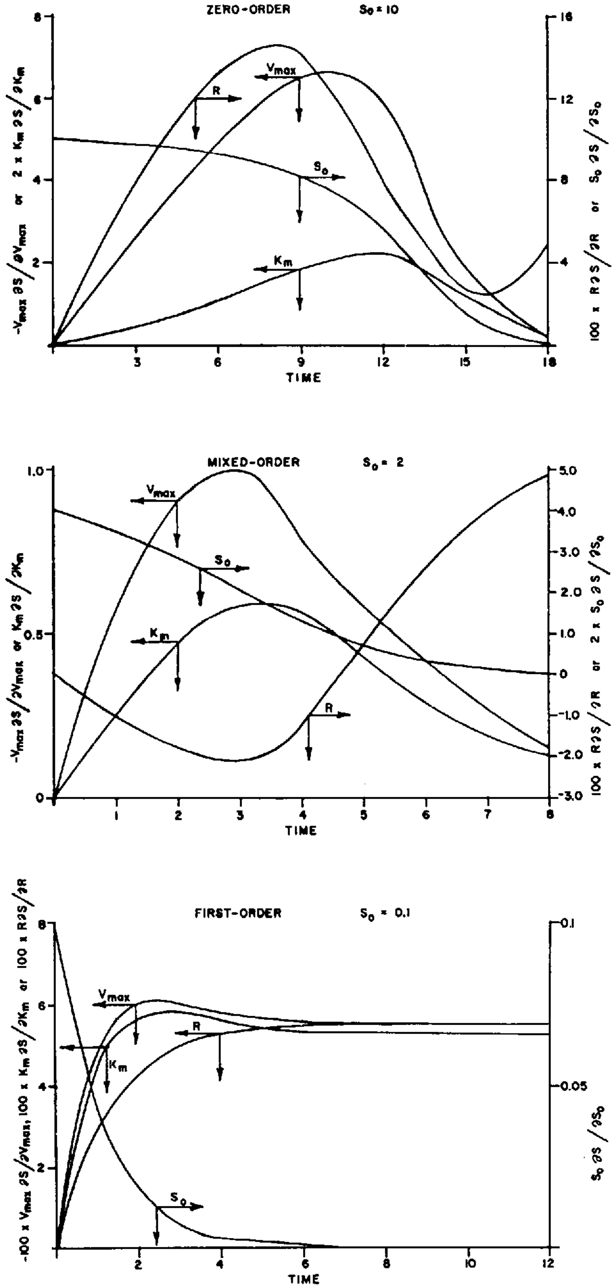

Fig. 3. The relative sensitivity equations for the parameters (V_{max}, K_m, S_0, R) of the integrated Michaelis-Menten equation that incorporates zero-order endogenous substrate production (Eq. 4) for the zero-, mixed-, and first-order regions. See the legend to Fig. 2 for more details.

Experimental Data

The fitting of experimental data to Eqs. 2 and 4 using nonlinear regression was also evaluated. H_2 depletion data obtained for diluted rumen fluid was fitted to these nonlinear models employing the same fitting procedure used to analyze the theoretical error-containing data. Details describing

Table 1. Affect of errors in the initial substrate concentration (S_0) on V_{max} and K_m estimates obtained using linearized forms of the integrated Michaelis-Menten equation[a]

	% Error in S_0[b]								
	0			−1			+1		
Eq[c]	[13]	[14]	[15]	[13]	[14]	[15]	[13]	[14]	[15]
V_{max}	0.92	0.92	0.93	0.83	0.84	0.86	1.03	1.01	1.01
K_m	0.78	0.80	0.84	0.56	0.60	0.66	1.08	1.00	1.02
r^2	0.97	0.77	0.88	0.96	0.60	0.73	0.97	0.84	0.93
rss[d]	0.04	0.03	0.03	0.07	0.05	0.04	0.02	0.02	0.02

[a] Values are means of analysis of 12 replicate progress curves containing simple errors (constant SD). The theoretical curves were generated by solving Eq. 2 for V_{max}, K_m, and S_0 values of 1, 1, and 4, respectively. Errors having a constant SD (= 0.004) were introduced into the solution curves using a pseudorandom number generator according to the procedure of Harbaugh and Bonham-Carter [9]

[b] Errors of 0, −1, and +1% in S_0 correspond to S_0 values of 4, 3.96, and 4.04, respectively

[c] Parameter estimates were obtained by transforming the theoretical error-containing data according to Eqs. 13–15 and applying linear unweighted least squares analysis

[d] rss = residual sum of squares, or the sum of the squared deviations

the incubation system and experimental methods used to obtain the H_2 progress curve data appeared in a previous publication [15].

Computer Methods

All regression analyses were done using an Apple II Plus microcomputer (Apple Computer, Inc., Cupertino, CA). Programs that fit data to Eqs. 2 (PROGCRV) and 4 (MMENDOGFIT) using Eq. 12 were written in Applesoft BASIC and are available from the authors upon request. Furthermore, programs for fitting transformed data to linearized forms of Eq. 2 using linear least squares analysis, and for estimating solution curves to Eqs. 2 and 4 are also available.

Results

Evaluation of Linearized Forms of the Integrated Michaelis-Menten Equation

Directly fitting data to nonlinear models like Eqs. 2 and 4 requires initial estimates of the parameters [3], which are then improved stepwise until the sum of the squared deviations reaches a minimum. Initial estimates of the parameters can be obtained either through guesswork or by using linearized forms of the nonlinear model. For Eq. 2, 3 linearized forms can be derived through algebraic manipulation and these are given below:

$$(1/t)\ln(S_0/S) = (1/V_{max})(S_0 - S)/\ln(S_0/S) + K_m/V_{max} \tag{13}$$

$$(S_0 - S)/t = V_{max} - K_m\ln(S_0/S)(1/t) \tag{14}$$

and

$$t/(S_0 - S) = (K_m/V_{max})\ln(S_0/S)/(S_0 - S) + 1/V_{max} \tag{15}$$

Table 2. Affect of errors in the initial substrate concentration (S_0) on V_{max} and K_m estimates obtained by fitting data directly to the integrated Michaelis-Menten equation[a]

	% Error in S_0[b]		
	0	−1	+1
V_{max}	0.97	0.94	1.02
K_m	0.96	0.88	1.08
rss	0.02	0.02	0.02

[a] Values are mean parameter estimates obtained from nonlinear regression analysis of the same data fitted to the 3 linearized forms of the Michaelis-Menten equation (Table 1). See the text and legend of Table 1 for additional details

In order to evaluate the above linearized forms of Eq. 2, simulated data containing simple errors were transformed and then fitted to Eqs. 13–15. This was accomplished using unweighted linear least squares analysis. Errors in S_0 of either −1% and +1% were used to test the reliability of Eqs. 13–15 when the initial substrate concentration is not error-free. Of the 3 linearizations, Eq. 15 produced estimates of V_{max} and K_m that on average were closest to the true values, although the r^2 values for fits to this expression were the lowest of the 3 linearizations used (Table 1). The linearization that yielded the best fits, according to the r^2 values, produced estimates of V_{max} and K_m furthest from the true values, i.e., those with the greatest bias (Table 1).

Influence of Errors in S_0 on V_{max} and K_m Determined via Nonlinear Regression

The same data sets fitted to Eqs. 13–15 were fitted to Eq. 2. Again, errors of −1% and +1% in S_0 were introduced to test the reliability of, in this case, fitting data directly to the nonlinear form of the integrated Michaelis-Menten equation. Although the residual sum of squares was essentially the same for fits of data to the best linearization (viz., Eq. 15 vs. Eq. 2), estimates of the Michaelis-Menten parameters obtained via nonlinear regression were less sensitive to −1% errors in S_0 (Table 2).

Although the results are not shown, evaluations of Eq. 2 and Eqs. 13–15 were also done for simulated data that contained relative errors. The conclusions that emerged from these analyses are identical to those given above for the data that contained simple errors.

Estimation of V_{max} and K_m in the Presence of Endogenous Substrate Production

When substrate production occurs concomitant with depletion of added substrate, then the integrated Michaelis-Menten equation can yield inaccurate

Table 3. Comparison of parameter estimates obtained from fitting progress curve data directly to Eqs. 2 vs. 4, where the endogenous substrate production rate is 1, 5, and 10% of V_{max}[a]

	Simple errors (SD = 0.04)					
	Eq [2]			Eq [4]		
	R = 0.01	R = 0.05	R = 0.10	R = 0.01	R = 0.05	R = 0.10
V_{max}	1.00	1.07	1.21	1.01	1.01	1.01
K_m	1.10	1.52	2.33	0.99	0.99	0.99
R	—	—	—	0.01	0.06	0.11
S_0	4.01	4.05	4.09	4.00	4.00	4.00
$10^{-3} \times$ rss	0.70	6.89	16.2	0.10*[b]	0.09*	0.09*
	Relative errors (CV = 0.01)					
	Eq [2]			Eq [4]		
	R = 0.01	R = 0.05	R = 0.10	R = 0.01	R = 0.05	R = 0.10
V_{max}	1.00	1.06	1.19	1.01	1.01	1.01
K_m	1.08	1.48	2.25	1.00	1.00	0.99
R	—	—	—	0.01	0.05	0.10
S_0	4.01	4.04	4.09	4.00	4.00	4.00
$10^{-4} \times$ rss	2.96	58.0	152	0.05*	0.04*	0.05*

[a] Parameter estimates are means obtained from analysis of 12 replicate progress curves; simulated data contain either simple (SD = 0.04) or relative errors (CV = 0.01). Simulated progress curves were generated by solving Eq. 4 for V_{max}, K_m, and S_0 values of 1, 1, and 4, respectively. R values used are given in the table
[b] Indicates a significant reduction in the rss value for incorporating R as an additional parameter (i.e., using Eq. 4 instead of Eq. 2) at the 5% level of statistical significance. The significance of the reduction in the rss was evaluated using an F-test based on equation 6.2.24 in Beck and Arnold [3]

estimates of V_{max} and K_m. The magnitudes of errors in these parameters depend on how great the endogenous substrate production rate is relative to V_{max} (i.e., R/V_{max}). To test the effects different ratios of R/V_{max} have on estimates of V_{max} and K_m obtained using the integrated Michaelis-Menten expression, simulated data were generated by solving Eq. 4 for V_{max}, K_m, and S_0 values of 1, 1, and 4, respectively. Three values of R were chosen, namely, 0.01, 0.05, and 0.10. The simulated data were fitted to Eq. 2 and 4 using nonlinear least squares analysis.

A rate of endogenous substrate production equal to 1% of V_{max} did not produce significant errors in V_{max}, when this parameter was estimated using Eq. 2, whereas slight positive biases were found for the K_m estimates obtained using Eq. 2 (Table 3). For an R value equal to 5% of V_{max}, again only slight errors were found for V_{max} values, estimated by fitting the progress curve data to the integrated Michaelis-Menten equation. In contrast, K_m estimates obtained using Eq. 2 were in error by about +50% (Table 3). For rates of endogenous substrate production equal to 10% of V_{max}, ignoring the deceleration of the progress curves due to endogenous substrate production resulted in errors for V_{max} and K_m of +10% and greater than +100%, respectively.

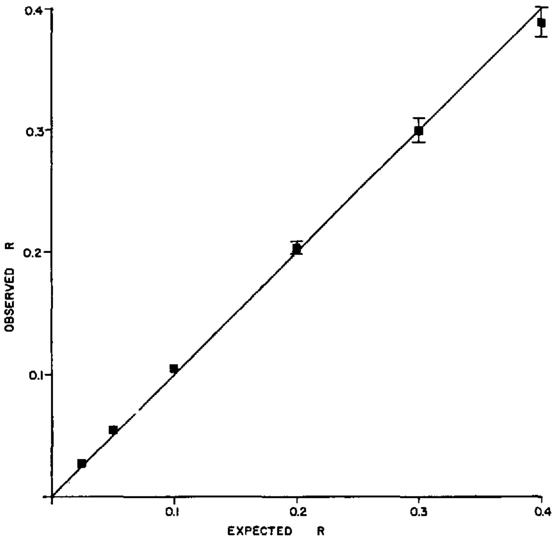

Fig. 4. Determination of the practical range for estimation of R from progress curve data. Simulated data containing simple errors (SD = 0.004) were fitted to Eq. 4 using Eq. 12. R values greater than those equal to 40% and less than 2.5% of V_{max} could not be reliably determined from progress curve data.

When the progress curve data were fitted to Eq. 4 using nonlinear regression, estimates of V_{max}, K_m, and R were all close to the values used to generate the data (Table 3). Further, the residual sums of squares were significantly lower for Eq. 4 than for Eq. 2 (Table 3), indicating that the former nonlinear model more accurately described the progress curves.

Limits to the Simultaneous Estimation of V_{max}, K_m, and R from Progress Curve Data

An examination of Eq. 9 reveals that when R = 1/2 V_{max}, then $dS/dK_m = 0$. Hence, when the rate of endogenous substrate production is one-half of the maximal rate of substrate consumption, then unique estimates of the parameters in Eq. 4 cannot be determined from progress curve data. Further, when R is greater than ½ V_{max}, Eq. 12 cannot be used to estimate the parameters in Eq. 4 (results not shown). Simultaneous estimation of V_{max}, K_m, and R from progress curve data is restricted to situations where R is less than ½ of V_{max}.

To establish the practical range over which R can be estimated from progress curve data, simulated data sets were obtained by solving Eq. 4 for different values of R, given V_{max}, K_m, and S_0 values of 1, 1, and 8, respectively. Accurate estimates of R could be obtained even when the rate of endogenous substrate production was only 2.5% of V_{max} (Fig. 4). The upper limit for accurate estimation of R was when this parameter equalled 40% of V_{max}. Using higher, and perhaps more realistic error levels, we found the practical range for estimation of R to be for values that equal 5–40% of V_{max}. For all values of R, estimates of V_{max} and K_m were obtained that were within 5% of the true values.

Table 4. Fits of H_2 progress curve data obtained for diluted rumen fluid to Eq. 2 vs. Eq. 4[a]

	Eq [2]	Eq [4]
V_{max} (μM min^{-1})	27.9	25.6
K_m (μM)	11.9	9.43
R (μM min^{-1})	—	0.49
S_0 (μM)	19.9	19.8
rss	0.26	0.06[b]

[a] H_2 depletion data were fitted to Eqs. 2 and 4 using Eq. 12. The V_{max} estimates represent H_2 consumed in the entire closed experimental system. The number of data points analyzed in this case was 56

[b] This reduction in the rss is significant at the 5% level. See Table 3 for the reference on the F-test used to evaluate the reduction in the rss value

Application of Eq. 4 to Experimental Data

The parameters of Eqs. 2 and 4 were estimated for H_2 consumption by diluted rumen fluid using the same techniques applied to the simulated data. The H_2 depletion data were obtained using a previously described experimental system [15]. The fit of the data to Eq. 4 was significantly better than the fit of the data to Eq. 2 (Table 4). The estimate of R for the H_2 progress curve data was about 2% of the H_2 V_{max} and ignoring this parameter resulted in positive biases in V_{max} and K_m of 9.0 and 26%, respectively (Table 4).

Discussion

In this study, simulated data were used to evaluate estimation of V_{max}, K_m, and a zero-order production term (R) from progress curve data. Analysis of simulated data containing known errors is useful since the different forms of a nonlinear model can be evaluated for their ability to provide reliable estimates of the parameters used to generate the data. An estimation scheme that does not yield accurate estimates of parameters for simulated data for which the true parameters are known, obviously will not provide reliable values of parameters for actual data. We found that the nonlinear form of the integrated Michaelis-Menten equation (Eq. 2) was superior to linearized forms of this model (Eqs. 13–15) for estimation of V_{max} and K_m when S_0 is not free of error.

Many kineticists and enzymologists have discussed the estimation of V_{max} and K_m from progress curve data [1, 2, 6, 8, 13], although in all of these cases S_0 was assumed to be known with certainty. Biochemists have, for the most part, used the product appearance form of the integrated Michaelis-Menten model since enzymological assays usually involve monitoring a given compound's appearance rather than its disappearance. If this form of the integrated Michaelis-Menten equation is used, then S_0 cannot be treated as another parameter—like V_{max} and K_m—to be iteratively improved through the application of Eq. 12. Only when the substrate disappearance form of the integrated Michaelis-Menten equation is used—viz., Eq. 2—can nonlinear regression analysis be used to update S_0 simultaneously with V_{max} and K_m. Assuming that S_0 is free from error is unrealistic in many cases, and as we have shown, errors in this initial condition can create biases in estimates of V_{max} and K_m, particularly when a linearized form of Eq. 2 is employed. In the program (PROGCRV) developed to fit data to Eq. 2, S_0 is treated as another parameter, thus avoiding the biases introduced into V_{max} and K_m estimates caused by ignoring errors in S_0.

In contrast to biochemists, microbial ecologists have largely applied the substrate disappearance form of the integrated Michaelis-Menten equation—viz., Eq. 2—to analysis of their progress curve data. Some investigators [4, 15] fitted their data to Eq. 2 using nonlinear regression, but most have employed a linearized form of Eq. 2 to estimate V_{max} and K_m. Strayer and Tiedje [18], Schauer et al. [16], and Lovley et al. [12] all used a linearized form of Eq. 2 similar to Eq. 14, the latter of which we found to be less reliable than Eq. 15 for estimating V_{max} and K_m (from linearized data). In a study on the anaerobic biodegradation of halogenated aromatics, Suflita et al. [19] used a more reliable linearized form of Eq. 2, namely, Eq. 13. Finally, other researchers [10, 11, 17] estimated V_{max} and K_m by calculating $-dS/dt$ pairs from their progress curve data and then fitting the calculated v-s pairs to the Lineweaver-Burk equation, a practice that is statistically unreliable [6].

Fitting transformed data to any linearization of the integrated Michaelis-Menten equation violates an assumption of least squares analysis: lack of error in the independent variable [3, 21]. In practice, this assumption is sufficiently met if errors in the independent variable are small (10-fold) relative to the errors in the dependent variable [21]. As pointed out by Beck and Arnold [3], the presence of nonnegligible errors in the independent variable makes the correct estimation of parameters in even a linear model (like $Y = BX + A$) a difficult proposition. Clearly, any linearized form of Eq. 2 violates the above assumption since the measured variable S appears in both the dependent and independent variables. For this reason alone, we recommend use of linearized forms of the integrated Michaelis-Menten equation—and then only Eq. 15—only when the experimenter lacks the option of fitting data directly to Eq. 2 using the method of nonlinear least squares.

The integrated Michaelis-Menten equation fails to produce reliable estimates of K_m, and V_{max} to a lesser extent, when endogenous substrate production occurs concomitant with the consumption of added substrate. The degree of bias introduced into these parameters depends on the rate of endogenous substrate production (R) relative to V_{max}. We found that even for R values equal to 5% of V_{max}, unacceptable biases were introduced into estimates of K_m (Table 3) obtained from fitting the progress curve data to Eq. 2. This problem was previously considered by Robinson and Tiedje [15] in their study on the kinetics of H_2 consumption by rumen fluid, digestor sludge, and eutrophic sediment. These authors appreciated that endogenously produced H_2 may have biased their H_2 K_m estimates but failed to recognize that a model like Eq. 4 could have been used to estimate the Michaelis-Menten parameters and R, thus eliminating biases in V_{max} and K_m introduced by assuming R was negligible.

In our study, we found that endogenous substrate production rates as low as

2.5% of V_{max} could be successfully estimated when the standard deviation in the dependent variable was 0.1% of S_0 (Fig. 4). With more realistic error levels of 1%, R can be estimated when the population mean of this parameter is 5–40% of V_{max}. For future kinetic investigations with substrates that are intermediates in natural metabolic cycles (e.g., H_2, nitrite, acetate), we recommend the use of an equation like Eq. 4 which accounts for both substrate consumption and endogenous substrate production. The major difficulty of using Eq. 4 is that the parameters must be estimated using nonlinear least squares techniques, since a linearized form of this model cannot be derived.

Including R in the integrated Michaelis-Menten model significantly reduced the residual sum of squares obtained for H_2 consumption by diluted rumen fluid (Table 4). Thus the additional parameter R, appearing in Eq. 4, is a term necessary for describing more completely the consumption of H_2 by diluted rumen fluid. This is reasonable since H_2 is an intermediate in the anaerobic degradation of organic matter in the rumen. Robinson and Tiedje [15] estimated that R was 5–10% of the H_2 V_{max} for diluted rumen fluid, several-fold higher than our estimate of 2%. These authors indirectly estimated R using endogenous rates of CH_4 production and the steady-state H_2 concentration. Although this approach is reasonable, estimation of R using an expression like Eq. 4 is advantageous since endogenous substrate production can be estimated directly from the H_2 disappearance data.

The biggest limitation to the use of Eq. 4 is that the parameters of this model cannot be estimated if R equals or exceeds ½ of V_{max}. This limitation may not be a serious one for many substrates of ecological interest (e.g., H_2, acetate) since the in situ concentrations of these substrates are often first-order. But the above limitation does not apply to Eq. 3, the differential form of Eq. 4; if $-dS/dt$ vs. S pairs are fitted to Eq. 3 using nonlinear least squares analysis, V_{max}, K_m, and R can all be estimated regardless of the ratio R/V_{max}. This illustrates how one form of a nonlinear model (Eq. 3 in this case) can be successfully applied under conditions for which a different mathematical representation (Eq. 4) of the same process fails.

In this study we developed BASIC programs to fit data to Eqs. 2 and 4 using the technique of nonlinear least squares. Similar canned programs for carrying out these analyses exist on mainframe campus computers. But it is necessary to understand the limitations of nonlinear regression analysis for wise application of this statistical tool. As is true for many statistical packages available on mainframe systems, there is a temptation to consider the limitations of the analyses after data collection is over. This practice, particularly for nonlinear regression analysis, can lead to a significant waste of experimental resources. If nothing else, it behooves an investigator to examine the sensitivity equations of the nonlinear model of interest to test a priori the possibility of obtaining unique parameter estimates from the data. By developing our own software for parameter estimation, we were forced to appreciate the limitations of estimating V_{max}, K_m, and R using nonlinear regression, which we might not have done had we used a mainframe routine for updating these parameters.

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