Simultaneous Estimation of $V_{\text{max}}$, $K_m$, 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 $V_{\text{max}}$ 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 $V_{\text{max}}$ 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 $V_{\text{max}}$, $K_m$, 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 $V_{\text{max}}$ 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 $V_{\text{max}}$ 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 $V_{\text{max}}$ 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 $V_{\text{max}}$ 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 $V_{\text{max}}$) to be estimated. One objective of the present study was to develop a procedure for estimating $V_{\text{max}}$, $K_m$, 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 $V_{\text{max}}$ 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 $V_{\text{max}}$, $K_m$, 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 $V_{\text{max}}$ 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 $V_{\text{max}}$, $K_m$, 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