Monod's Growth Kinetics. Is there a rationale for it?

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All that is known about microbial metabolism and growth would seem to demand a mathematical description of such unimaginable complexity that any form of conformity with Monod's simple growth kinetics should set the rationalists on the trail of some reasonable explanation. To air such a quest is to honor Rutherford Aris, the scholar, who constantly sought to find musicality in mathematical models of chemical systems. His expositions of the beauty of stoichiometry and its algebraic implications, the kinetics of continuous reaction mixtures, and issues connected with lumping of kinetics of large systems all represent scholarly accomplishments of exemplary character!

Appeal to the likeness of microbial metabolism to enzyme-substrate kinetics is too frail to serve as a rational base for their mathematical similarity, for the bewildering diversity of enzymes and substrates involved in metabolism could hardly yield to the simplicity of a single such reaction. It is the objective of this talk then to explore the circumstances, which will conceal the complexity of metabolism behind an ordinary single substrate enzyme kinetic expression.

The broad approach here to the quest for rationalization of Monod kinetics is based on viewing metabolism in terms of elementary modes representing various options for the utilization of external substrate each contributing to the production of intracellular metabolites, extracellular products, and biomass. Assuming that each reaction in a given elementary mode is in steady state with the uptake rate, estimation of its rate is enabled by stoichiometric coupling alone. The uptake rates are then modeled in terms of single substrate enzyme kinetics so that the rate of production of fresh biomass is eventually expressible as a convex combination of numerous single substrate enzyme kinetic expressions. Rationalization of Monod kinetics for the production of biomass then depends on how it can be obtained from a convex combination of numerous single substrate enzyme kinetic expressions. The various steps in this development are briefly described below.

Monod Kinetics for Growth

The kinetic expression due to Monod applies to the straitjacketing of metabolism into the following single step

$$B + S \rightarrow (1+Y)B + \cdots \qquad (1)$$

The foregoing reaction describes the yield of Y mass units of biomass from (a carbon, rate limiting) substrate with the aid of existing biomass. The Monod kinetic expression is designed to address the rate at which transformation (1) occurs without regard to the details of the mechanism by which metabolism functions to accomplish the same. The *specific* growth rate (i.e., the rate of synthesis of biomass per cell) is given by

$$r_G = \frac{\mu s}{K + s} \tag{2}$$

where μ is the maximum growth rate attained when the substrate concentration (defined per unit volume of culture, i.e., suspension of cells) is sufficiently larger than the Michaelis constant K.

Stoichiometric Formulation of Metabolism

Limiting ourselves to central carbon metabolism in which the carbon source is admitted into the metabolic network in many different ways, metabolism involves uptake of the substrate followed by numerous intracellular reactions. We assume that there are a total of R reactions numbered from 1 to R and represented by

$$-\sigma_i S + \sum_{j=1}^{n} \nu_{ij} M_j + \sum_{r=1}^{p} \pi_{ir} P_r = 0, \qquad i = 1, 2, \ldots, R \tag{3}$$

where S represents substrate, M_j's internal metabolites, P_r's fermentation products, and σ_i, ν_{ij}, and π_{ir} are their stoichiometric coefficients.

Mass Conservation with Steady State

The total consumption of substrate, r_s is obtained by summing the consumption rates $r_{s,i}$ of all reactions consuming substrate represented by the set U.

$$r_s = \sum_{i \in U} r_{s,i} \tag{4}$$

The assumption of pseudo-steady state for internal metabolites leads to

$$\mathbf{N}^T \mathbf{r} = \mathbf{0} \tag{5}$$

where $\mathbf{N}^T$ is the transpose of the matrix of stoichiometric coefficients $\{\nu_{ij}\}$ and $\mathbf{r}$ is the vector of all reaction rates in metabolism.

Decomposition into Elementary Modes

Elementary modes form a convex set and represent a convex basis of the null space of the matrix $\mathbf{N}^T$. They form a convex cone represented by $\left(\mathbf{N}^T \mathbf{r} = \mathbf{0};\quad r_i \geq 0, i = 1, 2, \ldots, n\right)$. Assuming m elementary modes, we represent them by reactions

$$-\sigma_i S + \sum_{j=1}^{n} \nu_{ij} M_j + \sum_{r=1}^{p} \pi_{ir} P_r = 0, \qquad i \in U_k, \qquad k = 1, 2, \ldots, m \tag{6}$$

Schuster et al. (1999, 2000) show the methodology for obtaining the reaction rate vectors in elementary modes, which are given by

$$\mathbf{r}^k \equiv \left[r_1^k, r_2^k, \ldots, r_R^k\right];\quad k = 1, 2, \ldots, m. \tag{7}$$

Growth Rate from Elementary Modes

The total uptake rate of substrate is then given by (8)

$$r_s = \sum_{k=1}^{m} w_k r_1^k$$

where w_k's are *known positive* weights. The entire reaction rate vector $\mathbf{r}$ can then be expressed in terms of the vector of substrate uptake rates, $\mathbf{r}_1^T \equiv \left[r_1^1, r_1^2, \ldots, r_1^m\right]$ given by

$$\mathbf{r} = \mathbf{Z}\mathbf{r}_1 \tag{9}$$

where the matrix $\mathbf{Z}$ is obtained from stoichiometry. The growth rate can be written in terms of the reaction rate vector as $r_G = \mathbf{h}^T\mathbf{r} = \mathbf{h}^T\mathbf{Z}\mathbf{r}_1$ in which the vector $\mathbf{h}$ is known. The crux of this work lies in assuming Michaelis-Menten kinetics for the substrate uptake rates through individual elementary modes. Thus we set

$$r_1^k = \frac{\mu_k' s}{K_k + s}, \quad k = 1, 2, \ldots, m \tag{10}$$

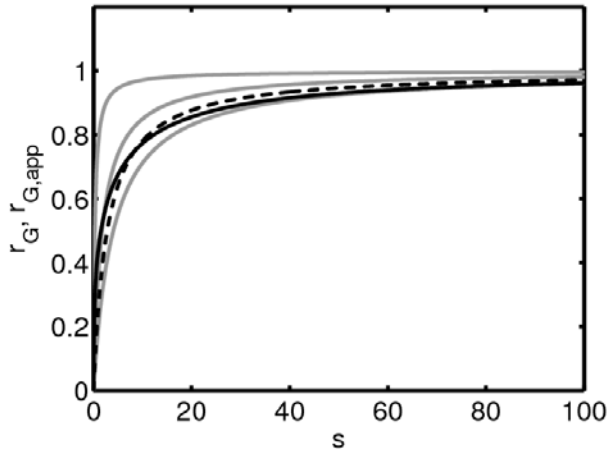
where μ_k' is the maximum uptake rate through the mode and K_k is the Michaelis constant. Clearly, the growth rate becomes

$$r_G = \sum_{k=1}^{m} \frac{\mu_k s}{K_k + s}, \qquad \mu_k \equiv \sum_i h_i z_{ik} \mu_k' \tag{11}$$

The rest of the development in this paper is to show how the sum of Michaelis-Menten expressions can be approximated by a single such expression for the growth rate thus providing a rationalization of Monod kinetics.

Comparison of Approximations

A sample of the comparison between the approximation and the sum kinetics is shown below.



The solid line in black represents the sum kinetics to be approximated, while the dotted line (with optimum values for the Michaelis-Menten kinetic constants) and the solid lines in grey represent various types of approximations with equivalent kinetic constants computed in various ways.

References

Schuster, S.; Dandekar, T.; Fell, D. A. Detection of elementary flux modes in biochemical networks: a promising tool for pathway analysis and metabolic engineering. *Trends in Biotechnol.* **1999**, *17*, 53

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