Implicit Analytic Solution of Michaelis–Menten–Monod Kinetics

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Supporting Information

ABSTRACT: An analytic solution to enzyme kinetics expressed by the Michaelis–Menten–Monod mathematical framework is presented. The analytic solution describes the implicit problem with the independent variable, normally time, substituted with the concentration of the reaction product. The analytic solution provides the substrate, enzyme, and microbial biomass concentration instantaneously and over all time domains without the use of numerical integration schemes or iterative solvers required to overcome transcendental functions. Experiments of NO$_2^-$ nitrification by Candidatus Nitrospira defluvii at temperatures ranging between 10 and 32 °C were used for validation tests with the numerical solution by finite differences and the implicit analytic solution presented here. Results showed that both finite differences and analytic solutions matched the experiments particularly well, with a correlation coefficient greater than 0.99 and residuals smaller than 2.75%.

INTRODUCTION

The Michaelis–Menten–Monod (MMM) framework is one of the most common reaction kinetics as it can describe biochemical reactions in various contexts. The MMM kinetics include only two chemical parameters, that is, the reaction rate constant $k$ and the Michaelis–Menten (affinity) constant $K$, and three biological parameters, that is, the biomass mortality rate $\delta$ and the biomass and enzyme yields $Y$ and $z$, respectively, which are combined as in the generalized equations for biochemical isotope kinetics (GEBIK$^{13}$).

An analytic solution to the Michaelis–Menten kinetics exists in an implicit form, that is, with the time expressed as a function of the substrate concentration.$^{21,24}$ and an explicit form has recently been achieved, with the use of the Lambert $W$ function.$^{23}$ Note, however, that the explicit $W$ form leads to a solving function that eventually is iterative in nature.$^{6,7}$ An analytic solution to the MMM kinetics when microbial biomass expresses a substantial effect on the reaction rate has not yet been attempted in either explicit or implicit form. The peculiarity is that two ordinary nonlinear differential equations have to be solved simultaneously in the MMM kinetics, one of which being the Michaelis–Menten equation for the chemicals and the other describing the microbial biomass Monod dynamics.

The aim of this study is to derive and test an implicit analytic solution of the coupled problem of substrate consumption according to Michaelis–Menten kinetics and biomass dynamics according to Monod kinetics. The advantage of having an analytic solution is that iterative solvers such as those required to solve transcendental functions or finite-difference methods are not involved. To test the validity of the proposed solution, the kinetic parameters were determined with a finite-difference solution and the analytic solution against the experimental data of NO$_2^-$ nitrification (oxidation) by Candidatus Nitrospira defluvii at temperatures ranging between 10 and 32 °C,$^{10}$ and reciprocity tests were carried out for the two solving schemes to measure the goodness of matching.

RESULTS AND DISCUSSION

Theory. Consider an enzymatic reaction that consumes a substrate $S$ and produces a product $P$ through an enzyme $E$. Using the 1913 Michaelis–Menten reaction framework,$^{11}$ $S$ is assumed to attach to $E$ to form an activated complex $C$ in equilibrium with $S$ and $E$, which releases $P$ and the free, unchanged enzyme $E$. During the consumption of $S$, the microbial species carrying out the reaction increases its biomass $B$ proportionally to the biomass yield coefficient $Y$, which expresses the biomass gain per mole of consumed substrate, and it decreases according to a first-order mortality rate $\delta$. Microorganisms, as a consequence of metabolic processes, produce the enzyme $E$ that feeds back the reaction as long as $S$ is available. These complexation reactions state the MMM kinetics and can be expressed as

$$S + E \xrightleftharpoons[k^-]{k^+} C \xrightarrow{k} P + E \quad (1a)$$

$$S \rightarrow B \xrightarrow{z} E \quad (1b)$$

where $k^+$ and $k^-$ are the rate constants of the forward and backward equilibrium reactions between reactants ($S$ and $E$).
and activated complex ($C^*$), and $k$ is the reaction rate constant. Here, it is assumed that the enzyme concentration is proportional to the microbial biomass by a factor $z$, that is, the enzyme is produced and degraded at the same rate as biomass growth and mortality as $E = zB$. Under these working hypotheses, the system of differential equations stating the MMM problem for $S$, $P$, $B$, and $E$ can be derived from the GEBIK equations under the quasi-steady-state assumption and take the form

$$\frac{dP}{dt} = kE(t) \frac{S(t)}{K + S(t)} \tag{2a}$$

$$\frac{dS}{dt} = -\frac{dP}{dt} \tag{2b}$$

$$\frac{dB}{dt} = Y - \frac{dP}{dt} - \delta B(t) \tag{2c}$$

$$\frac{dE}{dt} = z \frac{dB(t)}{dt} \tag{2d}$$

under the hypothesis that $k^-$ and $k^+$ are greater than $k$ and that the quasi-steady-state assumption is satisfied for $C^*$ (i.e., $dC^*/dt \approx 0$ and $C^* \approx 0$). In these circumstances, the Michaelis–Menten half-saturation concentration is $K = (k^- + k^+)/k^+$. In the system of eq 2, $S(t)$, $P(t)$, $B(t)$, and $E(t)$ are explicit functions of the independent variable time $t$. Note also that eq 2a and c are independent, whereas eq 2b is equivalent to eq 2a with the opposite sign and eq 2d is proportional to eq 2c by a factor $z$. For the solution proposed here, it is convenient to consider $P$ as the independent variable and to express $S(P)$, $B(P)$, $E(P)$, and $t(P)$ as functions of $P$. To this aim, eq 2a,c is rewritten as

$$\frac{dr(P)}{dP} = \left( \frac{kzB(P)}{S_0 - P} \right)^{-1} \tag{3a}$$

$$\frac{dB(P)}{dP} = Y - \delta B(P) \frac{dr(P)}{dP} \tag{3b}$$

where $S(t)$ is substituted by $S(P) = S_0 - P$ as well as the initial condition $S_0 = S(P)$ when $P = P_0 = 0$.

The analytic solution to eq 3b is (see Appendix A)

$$B(P) = B_0 + \left( Y - \frac{\delta}{zk} \right) P + \frac{\delta K}{zk} \ln \left( 1 - \frac{P}{S_0} \right) \tag{4}$$

with the initial biomass concentration $B_0 = B(P)$ when $P = 0$.

Equation 4 includes a logarithm of $P$ that, propagated into the system of eq 3, would lead to a transcendental problem. To circumvent this without the use of iterative solvers of transcendental functions either in their naked form or in the form of Lambert $W$ function, a polynomial Taylor expansion to the second-order in $P$ is used for $B(P)$ in eq 3a as

$$B(P) = B_0 + \left( Y - \frac{\delta}{zk} \right) P + \frac{\delta K}{2zkS_0} P^2 + O(P^3) \tag{5}$$

Equation 5 is valid as long as $B(P) > 0$; excluding values $P < 0$, this condition is satisfied if

$$0 \leq P < \frac{G + \sqrt{G^2 + j}}{2\delta K} \tag{6}$$

with $G = 2S_0(zkYS_0 - \delta S_0 - \delta K)$ and $J = 8zkB_0 \delta K S_0^2$. Note that $\lim dP/dt = \infty$ implies $t \to \infty$ as in the asymptotic approach to equilibrium of eqs 2 and is a case that can be excluded for practical applications. Substituting eq 5 into eq 3a and solving $t(P)$ with the initial value $t(P = 0) = 0$, one obtains the implicit solution for $t(P)$ as (see Appendix B)

$$t(P) = F \arctan \left( \frac{2HP + M}{\sqrt{-Q}} \right) - F \arctan \left( \frac{M}{\sqrt{-Q}} \right) - \frac{K}{2N'} \ln \left( \frac{(S_0 - P)^2}{HP^2 + MP + N} + \frac{K}{2N'} \ln \left( \frac{S_0^2}{N} \right) \right) \tag{7}$$

or, equivalently,

$$t(P) = -\frac{P'}{2} \ln \left( \frac{\sqrt{Q + 2HP + M}}{\sqrt{Q - 2HP - M}} \right) - \frac{K}{2N'} \ln \left( \frac{(S_0 - P)^2}{HP^2 + MP + N} + \frac{P'}{2} \ln \left( \frac{\sqrt{Q} + M}{\sqrt{Q} - M} \right) - \frac{K}{2N'} \ln \left( \frac{S_0^2}{N} \right) \right) \tag{8}$$

with the constants

$$H = -\frac{\delta K}{2S_0^2} < 0 \tag{9a}$$

$$M = kzY - \delta - \frac{\delta K}{S_0} N = kzB_0 > 0 \tag{9b}$$

$$M' = -(M + 2HS_0) N' = N + MS_0 + HS_0^2 \neq 0 \tag{9c}$$

$$-Q = 4HN - M^2 \neq 0 \tag{9d}$$

$$F = \frac{2}{\sqrt{-Q}} - \frac{KM'}{N'\sqrt{-Q}} \tag{9e}$$

$$F' = \frac{2}{\sqrt{Q}} - \frac{KM'}{N'\sqrt{Q}} \tag{9f}$$

under the condition

$$0 \leq P < \frac{-M - \sqrt{Q}}{2H} \tag{10}$$

Equations 4 and 8 represent the implicit analytic solutions for $B$ and $t$ as functions of $P$. The solutions for $S$ and $E$ can then be calculated as

$$S(P) = S_0 - P \tag{11}$$

$$E(P) = E_0 + zB(P) \tag{12}$$

with $B(P)$ as in eq 4.

**Methods of Testing.** To test the application of the implicit analytic solution of the MMM problem given by eqs 4, 8, 11, and 12, independent data sets of NO$_2^-$ nitration by C. N. deflavi at six temperatures between 10 and 32 °C were used. In using these experiments, O$_2$ and carbon sources were assumed to be not limiting.

The unknown parameters in the MMM kinetics (i.e., $k$, $K$, $Y$, and $B_0$) were determined by solving the inverse problem stated by eq 2 with an explicit finite-difference numerical scheme and with the implicit analytic solution proposed here against the
Table 1. Kinetic Parameters Estimated against the Experimental Data of NO$_2^-$ Nitrification by C. N. defluyii Using the Explicit Numerical Solution (Columns 2–5) and Implicit Analytic Solution (Columns 6–9)

| $T$ (°C) | k (1/s) | $K$ (μM) | $Y$ (g/mol) | $B_0$ (mg/L) | k (1/s) | $K$ (μM) | $Y$ (g/mol) | $B_0$ (mg/L) |
|----------|---------|----------|-------------|-------------|---------|----------|-------------|-------------|
| 10       | 1.48    | 38.59    | 10.45       | 0.415       | 1.59    | 37.86    | 9.69        | 0.392       |
| 15       | 1.49    | 16.33    | 10.45       | 0.931       | 1.54    | 13.24    | 9.69        | 0.950       |
| 17       | 2.13    | 7.11     | 10.45       | 1.004       | 1.90    | 7.89     | 9.69        | 1.466       |
| 22       | 2.50    | 39.71    | 10.45       | 0.926       | 2.65    | 38.33    | 9.69        | 0.891       |
| 28       | 8.41    | 384.92   | 10.45       | 0.354       | 8.70    | 374.46   | 9.69        | 0.355       |
| 32       | 22.10   | 848.00   | 10.45       | 0.309       | 22.76   | 824.07   | 9.69        | 0.309       |

| $T$ (°C) | R | NRMSD (%) | R | NRMSD (%) |
|----------|---|-----------|---|-----------|
| 10       | >0.99 | 2.19      | >0.99 | 2.19      |
| 15       | >0.99 | 1.25      | >0.99 | 1.32      |
| 17       | >0.99 | 1.24      | >0.99 | 2.75      |
| 22       | >0.99 | 0.63      | >0.99 | 0.59      |
| 28       | >0.99 | 0.54      | >0.99 | 0.44      |
| 32       | >0.99 | 0.46      | >0.99 | 0.32      |

Experimental data using the nonlinear least-square fitting algorithm implemented in PEST. The biomass mortality rate $\delta = 10^{-6} \text{s}^{-1}$ (refs 6 and 22) and enzyme yield coefficient $z = 10^{-16} \text{mol mg}^{-1}$, which was estimated assuming that 1% of the microbial biomass with an enzyme having $10^5 \text{g mol}^{-1}$ molar mass (i.e., 100 kDa = $1.66 \times 10^{-19} \text{g mol}^{-1}$) were used. In contrast to $k$ and $K$, the biomass yield $Y$ was assumed not to change with the temperature; hence, only one value was estimated and used at each experimental temperature.

For calculation of the numerical solution, an explicit finite-difference technique was implemented. The integration time step $dt = 0.5 \text{h}$ was used and verified to provide stable solution at all experimental temperatures. For calculation of the analytic solution, the constants in eq 9 were first determined (note that they depend only on the parameters $k$, $K$, and $Y$ and on the initial conditions $S_0$ and $B_0$) and then were used in eqs 4, 8, 11, and 12 with values of $P \in [0,1/(1-\epsilon)S_0]$, and with $\epsilon \ll 1$, as an arbitrary residual, to retrieve the time $t(P)$ at which $P$, $S(P)$, $B(P)$, and $E(P)$ occurred. The goodness of fit for both solving schemes against the experiments was measured with the correlation coefficient $R$ and normalized root-mean-square distance (NRMSD)

$$R = \frac{\sigma_{XY}}{\sigma_X \sigma_Y}$$

$$\text{NRMSD} = \left( \frac{1}{n} \sum_{i=1}^{n} (X_i - Y_i)^2 \right)^{1/2} \max(Y) - \min(Y)$$

where $X$ and $Y$ represent the modeled and experimental NO$_2^-$ concentrations over time, respectively, and $n$ is the number of experimental points.

The two sets of kinetic parameters were then used to cross-validate the implicit analytic solution provided by eqs 4, 8, 11, and 12 in reciprocity tests, and $R$ and NRMSD were used in this case to quantify the goodness of fit of the two solving schemes against each other.

**Experimentally Derived Kinetic Parameters.** Parameters estimated against experiments using the explicit numerical solution and implicit analytic solution are listed in Table 1.

The analytic solution calculated for the independent variable $P \in [0,1/(1-10^{-10})S_0]$ and with $S_0$ as per experiments was tested against the condition in eq 6 to verify its validity using parameters and initial conditions given in Table 1 at each experimental temperature. The upper boundary to $P$ in eq 6 turned out to be $(G + \sqrt{G^2 + J})/26K \approx 10^{-2} \text{mol/L}$, whereas the maximum $P$ used in the analytic solution was $P = (1 - 10^{-5})S_0 \approx 10^{-4} \text{mol/L}$ in all temperature ranges, thus eq 6 was always satisfied. Analogously, tests against the condition in eq 10 show that the upper boundary $(-M - \sqrt{Q})/2H$ ranged between $10^{-2}$ and $10^{-1} \text{mol/L}$, thus the used $P$ values also satisfied the condition given in eq 10. The testing approach, therefore, always satisfied the conditions for $P$ and allowed computation of the implicit solution of $t, S, B,$ and $E$.

Corresponding parameters determined with the two solving schemes were found to be close to each other at all tested temperatures, with variability $|\Delta t|/\bar{t}$ for each parameter $t$ ranging between 0.48% and 5.22% for kinetic parameters ($k$ and $K$) and between 0.01% and 1.9% for biological parameters ($Y$ and $B_0$). Note that the product $zY = \mu$ corresponds to the maximum specific biomass growth rate used in MMR kinetics when the enzyme dynamics is not explicitly accounted for and reflects typical values of similar biochemical $N$ reactions by soil and aquatic microorganisms found in earlier studies using numerical solutions, that is: $\mu = zY \approx 10^{-6}$ $1/\text{s}$ and $Y \approx 10^4 \text{mg/mol}$. Note also that these parameters were found to show a temperature dependence that aligns, at least over a limited temperature range, to an Arrhenius-like law, as already documented in earlier studies in ref 14 based on experiments in refs 3 15, and 16.

The explicit numerical solution matched experiments with a goodness of fit $R > 0.99$ and NRMSD $< 2.2%$ (Table 2).

**Table 2. Goodness of Fit of the Explicit Numerical Solution and Implicit Analytic Solution against the Experimental Concentration of NO$_2^-$ during Nitrification by C. N. defluyii at Various Temperatures for the Parameter Sets in Table 1**

| $T$ (°C) | R   | NRMSD (%) | R   | NRMSD (%) |
|----------|-----|-----------|-----|-----------|
| 10       | >0.99 | 2.19      | >0.99 | 2.19      |
| 15       | >0.99 | 1.25      | >0.99 | 1.32      |
| 17       | >0.99 | 1.24      | >0.99 | 2.75      |
| 22       | >0.99 | 0.63      | >0.99 | 0.59      |
| 28       | >0.99 | 0.54      | >0.99 | 0.44      |
| 32       | >0.99 | 0.46      | >0.99 | 0.32      |

Columns 2 and 3); the implicit analytic solution achieved a goodness of fit $R > 0.99$ and NRMSD $< 2.75%$ (Table 2, columns 4 and 5). Trends in parameter values and in goodness of fit were consistent in both solving schemes at the tested temperatures.

**Implicit Analytic Solution and Explicit Numerical Solution.** Along with the goodness of fit summarized in Table 2, visual inspection of NO$_2^-$ and $B$ concentrations in...
Figure 1 shows that absolute values and trends in NO$_2^-$ and $B$ concentrations calculated with the two solving schemes reflected those of experiments at all tested temperatures.

**Solution Reciprocity.** MMM kinetics obtained with independent parameter estimations in Figure 1 achieved a reciprocal matching quantified by NRMSD <2.7% in NO$_2^-$ and NRMSD <9.1% in $B$ (Table 3, columns 2 and 3). Reciprocal use of the best parameter set for one solving scheme with the other schemes resulted in NRMSD <0.7% in NO$_2^-$ and NRMSD <5.5% in $B$ (Table 3, columns 4–7). Reciprocity tests presented here suggest, therefore, that differences in solutions were small and within a typical experimental uncertainty of 5–10%. Results also suggest that either the explicit numerical solution or the implicit analytic solution can be used for the purposes of calibration of kinetic and biological parameters.

**CONCLUSIONS**

An experimentally and numerically validated implicit analytic solution to the MMM kinetics was presented. The analytic solution produced calculated values of substrate, product, and biomass concentration substantially equivalent to the numerical solution obtained by finite differences and observed to be stable and computable within its mathematical domain. This approach has the advantage of allowing fast calculation without the need to introduce numerical iterative solvers such as those used in the computation of transcendental functions. Additionally, it does not require validation of time integration (e.g., Courant criterion) such as in implicit and explicit finite-difference methods. The analytic solution proposed here can be used in the estimation of kinetic parameters from experimental and laboratory biochemical assays of nutrient and substrate consumption, but it may be particularly suited for application to large-scale reactive transport models used in climate and earth sciences, which may account for a large number of kinetic reactions over dense computational meshes. Implementations into large-scale computational tools, however, may have to be investigated further depending on the numerical schemes used.
to solve over a finite-time-step multiple processes occurring at a range of temporal and spatial scales.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.6b00174. Appendix A: Analytic solution for the microbial biomass dynamics described by the Monod kinetics. Appendix B: Analytic solution for the dynamics of chemicals described by the Michaelis–Menten kinetics under the quasi-steady-state assumption. Appendix C: Matlab2011b script of analytic solution used to generate Figure 1 (PDF)

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**Notes**

The authors declare no competing financial interest.

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