A Combination Formula of Michaelis-Menten-Monod Type

E. BERTOLAZZI
Dip. Ingegneria Meccanica e Strutturale
Università di Trento
via Mesiano 77, Trento, Italy

Abstract—The purpose of this paper is to present a general formula for the growth rate of unicellular microorganisms. A generalization of the Michaelis-Menten-Monod formula for the growth rate of microorganisms is derived and discussed. If nutrients and inhibitors are considered as limiting substances and assumed to be noninteracting, this generalized formula works for more than one limiting substance. Both competitive and noncompetitive inhibitors are included. This general form of Michaelis-Menten law is analytically derived from a few basic hypotheses, it yields both multiplicative and minimum formulas as limiting cases © 2005 Elsevier Ltd. All rights reserved

Keywords—Michaelis-Menten, Monod, Competitive inhibitor, Noncompetitive inhibitor, Huxley's allometric law

1. INTRODUCTION

The growth of microorganisms may be described by differential equations of the form [1–3]

$$\frac{dP}{dt} = G_{max}GP + (\text{other terms}),$$

where $P = P(t)$ stands for the microorganisms mass and the function $G = G(S_1, \ldots, S_n)$, $(0 \le G \le 1)$ describes the effects of generic limiting substrates. This formulation is the well-known extension to microorganisms growth of Michaelis-Menten enzyme kinetic [4]. Mathematically the effect of a single nutrient substrate $A = S_1$ is expressed by the Michaelis-Menten-Monod law (shortened as MMM)

$$G(A) = \frac{A}{K + A}. \tag{1}$$

Similarly, the effect of a single inhibitor substrate, $B = S_1$, is expressed by

$$G(B) = \frac{L}{L + B}, \tag{2}$$

I am deeply indebted to G. Pecenik who provided many hours of helpful advice and assistance on the biological aspects of this paper

where K and L are the half-saturation and inhibition constant, respectively. Formulas (1) and (2) may be used only when the limiting function G depends on a single substrate, but there is not a unique generalization when G depends on multiple substrate. Possible interactions for substrates are usually described by combination law. For example, common generalizations for formula (1) in presence of two substrates A_1 and A_2 fall into one of these formulations [1,5].

(I) Minimum

$$G^{\mathrm{I}}(A_1, A_2) = \min\left\{\frac{A_1}{K_1 + A_1}, \frac{A_2}{K_2 + A_2}\right\}.$$

(II) Multiplicative

$$G^{\mathrm{II}}(A_1, A_2) = \frac{A_1}{K_1 + A_1} \times \frac{A_2}{K_2 + A_2}.$$

(III) Harmonic mean

$$G^{\mathrm{III}}(A_1, A_2) = 2\left(\frac{K_1 + A_1}{A_1} + \frac{K_2 + A_2}{A_2}\right)^{-1} = 2\left(2 + \frac{K_1}{A_1} + \frac{K_2}{A_2}\right)^{-1}.$$

(IV) Simple average

$$G^{\mathrm{IV}}(A_1, A_2) = \frac{1}{2}\left(\frac{A_1}{K_1 + A_1} + \frac{A_2}{K_2 + A_2}\right).$$

Similar generalizations correspond to formula (2).

Evidence for the multiplicative model has been brought forward by Baule [6] and O'Brian [7], while the minimum or threshold model better described the experimental results of Droop [8] and Rhee [9]

Formulations (I)–(IV) are derived by some intuitive approach; for example (I) reflects the well-known Liebig's minimum principle. The adoption of one of these formulations instead of the other clearly depends on the type of system under investigation The multiplicative model is more appropriate when dealing with microbiological population exhibiting age structure and physiological conditions changing in function of the growth stage [10]. The minimum model better reflects instead the growth features for specialized synchronous populations.

Formulations (I)–(IV) share some properties. First, if the concentration of substrates A_1 and A_2 saturates, i.e., they take the limit value ∞, then all the growth rates (I)–(IV) are maximum, i.e., take the value 1 This property shared by all (I)–(IV) will be referred to as *Property a* in what follows.

Second, if the concentration of nutrient A_2 takes the limit value ∞, then the single substrate limitation due to A_1 takes the form

$$G^{\mathrm{I}}(A_1, \infty) = G^{\mathrm{II}}(A_1, \infty) = \frac{A_1}{K_1 + A_1} \tag{3}$$

only for (I) and (II), while for (III) one has

$$G^{\mathrm{III}}(A_1, \infty) = \frac{A_1}{K_1/2 + A_1}$$

and for (IV) one has

$$G^{\mathrm{IV}}(A_1, \infty) = \frac{1}{2}\left(\frac{A_1}{K_1 + A_1} + 1\right)$$

This means that in (III) the half-saturation constant changes, while (IV) does not follow the MMM law any more. The property expressed by formula (3) will be referred to as *Property b*.

Third, if the concentration of A_2 is kept fixed, (II) becomes

$$G^{\mathrm{II}}(A_1, A_2) = G^{\mathrm{II}}_{\max}(A_2)\frac{A_1}{K_1 + A_1}, \qquad G^{\mathrm{II}}_{\max}(A_2) = \frac{A_2}{K_2 + A_2}, \tag{4}$$

while (III) becomes

$$G^{\mathrm{III}}(A_1, A_2) = G_{\max}^{\mathrm{III}}(A_2) \frac{A_1}{K(A_2) + A_1},$$

$$G_{\max}^{\mathrm{III}}(A_2) = \frac{2}{2 + K_2/A_2}, \qquad K(A_2) = K_1 \left(2 + \frac{K_2}{A_2} \right). \tag{5}$$

Thus, (4) and (5) are again an MMM growth rate, while under the same Conditions (I) and (IV) lose their MMM character. This property will be referred to as *Property c*.

Fourth, regarding $a = A_1 + A_2$ as a unique substrate where the relative concentrations of A_1 and A_2 are kept fixed with respect to a, and setting

$$A_1^0 = \frac{A_1}{A_1 + A_2}, \qquad A_2^0 = \frac{A_2}{A_1 + A_2},$$

one has $A_1 = aA_1^0$ and $A_2 = aA_2^0$. Then (I) in terms of a becomes

$$G^{\mathrm{I}}\left(aA_1^0, aA_2^0\right) = \begin{cases} \dfrac{a}{K_1/A_1^0 + a}, & \text{if } \dfrac{K_2}{A_2^0} < \dfrac{K_1}{A_1^0}, \\[2mm] \dfrac{a}{K_2/A_2^0 + a}, & \text{otherwise,} \end{cases} \tag{6}$$

and (II) becomes

$$G^{\mathrm{II}}\left(aA_1^0, aA_2^0\right) = \frac{a}{(1/2)\left(K_1/A_1^0 + K_2/A_2^0\right) + a}. \tag{7}$$

Thus, (6) and (7) are again an MMM growth rate but this does not happen with (II) and (IV). This property will be referred to as *Property d*. The four properties are resumed as follows:

Property *a*: $G(\infty, \infty) = 1,$

Property *b*: $G(A_1, \infty) = \dfrac{A_1}{K_1 + A_1},$

$\qquad\qquad\quad G(\infty, A_2) = \dfrac{A_2}{K_2 + A_2},$

Property *c*: $G(A_1, A_2) = G(\infty, A_2) \dfrac{A_1}{K_1(A_2) + A_1},$

$\qquad\qquad\quad G(A_1, A_2) = G(A_1, \infty) \dfrac{A_2}{K_2(A_1) + A_2},$

Property *d*: $G(tA_1, tA_2) = \dfrac{t}{K(A_1, A_2) + t}.$

In words Property *a* means that, in presence of excess of all substrates, the growth rate takes its maximum value. Property *b* means that if only one substrate is in excess an MMM is still respected. Property *c* guarantees that an MMM law is respected even when all substrates are limiting. Property *d* means that the growth rate is an MMM law depending on the concentration of mixture of all substrates. These facts are summarized in Table 1 Analogous property follows for MMM law for inhibitors by noticing that setting $K_i = 1/L_i$ and $A_i = 1/B_i$, equation (2) becomes (1)

Table 1. Comparison between combination laws

	Property *a*	Property *b*	Property *c*	Property *d*
Minimum	yes	yes	no	yes
Multiplicative	yes	yes	yes	no
Harmonic Mean	yes	yes	no	yes
Simple Average	yes	no	no	no

Additional bibliography, methodological topics on model building, as well as alternative mathematical structure (toward modelling) on the dynamics of multicellular organisms (and large biological systems) can be recovered in the book by Diekmann and Heesterbeek [11] as in the review paper by Bellomo et al. [12].

2. PURPOSE OF THE PAPER

If Formulations (I)–(IV) represent a combination law generalizing the MMM law, they should conserve the MMM properties in some mathematical sense. Formulations (I)–(IV) are not true generalization of the original MMM law, because restriction in a linear one-dimensional subspace through the origin implies the loss the original MMM shape. The purpose of this paper is to prove that a unique generalization of Michaelis-Menten-Monod law exists and has the general form

$$G(A_1, \ldots, A_n, B_1, \ldots, B_n) = \frac{1}{1 + \sum\limits_{i=1}^{n} (K_i/A_i + (B_i/L_i)(\ell_i + K_i/A_i))},$$

where ℓ_i is 0 if B_i is a competitive inhibitor while ℓ_i is 1 if B_i is a noncompetitive inhibitor, and K_i and L_i are the half-saturation and inhibition constant of A_i and B_i, respectively.

The paper is organized as follows: in Section 3 the growth rate is derived without considering inhibitors contribution. In Section 4 growth rate is extended to consider inhibitors contributions. In Section 5 an analytic model is used to derive the same formula. In Section 6 the proposed growth rate is compared with multiplicative and minimum formulations. Final remarks and conclusions are offered in Section 7.

3. THE GROWTH RATE WITHOUT INHIBITORS

In this section, we assume that inhibitors are not present. The more general case with inhibitors will be considered later.

Suppose that G, the growth rate, depends on n substrates denoted by $A_1, A_2, \ldots, A_n$ acting as nutrients. G is then a function of n variables

$$(A_1, A_2, \ldots, A_n) \mapsto G(A_1, A_2, \ldots, A_n),$$
$$\mathbb{R}_+^n \mapsto [0, 1].$$

Now, we postulate the following requirement.

(A) The function G and the function $\hat{G}$

$$\hat{G}(A_1, A_2, \ldots, A_n) = G\left(\frac{1}{A_1}, \frac{1}{A_2}, \ldots, \frac{1}{A_n}\right), \qquad \forall A_k \in \mathbb{R}_+,$$

are continuous functions. This means that the limit value $G(\infty, A_2, \ldots, A_n)$ exists and it is defined as

$$G(\infty, A_2, \ldots, A_n) = \lim_{A_1 \mapsto \infty} G(A_1, A_2, \ldots, A_n).$$

By the same token, the limit values for multiple substrate are recursively defined as

$$G(\infty, \infty, A_3, \ldots, A_n) = \lim_{A_1, A_2 \mapsto \infty} G(A_1, A_2, \ldots, A_n)$$
$$= \lim_{A_2 \mapsto \infty} \lim_{A_1 \mapsto \infty} G(A_1, A_2, \ldots, A_n)$$
$$= \lim_{A_1 \mapsto \infty} \lim_{A_2 \mapsto \infty} G(A_1, A_2, \ldots, A_n),$$

and by continuity of $\hat{G}$ the limiting procedure is independent of the order. These limits reflect saturation phenomena and imply that saturation values are independent of how this saturation is attained.

(B) When only A_j concentration is allowed to vary, G is considered to be a function of only one variable

$$f(z) = G(\ldots, A_{j-1}, z, A_{j+1}, \ldots),$$

and it must follow the MMM law

$$f(z) = C\frac{z}{K+z},$$

where both K and C are independent of z. Obviously, C must assume the value $f(\infty)$. Thus, $f(z)$ becomes

$$f(z) = \frac{f(\infty)z}{K+z}.$$

By denoting with $K_j(\ldots, A_{j-1}, A_{j+1}, \ldots)$ the half-saturation constant of function $f(z)$ and by changing z to A_j the requirement becomes

$$G(A_1, A_2, \ldots, A_n) = \frac{G(\ldots, A_{j-1}, \infty, A_{j+1}, \ldots)A_j}{K_j(\ldots, A_{j-1}, A_{j+1}, \ldots) + A_j}.$$

(C) Consider now a mixture, made up of substrates in a fixed proportion, which acts itself as a substrate. By setting

$$A_j^0 = \frac{A_j}{A_1 + A_2 + \cdots + A_n},$$

for this mixture, it follows that $A_j^0 = $ constant and consequently A_j must satisfy

$$A_j = aA_j^0, \qquad j = 1, 2, \ldots, n$$

The requirement for G is the same as for equation (1), i.e.,

$$G\left(aA_1^0, aA_2^0, \ldots, aA_n^0\right) = \frac{a}{K\left(A_1^0, A_2^0, \ldots, A_n^0\right) + a},$$

for all $a \geq 0$, where $K(A_1^0, A_2^0, \ldots, A_n^0)$ becomes the half-saturation constant of the mixture $A_1^0, A_2^0, \ldots, A_n^0$. Again,

$$\sum_{j=1}^{n} A_j = a\sum_{j=1}^{n} A_j^0 = a,$$

because $1 = A_1^0 + \cdots + A_n^0$, and a becomes the concentration of the mixture.

REMARK 1. Assumption (B) is a generalization of Property (c) and Assumption (C) is a generalization of Property (d). From Assumptions (A)–(C), if all the substrates take the limit value ∞, the growth rate attains its maximum value

$$G(\infty, \infty, \ldots, \infty) = 1,$$

and this is a generalization of Property (a). The generalization of Property (b) is obtained from (A)–(C) if all but one substrate take the limit value ∞, G is expressed by

$$G(\ldots, \infty, A_j, \infty, \ldots) = \frac{A_j}{K_j + A_j}, \qquad j = 1, 2, \ldots, n,$$

where $K_j = K_j(\infty, \ldots, \infty)$ are the half-saturation constants of the substrate A_j.

The general form for G satisfying Requirements (A)–(C) is now derived.

THEOREM 1. *The function $G \in C^1(\mathbb{R}_+^n)$ which satisfies Properties (A)–(C) exists, is unique, and is given by*

$$G(A_1, A_2, \ldots, A_n) = \frac{1}{1 + K_1/A_1 + K_2/A_2 + \cdots + K_n/A_n}. \tag{8}$$

PROOF. From Requirement (C), the function $H = 1/G - 1$ becomes

$$H(A_1, A_2, \ldots, A_n) = \frac{K\left(A_1^0, A_2^0, \cdots, A_n^0\right)}{A_1 + A_2 + \cdots + A_n}, \tag{9}$$

and from Requirement (B) it follows that

$$H(A_1, A_2, \ldots, A_n) = H(\ldots, A_{j-1}, \infty, A_{j+1}, \cdots) \\ + \frac{K_j(\ldots, A_{j-1}, A_{j+1}, \ldots)\left(H(\ldots, A_{j-1}, \infty, A_{j+1}, \ldots) + 1\right)}{A_j}.$$

Saturating the first $j - 1$ nutrients (i.e., $A_i \mapsto \infty$)

$$H(\infty, \ldots, \infty, A_j, \ldots, A_n) = H(\infty, \ldots, \infty, A_{j+1}, \ldots, A_n) \\ + \frac{K_j(\infty, \ldots, \infty, A_{j+1}, \ldots, A_n)(H(\infty, \ldots, \infty, A_{j+1}, \ldots, A_n) + 1)}{A_j}, \tag{10}$$

and by setting

$$g_j(A_{j+1}, \ldots, A_n) = K_j(\infty, \ldots, \infty, A_{j+1}, \ldots, A_n)(H(\infty, \ldots, \infty, A_{j+1}, \ldots, A_n) + 1),$$

we can write

$$H(A_1, \ldots, A_n) = \frac{g_1(A_2, \ldots, A_n)}{A_1} + \frac{g_2(A_3, \ldots, A_n)}{A_2} + \cdots + \frac{g_{n-1}(A_n)}{A_{n-1}} + \frac{g_n}{A_n} \tag{11}$$

where (10) is repeatedly used and $H(\infty, \infty, \ldots, \infty) = 0$ from Remark 1 was used. Multiplying all A_is in (9) by the same λ results in

$$H(\lambda A_1, \lambda A_2, \ldots, \lambda A_n) = \lambda^{-1} H(A_1, A_2, \ldots, A_n),$$

so H is a homogeneous function of degree -1 and, by Euler's theorem on homogeneous functions [13], it must satisfy the following differential equation·

$$-H = \frac{\partial H}{\partial A_1} A_1 + \frac{\partial H}{\partial A_2} A_2 + \cdots + \frac{\partial H}{\partial A_n} A_n. \tag{12}$$

By combining (12) with (11) the following expression holds:

$$0 = \underbrace{\frac{\sum_{i=2}^{n} \frac{\partial g_1}{\partial A_i} A_i}{A_1}}_{(1)} + \underbrace{\frac{\sum_{i=3}^{n} \frac{\partial g_2}{\partial A_i} A_i}{A_2}}_{(2)} + \cdots + \underbrace{\frac{\sum_{i=n-1}^{n} \frac{\partial g_{n-1}}{\partial A_i} A_i}{A_{n-2}}}_{(n-2)} + \cdots + \underbrace{\frac{\frac{\partial g_{n-1}}{\partial A_n} A_n}{A_{n-1}}}_{(n-1)}.$$

Observe that the numerator of fraction 1 and all the fractions from 2 to $n - 1$ are independent of A_1 so that

$$\sum_{i=2}^{n} \frac{\partial g_1}{\partial A_i} A_i = 0,$$

and by repeatedly applying this argument it follows that

$$\sum_{i=k+1}^{n} \frac{\partial g_k}{\partial A_i} A_i = 0, \qquad \text{for } k = 1, 2, \ldots, n,$$

so $g_1, g_2, \ldots, g_n$ are homogeneous functions of degree 0. Then

$$g_k(A_{k+1}, \ldots, A_n) = g_k(\lambda A_{k+1}, \ldots, \lambda A_n),$$

and the only issue to prove is that the limit

$$\lim_{\lambda \to \infty} g_k(\lambda A_{k+1}, \ldots, \lambda A_n)$$

exists and is independent from $A_1, A_2, \ldots, A_n$. From (11)

$$A_k H(A_1, \ldots, A_n) = \frac{g_1(A_2, \ldots, A_n) A_k}{A_1} + \frac{g_2(A_3, \ldots, A_n) A_k}{A_2} + \cdots + \frac{g_{n-1}(A_n) A_k}{A_{n-1}} + \frac{g_n A_k}{A_n},$$

and by setting all $A_i's = \infty$ but A_k, by using Requirement (B) it follows

$$g_k(\infty, \ldots, \infty) = A_k H(\ldots, \infty, A_k, \infty, \ldots),$$

$$= A_k \left(\frac{1}{G(\ldots, \infty, A_k, \infty, \ldots)} - 1 \right),$$

$$= K_k, \qquad k = 1, 2, \ldots, n,$$

so equation (8) is obtained

4. THE GROWTH RATE WITH INHIBITORS

Let $G(B_1, B_2, \ldots, B_n)$ be the limiting function for n inhibitors. Now, since inhibitors are assumed to be noninteracting and nutrients are assumed to be fixed, G is required to satisfy the following conditions similar to Conditions (A)–(C).

(A′) The function G and the function $\hat{G}$

$$\hat{G}(B_1, B_2, \ldots, B_n) = G\left(\frac{1}{B_1}, \frac{1}{B_2}, \ldots, \frac{1}{B_n} \right)$$

are continuous functions.

(B′) When only B_j concentration is allowed to vary,

$$G(B_1, \ldots, B_n) = \frac{G(\ldots, B_{j-1}, 0, B_{j+1}, \ldots) L_j(\ldots, B_{j-1}, B_{j+1}, \ldots)}{L_j(\ldots, B_{j-1}, B_{j+1}, \ldots) + B_j}.$$

(C′) By setting $B_j^0 = B_j/(B_1 + \cdots + B_n)$ we have

$$G\left(b B_1^0, b B_2^0, \ldots, b B_n^0 \right) = \frac{L\left(B_1^0, B_2^0, \ldots, B_n^0 \right)}{L\left(B_1^0, B_2^0, \ldots, B_n^0 \right) + b},$$

for all $b \geq 0$.

Now we consider a complete growth function $G(A_1, A_2, \ldots, A_n, B_1, B_2, \ldots, B_n)$ and set up a first problem which characterizes MMM growth rate. Here we consider inhibitors that affect growth rate either by modifying the half-saturation constants or by modifying the maximum growth rate. The first type of inhibitors is referred to as *competitive*, the second type as *noncompetitive* [1,2,14]. Growth rate for a competitive inhibitor takes the form

$$G(A, B) = \frac{1}{1 + K/A + (K/L)(B/A)}. \tag{13}$$

Growth rate for a noncompetitive inhibitor takes the form

$$G(A, B) = \frac{1}{1 + K/A + B/L + (K/L)(B/A)} \tag{14}$$

For details on growth rates (13) and (14) refers to references [1,2,14].

PROBLEM 1. *Find $G \in C^1(\mathbb{R}^{2n}_+)$ such that·*

P1. *Whenever $B_1, \ldots, B_n$ are kept fixed the function*

$$G\frac{(A_1, \ldots, A_n, B_1, \ldots, B_n)}{G(\infty, \ldots, \infty, B_1, \quad , B_n)}$$

satisfies Conditions (A)–(C)

P2. *Whenever $A_1, \ldots, A_n$ are kept fixed the function*

$$\frac{G(A_1, \ldots, A_n, B_1, \ldots, B_n)}{G(A_1, \ldots, A_n, 0, \ldots, 0)}$$

satisfies Conditions (A')–(C').

P3. $G(\infty, \ldots, \infty, 0, \ldots, 0) = 1.$

Property P1 means that when inhibitors are fixed, the growth rate is an MMM law. Similarly, P2 means that when nutrients are fixed, the growth rate is an MMM law. Property P3 means that when no inhibitors are present and nutrients are abundant the growth is maximum.

With these properties it is possible to characterize the growth rate.

LEMMA 1. *All the functions $G \in C^1(\mathbb{R}^{2n}_+)$ that solve Problem 1 are given by*

$$G(A_1, \ldots, A_n, B_1, \ldots, B_n) = \frac{1}{1 + \sum_{i=1}^{n}(K_i/A_i) + \sum_{i=1}^{n}\ell_i(B_i/L_i) + \sum_{i,j=1}^{n}\alpha_{ij}(B_j/A_i)}, \quad (15)$$

where α_{ij} are independent of $A_1, A_2, \ldots, A_n$ and $B_1, B_2, \ldots, B_n$. Moreover $\ell_j = 0$ if B_j is a competitive inhibitor while $\ell_j = 1$ if B_j is a noncompetitive inhibitor, K_i and L_j are the half-saturation and inhibition constant of A_i and B_j, respectively.

PROOF. By using Property P1 from Theorem 1 it follows

$$G(A_1, \quad , A_n, B_1, \ldots, B_n) = \frac{G(\infty, \ldots, \infty, B_1, \ldots, B_n)}{1 + \sum_{i=1}^{n}(K_i(B_1, \ldots, B_n)/A_i)} \quad (16)$$

By using Property P3 and by taking $K_j = 1/L_j$ and $A_j = 1/B_j$ and noticing that if B_i is a competitive inhibitor, then we can use Theorem 1 to prove

$$G(\infty, \ldots, \infty, B_1, \ldots, B_n) = \left(1 + \sum_{i=1}^{n}\ell_i\frac{B_i}{L_i}\right)^{-1}, \quad (17)$$

since reciprocal transformation exchanges the role of inhibitors and nutrients, and transforms Conditions (A')–(C') into Conditions (A)–(C). $G(\infty, \ldots, \infty, B_1, \ldots, B_n)$ do not depend on B_i. Substituting (17) in (16) we have

$$G(A_1, \quad , A_n, B_1, \ldots, B_n) = \left(1 + \sum_{i=1}^{n}\ell_i\frac{B_i}{L_i} + \sum_{i=1}^{n}\frac{\tilde{K}_i(B_1, \ldots, B_n)}{A_i}\right)^{-1}, \quad (18)$$

where

$$\tilde{K}_i(B_1, \ldots, B_n) = K_i(B_1, \ldots, B_n)\left(1 + \sum_{i=1}^{n}\ell_i\frac{B_i}{L_i}\right).$$

From (18) it follows

$$G(A_1, \ldots, A_n, 0, \ldots, 0) = \left(1 + \sum_{i=1}^{n}\frac{K_i}{A_i}\right)^{-1}, \quad (19)$$

where

$$K_i \equiv K_i(0, \ldots, 0).$$

From Property P1 by taking $K_j = 1/L_j$ and $A_j = 1/B_j$ we can use Theorem 1 to prove

$$G(A_1, \ldots, A_n, B_1, \ldots, B_n) = \frac{G(A_1, \ldots, A_n, 0, \ldots, 0)}{1 + \sum_{i=1}^{n}(B_i/L_i(A_1, \quad , A_n))}, \tag{20}$$

since reciprocal transformation exchanges the role of inhibitors and nutrients, and transforms Conditions (A')–(C') into Conditions (A)–(C). Substituting equation (19) in (20) we have

$$G(A_1, \ldots, A_n, B_1, \quad , B_n) = \left(1 + \sum_{i=1}^{n}\frac{K_i}{A_i} + \sum_{i=1}^{n}\frac{B_i}{\tilde{L}_i(A_1, \ldots, A_n)}\right)^{-1}, \tag{21}$$

where

$$\tilde{L}_i(A_1, \ldots, A_n) = L_i(A_1, \ldots, A_n)\left(1 + \sum_{i=1}^{n}\frac{K_i}{A_i}\right)^{-1}.$$

By equating the right-hand side of equations (18) and (21) we have

$$\sum_{i=1}^{n}\ell_i\frac{B_i}{L_i} + \sum_{i=1}^{n}\frac{\tilde{K}_i(B_1, \ldots, B_n)}{A_i} = \sum_{i=1}^{n}\frac{K_i}{A_i} + \sum_{i=1}^{n}\frac{B_i}{\tilde{L}_i(A_1, \ldots, A_n)} \tag{22}$$

and applying the operator $\frac{\partial}{\partial B_j}$ on (22),

$$\frac{1}{\tilde{L}_j(A_1, \ldots, A_n)} - \frac{\ell_j}{L_j} = \sum_{i=1}^{n}\frac{\partial \tilde{K}_i(B_1, \ldots, B_n)}{\partial B_j}\frac{1}{A_i} \tag{23}$$

and by saturating all nutrients but A_i (i.e., $A_j \mapsto \infty$) in (23) the following expression is obtained·

$$\frac{A_i}{\tilde{L}_j(\ldots, \infty, A_i, \infty, \ldots)} - \frac{\ell_j A_i}{L_j} = \frac{\partial \tilde{K}_i(B_1, \ldots, B_n)}{\partial B_j}. \tag{24}$$

Observe that the left-hand side of equation (24) does not depend on inhibitors, while the right-hand side does not depend on nutrients, therefore both sides are constant, i.e.,

$$\frac{\partial \tilde{K}_i(B_1, \ldots, B_n)}{\partial B_j} = \alpha_{ij},$$

and therefore, because $\tilde{K}_i(0, \ldots, 0) = K_i(0, \ldots, 0) = K_i$

$$\tilde{K}_i(B_1, \ldots, B_n) = K_i + \sum_{j=1}^{n}\alpha_{ij}B_j, \tag{25}$$

and substitution of (25) into (18) yields (15).

To characterize the constants α_{ij} in Lemma 1 we must specify how the inhibitors interact with the nutrients

PROBLEM 2. *Find G which solve Problem 1 which also satisfy the following.*

P4. *When all but A_i are saturated and all but B_i are absent, then growth rates must be of the type (13) if B_i is competitive or (14) if B_i is not competitive.*

P5. *When all but A_i are saturated and all but B_j are absent with $i \neq j$, then the half-saturation constant of A_i does not depend on B_j*

THEOREM 2. *There exists a unique $G \in C^1(\mathbb{R}_+^{2n})$ that solves Problem 2 and is given by*

$$G(A_1, \ldots, A_n, B_1, \ldots, B_n) = \frac{1}{1 + \sum\limits_{i=1}^{n} (K_i/A_i + (B_i/L_i)(\ell_i + K_i/A_i))}.$$

PROOF. From Lemma 1, G is of the form (15), moreover

$$G(\infty, \ldots, A_i, \ldots, \infty, 0, \ldots, B_j, \ldots, 0) = \frac{1}{[1 + \ell_j(B_j/L_j)] + (K_i + \alpha_{ij}B_j)/A_i}, \qquad (26)$$

when $i = j$ comparing (26) with (13) and (14) follows that $\alpha_{ii} = K_i/L_i$. When $i \neq j$ the half-saturation constant of A_i is $K_i + \alpha_{ij}B_j$, but from Property P5 follows $\alpha_{ij} = 0$.

5. DERIVATION OF GROWTH RATE BY A SIMPLE CELL MODEL

In this section, a simple cell growth model is introduced. The growth process of a cell is modeled as a two stages process as follows.

- Stage (1) growth of the cell by nutrients adsorption;
- Stage (2) split of the cell in two equal ones.

In the model, the cell at Stage 1 starts with a minimum mass $\mathcal{M}^0$ and continues to grow until it reaches the critical mass $\mathcal{M}^1$. When the cell reaches the critical mass $\mathcal{M}^0$ then it stops growing and goes to Stage 2 starting to split. After splitting, two equal cells are created with equal minimum mass $\mathcal{M}^0$. So the critical mass $\mathcal{M}^1$ must be equal to $2\mathcal{M}^0$. Graphically this process is illustrated in Figure 1.
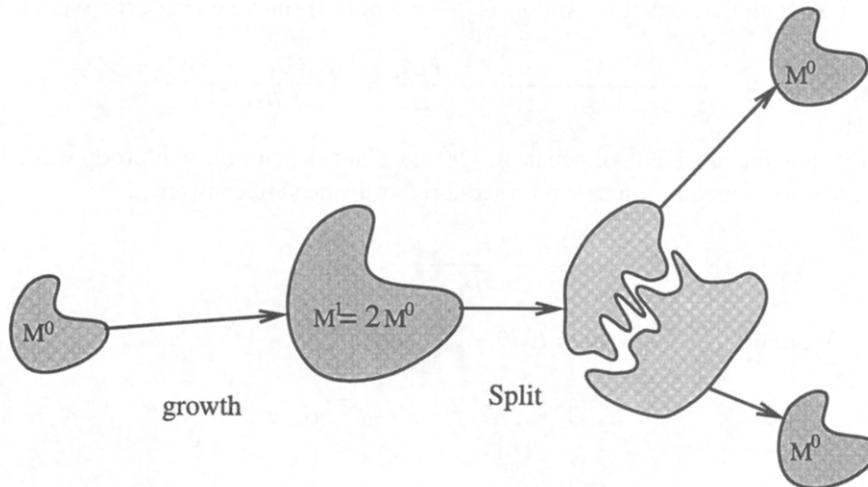

Figure 1

At present, we consider cell growth based on a unique nutrient of concentration A. In Stage 1, while the cell is growing, the change of mass is proportional to nutrient concentration. Again, the change of mass is directly proportional to the surface of cell membrane because the adsorption is performed by this last one. In formula this becomes

$$\frac{dM}{dt} = \frac{dP}{dt}, \qquad \frac{dP}{dt} = f(A, B)S, \qquad (27)$$

where P represents the total amount of nutrient that was adsorbed, $f(A,B)$ is a function that determines the adsorption rate by unit surface, and S is the total surface of cell membrane. Incidentally $f(A,B)$ takes the form [1]

$$f(A,B) = \frac{f_{\max}}{1 + (K/A)(1 + B/L) + \ell(B/L)}$$

where $\ell = 0$ if B is competitive and $\ell = 1$ if B is noncompetitive.

5.1. Growth in the Case of One Nutrient

The relation of cell surface and volume is assumed to follow the Huxley's allometric law [15]

$$S = kV^{\gamma} \qquad (S = \text{Surface}, V = \text{Volume}), \tag{28}$$

where k and γ are constants characteristic of the cell. For example, if the cell is spheric then $S = 4\pi R^2$, $V = (4\pi R^3)/3$ where R is the cell radius, so the constants for allometric law become $k = \sqrt[3]{12\pi}$ and $\gamma = 2/3$. Assuming that the density of the cell is constant during the growth, it follows that the mass is linearly proportional to volume so equation (28) can be substituted with $S = k'M^{\gamma}$ with suitable k'. By using equation (27) then

$$\frac{d\mathcal{M}}{dt} = f(A,B)k'\mathcal{M}^{\gamma} \tag{29}$$

is obtained. Now the constancy of nutrient and inhibitor concentration while the cell is growing is assumed, so equation (29) has the solution

$$\mathcal{M}(t) = \mathcal{M}(0)\begin{cases} [(1-\gamma)f(A,B)k']^{1/(1-\gamma)}, & \text{if } \gamma \neq 1, \\ \exp(f(A,B)k't), & \text{if } \gamma = 1, \end{cases} \tag{30}$$

and growth law for Stage 1 becomes equation (30). The splitting process starts after a time Δt_g when all cells have doubled their masses. Thus Δt_g must satisfy

$$\mathcal{M}(\Delta t_g) = 2\mathcal{M}(0). \tag{31}$$

Δt_g is easily obtained from equations (30) and (31) and takes the following value:

$$\Delta t_g = \frac{\kappa_{\gamma}}{f(A,B)k'}, \qquad \text{where } \kappa_{\gamma} = \begin{cases} \dfrac{2^{1-\gamma}}{1-\gamma}, & \text{if } \gamma \neq 1, \\ \log(2), & \text{if } \gamma = 1. \end{cases}$$

After Δt_g the cell become to split and terminate after a fixed time Δt_s. So the time Δt_t spent in the complete process becomes

$$\Delta t_t = \Delta t_s + \Delta t_g = \Delta t_s + \frac{\kappa_{\gamma}}{f(A,B)k'}.$$

Consider now a continuum of cells $\mathcal{M}(t)$ where $\mathcal{M}(t)$ represents the total mass of the continuum at time t. If the continuum grows with constant nutrient concentration then the growth satisfies $\mathcal{M}(n\Delta t_t) = \mathcal{M}(0)2^n$ becoming exponential. The following differential equation:

$$\frac{d\mathcal{M}}{dt} = k\mathcal{M} \tag{32}$$

has the exponential solution $\mathcal{M}(t) = \mathcal{M}(0)\exp(kt)$. So if the condition $\mathcal{M}(n\Delta t_t) = \mathcal{M}(0)2^n$ is forced, then k must be equal to $\log(2)/\Delta t_t$ which substituted in equation (32) becomes

$$\frac{d\mathcal{M}}{dt} = \mathcal{M}g(A,B)$$

where the growth rate g is again an MMM law

$$g(A,B) = \frac{g_{\max}}{1 + \left(\tilde{K}/A\right)\left(1 + B/\tilde{L}\right) + \ell\left(B/\tilde{L}\right)}$$

with

$$C = \left(\Delta t_s + \frac{\kappa_{\gamma}}{f_{\max}k'}\right)^{-1}, \qquad g_{\max} = \log(2)C, \qquad \tilde{K} = \frac{K}{C}, \qquad \tilde{L} = LC.$$

5.2. The Case of More Nutrients

In the case of more nutrients, we suppose that the cell is composed by n nutrients $A_1, A_2, \ldots, A_n$ in a some fixed proportions. When the cell goes from minimal mass to critical mass it must duplicate the mass of each nutrient contained in the cell. While the cell grows, the mass $\mathcal{M}_i$ composed by product p_i increases by the assimilation of this last one.

$$\frac{d\mathcal{M}_i}{dt} = \frac{dp_i}{dt}, \qquad i = 1, 2, \ldots, n.$$

This growth is not free because the ratio

$$\frac{\mathcal{M}_i(t)}{\mathcal{M}(t)} = \alpha_i, \qquad \text{with } \sum_{i=1}^{n} \alpha_i = 1, \tag{33}$$

is assumed constant. This means that in the growth, all the nutrients are used at the same time, and the composition of the cell (in the sense of nutrients composition) is constant. Another assumption is that the surface element dS can assimilate only one nutrient at time. So the growth of $\mathcal{M}_i$ due to the assimilations of nutrient A_i becomes

$$\frac{d\mathcal{M}_i}{dt} = \frac{dp_i}{dt} = f_i(A_i, B_i)\beta_i k' \mathcal{M}^\gamma, \qquad \text{with } \sum_{i=1}^{n} \beta_i = 1, \tag{34}$$

where β_i represents the surface fraction which is assimilating nutrient A_i. Again by similar arguments of the previous section [1]

$$f_i(A_i, B_i) = \frac{f_{\max,i}}{1 + (K_i/A_i)(1 + B_i/L_i) + \ell_i(B_i/L_i)}.$$

Because of equation (33) we have

$$\frac{d\mathcal{M}_i}{dt} = \frac{\mathcal{M}_i}{\mathcal{M}} \frac{d\mathcal{M}}{dt} = \alpha_i \frac{d\mathcal{M}}{dt},$$

and equation (34) becomes

$$\frac{d\mathcal{M}}{dt} = w_i \beta_i \mathcal{M}^\gamma, \qquad \text{where } w_i = \frac{k'}{\alpha_i} \times \frac{f_{\max,i}}{1 + (K_i/A_i)(1 + B_i/L_i) + \ell(B_i/L_i)}. \tag{35}$$

From (35) it follows that $w_i \beta_i = w_j \beta_j$ and in particular $w_i \beta_i = w_1 \beta_1$ so by

$$\beta_i = \beta_1 \frac{w_1}{w_i} \qquad \text{and} \qquad \sum_{i=1}^{n} \beta_i = 1$$

follows that

$$\beta_i = \frac{1}{w_i} \cdot \frac{1}{\left(\sum_{j=1}^{n}(1/w_j)\right)} \tag{36}$$

and putting equation (36) in equation (35) then

$$\frac{d\mathcal{M}}{dt} = \frac{\mathcal{M}^\gamma}{\left(\sum_{i=1}^{n}(1/w_i)\right)}. \tag{37}$$

Equation (37) has the following solution:

$$
\mathcal{M}(t) = \mathcal{M}(0)
\begin{cases}
\left(\displaystyle\sum_{j=1}^{n}\frac{t}{w_j}\right)^{-1/(1-\gamma)}, & \text{if } \gamma \neq 1, \\[2em]
\exp\left(-\displaystyle\sum_{j=1}^{n}\frac{t}{w_j}\right), & \text{if } \gamma = 1,
\end{cases}
$$

so following the same arguments of one nutrient case, the time Δt_g spent to double the mass of the cell must satisfy $M(\Delta t_g) = 2M(0)$. So the time spent in the full process becomes

$$
\Delta t_t = \Delta t_s + \Delta t_g = \Delta t_s + \kappa_\gamma \sum_{j=1}^{n}\frac{1}{w_j}.
$$

As in the one nutrient case, consider a continuum of cells $\mathcal{M}(t)$. If the continuum grows with constant nutrient concentration then the growth satisfies $\mathcal{M}(n\Delta t_t) = \mathcal{M}(0)2^n$ becoming exponential. The differential equation (32) has the exponential solution $\mathcal{M}(t) = \mathcal{M}(0)\exp(kt)$. So if the condition $\mathcal{M}(n\Delta t_t) = \mathcal{M}(0)2^n$ is forced, then k must be $\log(2)/\Delta t_t$ which substituted in equation (32) becomes

$$
\frac{\mathrm{d}\mathcal{M}}{\mathrm{d}t} = \frac{\mathcal{M}\log(2)}{\Delta t_t} = \mathcal{M}G_{\max}G(A_1,\ldots,A_n,B_1,\ldots,B_n)
$$

and growth is

$$
G(A_1,\ldots,A_n,B_1,\ldots,B_n) = \frac{1}{1 + \sum\limits_{i=1}^{n}\left(\tilde{K}_i/A_i + \left(B_i/\tilde{L}_i\right)\left(\ell_i + \tilde{K}_i/A_i\right)\right)},
$$

with

$$
C = \left(\Delta t_s + \sum_{i=1}^{n}\frac{\alpha_i}{f_{\max,i}k'}\right)^{-1}, \qquad G_{\max} = \log(2)C, \qquad \tilde{K}_i = \frac{K_i}{C}, \qquad \tilde{L}_i = L_i C.
$$

6. CONNECTION WITH MULTIPLICATIVE AND MINIMUM FORMULATION

The asymptotic connection of our formula with the multiplicative and minimum formulation is now briefly investigated. For simplicity the case with only two nutrients is considered. First, the multiplicative law (II) is written as follows:

$$
\frac{A}{K_A + A} \times \frac{B}{K_B + B} = \frac{1}{K_A/A + 1} \times \frac{1}{K_B/B + 1} = \frac{1}{K_A K_B/AB + K_A/A + K_B/B + 1}.
$$

Next, the proposed formulation is

$$
G(A,B) = \frac{1}{K_A/A + K_B/B + 1},
$$

so the difference between the two formulations is only in the term $K_A K_B/(AB)$. Observe that this term for both $A \gg K_A$ and $B \gg K_B$ becomes negligible and thus in this condition the two formulations give almost the same limitation. In the case of $B \approx K_B$ the situation becomes

$$
\frac{1}{K_A K_B/AB + K_A/A + K_B/B + 1} \approx \frac{1}{2(K_A/A) + 2} = \frac{1}{2}\frac{A}{K_A + A} \qquad \text{(multiplicative law)},
$$

$$
\frac{1}{K_A/A + K_B/B + 1} \approx \frac{1}{K_A/A + 2} = \frac{1}{2}\frac{A}{K_A/2 + A} \qquad \text{(proposed formulation)},
$$

where it is clear that the new formulation is affected by the nutrient B also in the half-saturation constant. The modification of the half-saturation constant becomes important in very low concentration of A ($A \ll K_A$). In this situation growth can be approximately expressed by

$$\frac{1}{K_A K_B / AB + K_A / A + K_B / B + 1} \approx \frac{A}{2K_A},$$

$$\frac{1}{K_A / A + K_B / B + 1} \approx \frac{A}{K_A},$$

so in the proposed formulation if the nutrient A is very diluted, the growth depends essentially and linearly on A.

Thus, when the concentration of one nutrient is very small the difference between the multiplicative and the proposed formulation becomes considerable. In this range the proposed formulation becomes similar to the minimum formulation, in fact, if $A \ll K_A$ and $B \approx K_B$ or $B \gg K_B$ then

$$\min\left(\frac{1}{K_A/A + 1}, \frac{B}{K_B/B + 1}\right) = \frac{1}{K_A/A + 1} \approx \frac{A}{K_A}.$$

The case of $A \approx K_A$ and $B \approx K_B$ gives instead

$$\frac{1}{K_A K_B / AB + K_A / A + K_B / B + 1} \approx \frac{1}{4},$$

$$\min\left(\frac{1}{K_A/A + 1}, \frac{B}{K_B/B + 1}\right) \approx \frac{1}{2},$$

$$\frac{1}{K_A / A + K_B / B + 1} \approx \frac{1}{3}.$$

Thus, the proposed formulation stands between the multiplicative and minimum law. Furthermore, it is asymptotically similar to the minimum formulation (I) in the case of low concentration and similar to the multiplicative formulation (II) in the case of high concentration.

7. CONCLUSIONS

The formulation derived by an axiomatic approach is a generalization of Michaelis-Menten-Monod growth rate in the function of multiple substrates. In fact, the restriction of the formula on a linear subspace through the origin is again Michaelis-Menten-Monod law.

The formula is quite general and considers the case of more than one limiting substance. Both nutrients and inhibitors are considered as limiting substances and are assumed to be noninteracting. Both competitive and noncompetitive inhibitors are considered. The formula is determined by analytical arguments based on few hypotheses. Comparison of this formulation with other formulas (namely multiplicative or minimum formula) shows that this formula becomes close to the multiplicative formula in the case of nearly equal nutrient concentrations and becomes close to the minimum formula in the case of very spread nutrient concentrations.

REFERENCES

1 J E Bailey and D.F Ollis, *Biochemical Engineering Fundamentals*, McGraw-Hill, (1977).

2. J D Spain, *BASIC Microcomputer Models in Biology*, Addison-Wesley, (1982).

3 L.A Segel, *Modeling Dynamics Phenomena in Molecular and Cellular Biology*, Cambridge University Press, (1984).

4 J Monod, *Recherches Sur la Croissance des Cultures Bacteriennes*, Herman and Cie, (1942)

5. G L Bowie, W.B. Mills, D B Porcella, C.L. Campbell, J.R. Pagenkopf, G L Rupp, K M. Johnson, P.W H Chan, S.A. Gherini and C.E. Chamberlain, Rates, constant, and kinetics formulations in surface water quality modeling, Tech Rep , United States Environmental Protection Agency/600/3-85/040, (1985)

6 B Baule, Zu Mitscherlichs Gesetz der physiologischen Beziehungen, *Landw Jahrb* **51**, 363–385, (1917)

7 W J O'Brian, The dynamics of nutrient limitation of phytoplankton algae: A model reconsidered, *Ecology* **55**, 135–141, (1974)

8 M R Droop, The nutrient status of algal cells in continuous culture, *J. Mar Biol Ass* **54**, 825–855, (1974)

9 G Y. Rhee, Effects of N:P atomic ratios and nitrate limitation on algal growth composition and nitrate uptake, *Limnol. Oceanogr* **23**, 10–25, (1978).

10. C.C. Chen and E R Christensen, A unified theory for microbial growth under multiple nutrient limitation, *Water Research* **19** (6), 791–798, (1985)

11. O Diekmann and J.A P Heesterbeek, *Mathematical Epidemiology of Infectious Diseases—Model Building, Analysis and Interpretation*, Wiley, (2000)

12 N Bellomo, A Bellouquid and M. Delitala, Mathematical topics on the modelling complex multicellular systems and tumor immune cells competition, *Math. Models Methods Appl Sci.* **14** (12), (2004)

13 R Courant and D. Hilbert, *Methods of Mathematical Physics, Volume II*, John Wiley and Sons, (1989)

14. I. Segel, *Enzyme Kinetics*, John Wiley and Sons, (1975).

15. P.L. Antonelli, Non-Euclidean allometry and the growth of forest and coral, In *Mathematical Essay on Growth and the Emergence of Form*, University of Alberta Press, (1985).