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Mathematical Models for Continuous Culture Growth Dynamics of Mixed Populations Subsisting on a Heterogeneous Resource Base: I. Simple Competition*

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The classical Monod model for bacterial growth in a chemostat, based on a Michaelis–Menten kinetic analog, is restated in terms of an approximate Lotka–Volterra formulation. The parameters of these two formulations are explicitly related; the new model is easier to work with, but yields the same results as the original. The model is then extended to the case where multiple *alternate* substrates may be growth limiting, using the corresponding kinetic analogs for multiple-substrate enzymes. Again, one is led to a Lotka–Volterra analog. In the multiple-substrate model, however, coexistence of multiple genotypes is possible, in contrast to the single-substrate model. The usual Lotka–Volterra conditions for existence and stability of pure or mixed equilibria may all be translated into corresponding statements about the parameters of the chemostat system. Possible extensions to deal with metabolic inhibition, cross-feeding, and predation are indicated.

INTRODUCTION

Population biologists have begun to develop an avid interest in the dynamics and statics of mixed populations subsisting on a heterogeneous resource base. The mathematical theory for such systems is fairly elaborate (cf. Schoener, 1973, 1974), though still a bit ad hoc. Our useful field data are rather more limited than our theory, and we are badly in need of some careful experimental work. Natural ecosystems are, of course, far more complex than we can cope with in the laboratory, but our knowledge is still fragmentary enough that we may profit from judicious experimental reductionism. For a variety of reasons, microbial populations maintained under continuous culture growth conditions in a chemostat represent an attractive modeling system for the study of some of the simpler natural phenomena. The real beauty of the chemostat system is that one may hope to compare results with theoretical predictions under a variety of conditions.

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The classical theory of continuous culture growth dynamics is predicated on the assumption that a single limiting substrate determines the growth of each and every organism (genotype) in the culture. The inevitable theoretical consequence of this assumption is that one strain (genotype) will displace all the others (e.g., Monod, 1949; Herbert *et al.*, 1956; Powell, 1958; Fencl, 1966). An examination of the experimental literature (cf. Bungay and Bungay, 1968; Veldkamp and Jannasch, 1972; Veldkamp and Kuenen, 1973; Jannasch and Mateles, 1974) will yield numerous observable exceptions to this predicted outcome. Some of the exceptions are attributable to failure of the "perfect mixing" assumption implicit in mass action models of the Monod type, due to wall growth (cf. Larsen and Dimmick, 1964; Munson and Bridges, 1964; Topiwala and Hamer, 1971). Other cases may be attributed to metabolic cross-feeding (e.g., Powell, 1958; Shindala *et al.*, 1965; Contois and Yango, 1964). Experiments in several laboratories suggest that the assumption of a single limiting substrate may itself be qualitatively inadequate (cf. Chian and Mateles, 1968; Mateles and Chian, 1969; Megee *et al.*, 1972; Smouse and Kosuda, 1977).

The purpose of this paper is to extend existing theory to the case where any one of several substrates may be limiting. I shall not deal here with failure of the "perfect mixing" assumption nor with metabolic cross-feeding. Both of these matters must be dealt with, but are better deferred to later communications. The basic formulation used here is an extension of the classic Michaelis–Menten model of Monod (1949), based on an analog of competitive inhibition kinetics. The model is translated into an analogous Lotka (1925)–Volterra (1926) form, using a mathematical approach first described by Waldon (1975). The Lotka–Volterra model is more familiar to population biologists than are its more precise chemostat analogs, but the parameters are low on information content. In the present context, the traditional parameters may be explicitly related to the underlying experimental inputs and convey useful information about the system. There are three questions of particular interest here: (1) In what sense can more than one substrate be limiting? (2) How do the parameters of the Lotka–Volterra formulation relate to the parameters and inputs of the chemostat system? (3) What are the conditions for existence and stability of polymorphic (mixed population) equilibria? I shall attempt to answer each of these questions in turn.

The One-Substrate Model

It is appropriate to set the stage with a brief recapitulation of the classical (one substrate) model, because the notation used here is a bit unusual. [The notational change is necessary to avoid very cumbersome subscripting problems later. The reader familiar with traditional notation will find the translation straightforward. The results of this section are, of course, the traditional results.] The basic chemostat growth system is shown in Fig. 1. The organisms in the

culture vessel are maintained in a continuous state of growth and reproduction by a steady flow of medium from the reservoir, through the culture vessel, and into the trap. Excess organisms, metabolic waste products, etc. are also washed out of the culture vessel at a constant rate. The medium contains several nutrients, one of which will be limiting; the others are present in excess. The steady-state population size in the culture vessel is entirely determined by the reservoir concentration of this single limiting substrate, given a flow rate, and one may ignore all other nutrients for theoretical purposes. I shall deal, in sequence, with the growth dynamics and fate of one, two, and multiple genotypes.

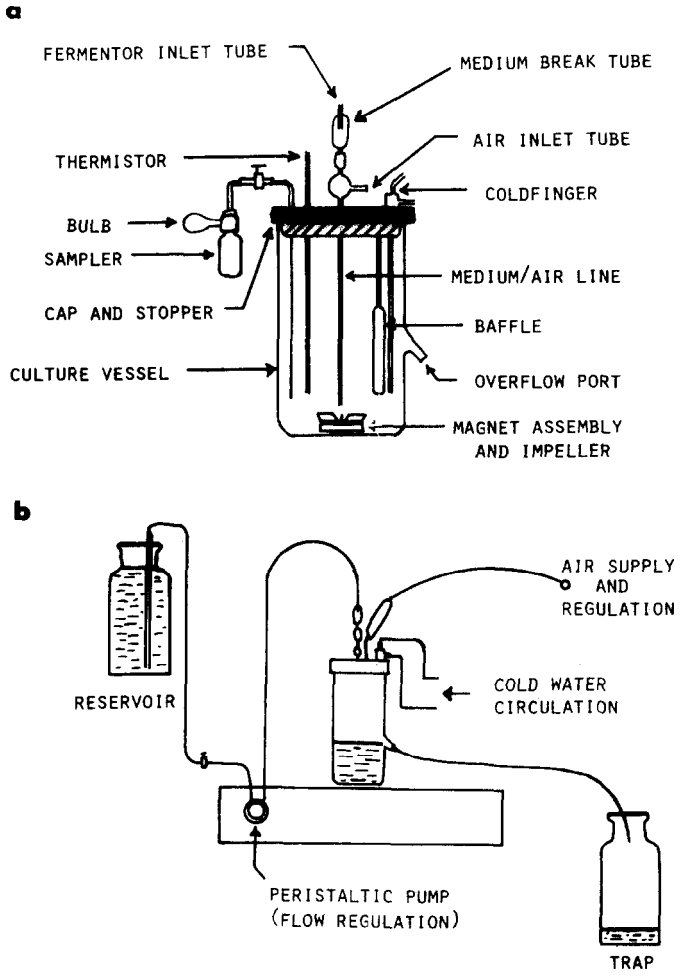

FIG. 1. General schematic of a continuous-flow chemostat: (a) Culture vessel with various control and assay features; (b) overview of the continuous-flow system, with reservoir, culture vessel, and trap.

One Genotype

Consider a single genotype (G_1), and denote its biomass (or numbers) by M_1 . Denote the limiting substrate by S_2 and its concentration by C_2 . The convention of numbering genotypes and substrates in sequence will help to avoid confusion below. The dynamics of M_1 may be described by the Monod model, based on an autocatalytic analog shown in Fig. 2a, and formally described by

$$\dot{M}_1 = (\mu_{12} - D)\, M_1 = \left[\frac{V_{12}C_2}{K_{12} + C_2} - D\right] M_1 , \tag{1}$$

where μ_{12} is the instantaneous growth rate, D is the dilution rate [the flow rate (ml/hr) divided by the volume of the culture vessel (ml)], V_{12} is the maximum growth rate achievable by G_1 , grown on S_2 , and K_{12} is the "half-maximum" substrate concentration, i.e., the concentration of S_2 yielding $\mu_{12} = \frac{1}{2}V_{12}$. Since $(K_{12} + C_2) = K_{12}[1 + K_{12}^{-1}C_2] = K_{12}\phi_1$, Eq. (1) may also be written as

$$\dot{M}_1 = \left[\frac{V_{12}C_2}{K_{12}} - D\left(1 + \frac{C_2}{K_{12}}\right)\right]\phi_1^{-1}M_1 = \left[\frac{V_{12} - D}{K_{12}}\, C_2 - D\right]\phi_1^{-1}M_1 , \tag{2}$$

a more convenient form for much of what follows. The dynamics of C_2 are routinely described by

$$\dot{C}_2 = (R_2 - C_2)\, D - \mu_{12}\lambda_{12}M_1 , \tag{3}$$

where R_2 is the reservoir (input) concentration of S_2 , and λ_{12} is the amount of S_2 consumed in producing one unit of G_1 (Waldon, 1975). The parameter λ_{12} is the reciprocal of the usual yield constant Y_{12} = biomass of G_1 produced/unit of S_2 .

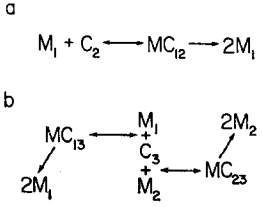

FIG. 2. Autocatalytic growth models for systems limited by the concentration of a single substrate: (a) M_1 = mass of G_1 , C_2 = concentration of S_2; (b) M_1 = mass of G_1 , M_2 = mass of G_2 , C_3 = concentration of S_3 .

Together, Eqs. (1) and (3) describe the dynamics of the system. Now consider the weighted sum of (1) and (3), which becomes

$$[\dot{C}_2 + \lambda_{12}\dot{M}_1] = \cdots = [R_2 - C_2 - \lambda_{12}M_1]D. \tag{4}$$

Equation (4) is determined essentially by the physical properties of the system (Waldon, 1975), and constitutes a "state" equation which describes changes in the total substrate pool (measured either directly or in terms of biomass equivalents). If $C_2 \approx R_2$ and $M_1 \approx 0$ initially (as will almost always be the case), then (4) remains very close to zero, i.e., $(C_2 \approx R_2 - \lambda_{12}M_1)$. Substitution into (2) yields

$$\dot{M}_1 = [\alpha_1 - \beta_{11}M_1]\,\phi_1^{-1}M_1\,, \tag{5}$$

where α_1 and β_{11} are defined by

$$\alpha_1 = \frac{V_{12} - D}{K_{12}}R_2 - D = \theta_{12}R_2 - D, \qquad \beta_{11} = \theta_{12}\lambda_{12}\,. \tag{6}$$

Equation (5) is approximately the logistic equation of Verhulst (1838) and Pearl and Reed (1920). The parameter α_1 is generally described as the "intrinsic rate of increase," and β_{11} is usually described as the "density dependent damping" parameter. In the usual situation, these measures are simply invoked. Here, they may be explicitly related to R_2, D, and the capabilities of the organism (V_{12}, K_{12}, λ_{12}). The constant θ_{12} contains elements (V_{12} and K_{12}) intrinsic to the organism, as well as an element (D) under the control of the experimenter. The outcome of competition will be seen below to depend on both types of elements. Since C_2 is not a constant (except at equilibrium), ϕ_1 is not a constant either, and the model is only *approximately* logistic.

One may obtain the steady-state solutions by setting (2) or (5) equal to zero and solving for $\hat{C}_2$. This solution is substituted into the "state" equation (4), which in turn is set to zero and solved for $\hat{M}_1$. Since ϕ_1 is always positive, it drops out of the equilibrium argument, and the various steady states may be compared in terms of α and β parameters. For the case at hand, there are two possible steady states.

$$\begin{aligned} &\textit{Case} \text{ I:} \quad \hat{M}_1 = 0, && \hat{C}_2 = R_2\,. \\ &\textit{Case} \text{ II:} \quad \hat{M}_1 = \frac{\alpha_1}{\beta_{11}} = \frac{R_2 - \hat{C}_2}{\lambda_{12}}\,, && \hat{C}_2 = \frac{DK_{12}}{V_{12} - D} = D\theta_{12}^{-1} \end{aligned}$$

Now, if $\theta_{12}R_2 < D$ (i.e., if $R_2 < D\theta_{12}^{-1}$), then Case II cannot exist. This constitutes the washout condition, i.e., the case where D is so large that the organism cannot reproduce fast enough to maintain itself. If $\theta_{12}R_2 > D$, then Case II exists. [We shall ignore the trivial case where $\theta_{12}R_2 = D$.]

The existence of a solution does not guarantee its stability. For the models presented here, it is possible to examine local stability by means of the Liapanov criterion (cf. Andronov *et al.*, 1966). One first defines a Jacobian matrix $\mathbf{J}_{MC}$

$$\mathbf{J}_{MC} = \begin{bmatrix} \partial\dot{M}_1/\partial M_1 & \partial\dot{M}_1/\partial C_2 \\ \partial\dot{C}_2/\partial M_1 & \partial\dot{C}_2/\partial C_2 \end{bmatrix} \tag{7}$$

and then extracts a pair of characteristic roots (w) by solving the eigenequation

$$\det[\mathbf{J}_{MC} - w\mathbf{I}] = 0. \tag{8}$$

To evaluate the stability of any particular equilibrium, one substitutes the steady-state values of M_1 and C_2 into the eigenequation (8). The steady state is stable iff all the roots (w) have negative real parts.

It develops that the stability criteria for the chemostat parameters (V's, K's, λ's, and D) can always be translated into statements about the Lotka–Volterra analogs (α's, β's, and ϕ's). These latter criteria are readily seen to be very similar to those of the classic Lotka–Volterra model. I shall indicate the nature of the translation for this model and for a selected subset of those which follow in the Appendix, and will merely indicate the results in the text. For the present model, it turns out that:

$$\textit{Case} \text{ I: Stable iff } \theta_{12}R_2 < D \Leftrightarrow \alpha_1 < 0.$$

$$\textit{Case} \text{ II: Stable iff } \theta_{12}R_2 > D \Leftrightarrow \alpha_1 > 0.$$

Thus, under washout conditions, only solution set I exists, and it is stable. Under growth conditions, both solution sets exist, but only the second ($\hat{M}_1 > 0$, $R_2 > \hat{C}_2$) is stable. These are the usual results (cf. Fencl, 1966), as indicated at the outset.

Two Genotypes

Next, consider a pair of genotypes (G_1 and G_2), whose masses (or numbers) are denoted by M_1 and M_2, and a single substrate (now denoted S_3), whose concentration is denoted by C_3. This situation is depicted in Fig. 2b and the corresponding equations are

$$\begin{aligned}
\dot{M}_1 &= (\mu_{13} - D)\, M_1 = \left[\frac{V_{13}C_3}{K_{13}\phi_1} - D\right] M_1 = (\theta_{13}C_3 - D)\,\phi_1^{-1}M_1\,, \\
\dot{M}_2 &= (\mu_{23} - D)\, M_2 = \left[\frac{V_{23}C_3}{K_{23}\phi_2} - D\right] M_2 = (\theta_{23}C_3 - D)\,\phi_2^{-1}M_2\,, \\
\dot{C}_3 &= (R_3 - C_3)\, D - \mu_{13}\lambda_{13}M_1 - \mu_{23}\lambda_{23}M_2\,,
\end{aligned} \tag{9}$$

where $\phi_i = [1 + K_{i3}^{-1}C_3]$. The "state" equation is given by

$$[\dot{C}_3 + \lambda_{13}\dot{M}_1 + \lambda_{23}\dot{M}_2] = [R_3 - C_3 - \lambda_{13}M_1 - \lambda_{23}M_2]D \approx 0. \tag{10}$$

Substitution of (10) into (9) yields an approximation to the Lotka–Volterra model

$$\begin{aligned}
\dot{M}_1 &= [\alpha_1 - \beta_{11}M_1 - \beta_{12}M_2]\,\phi_1^{-1}M_1\,, \\
\dot{M}_2 &= [\alpha_2 - \beta_{21}M_1 - \beta_{22}M_2]\,\phi_2^{-1}M_2\,,
\end{aligned} \tag{11}$$

where the α and β parameters are defined as

$$\alpha_i = \theta_{i3}R_3 - D, \qquad \beta_{ii'} = \theta_{i3}\lambda_{i'3}, \qquad i, i' = 1, 2. \tag{12}$$

With two genotypes, there are four possible steady states; these are obtained by setting (11) equal to zero and solving for M_1 and M_2. [Again, ϕ_1 and ϕ_2 drop out of consideration.] The stability of each solution may be evaluated by recourse to (8). The steady states are listed below, along with the criteria for existence and stability.

Case I: Null State

$$\hat{M}_2 = 0, \qquad \hat{M}_2 = 0, \qquad \hat{C}_3 = R_3.$$

Exists: Always.
Stable if: $(\alpha_1 < 0 > \alpha_2) \Leftrightarrow (\theta_{13}R_3 < D > Q_{23}R_3)$.

Case II: Competitive Exclusion of G_2

$$\hat{M}_1 = \frac{\alpha_1}{\beta_{11}} = \frac{R_3 - \hat{C}_3}{\lambda_{13}}, \qquad \hat{M}_2 = 0, \qquad \theta_{13}\hat{C}_3 = D.$$

Exists: $(\alpha_1 > 0) \Leftrightarrow (\theta_{13}R_3 > D)$.

Stable if: $\frac{\alpha_2}{\beta_{21}} < \frac{\alpha_1}{\beta_{11}} > 0 \Leftrightarrow (\theta_{23}R_3 < \theta_{13}R_3 > D)$.

Case III: Competitive Exclusion of G_1

$$\hat{M}_1 = 0, \qquad \hat{M}_2 = \frac{\alpha_2}{\beta_{22}} = \frac{R_3 - \hat{C}_3}{\lambda_{23}}, \qquad \theta_{23}\hat{C}_3 = D.$$

Exists: $(\alpha_2 > 0) \Leftrightarrow (\theta_{23}R_3 > D)$.

Stable if: $\frac{\alpha_1}{\beta_{12}} < \frac{\alpha_2}{\beta_{22}} > 0 \Leftrightarrow (\theta_{13}R_3 < \theta_{23}R_3 > D)$.

Case IV: Competitive Coexistence of G_1 and G_2

$$\hat{M}_1 = \frac{\alpha_1\beta_{22} - \alpha_2\beta_{12}}{\beta_{11}\beta_{22} - \beta_{12}\beta_{21}} > 0 < \frac{\alpha_2\beta_{11} - \alpha_1\beta_{21}}{\beta_{11}\beta_{22} - \beta_{12}\beta_{21}} = \hat{M}_2,$$

$$\hat{C}_3 = R_3 - \lambda_{13}\hat{M}_1 - \lambda_{23}\hat{M}_2$$

It develops that Case IV, competitive coexistence, cannot exist with a single substrate (Appendix). In the parlance of the Lotka–Volterra model, we see that

$$(\beta_{11}\beta_{22} - \beta_{12}\beta_{21}) = 0 = (\theta_{13}\lambda_{13} \cdot \theta_{23}\lambda_{23} - \theta_{13}\lambda_{23} \cdot \theta_{23}\lambda_{13}), \tag{13}$$

which precludes a stable solution to Case IV. In summary then, the two genotype–one substrate system proceeds to the null state under generalized washout conditions ($\theta_{13}R_3 < D > \theta_{23}R_3$) and to either pure G_1 or pure G_2, depending on whether ($\theta_{23}R_3 < \theta_{13}R_3 > D$) or ($\theta_{13}R_3 < \theta_{23}R_3 > D$), respectively.

Multiple Genotypes

The generalization to multiple genotypes (G_1,..., G_I) and one substrate ($S_{I+1=J}$) is obvious. The dynamic system is described by

$$\begin{aligned} \dot{M}_i &= (\mu_{iJ} - D)\, M_i = (\theta_{iJ}C_J - D)\,\phi_i^{-1}M_i\,, \qquad i = 1,...,I, \\ \dot{C}_J &= (R_J - C_J)\, D - \sum_i \mu_{iJ}\lambda_{iJ}M_i\,, \end{aligned} \tag{14}$$

with $\theta_{iJ} = (V_{iJ} - D)/K_{iJ}$ and $\phi_i = [1 + K_{iJ}^{-1}C_J]$. The "state" equation is given by

$$\left[\dot{C}_J + \sum_i \lambda_{iJ}\dot{M}_i\right] = \left[R_J - C_J - \sum_i \lambda_{iJ}M_i\right] D \approx 0, \tag{15}$$

which upon substitution into (14), yields the generalized Lotka–Volterra analog

$$\dot{M}_i = \left[\alpha_i - \sum_{i'} \beta_{ii'}M_{i'}\right]\phi_i^{-1}M_i\,, \qquad i = 1,...,I. \tag{16}$$

The α_i and $\beta_{ii'}$ parameters are defined as in (12).

There are 2^I potential steady states, but for a single substrate, only $(I + 1)$ steady states actually exist. Of these, only two are of interest. If we number the genotypes so that $\theta_{1J} > \theta_{2J} > \cdots > \theta_{IJ}$, the two steady states of interest are

Case I:

$$\hat{M}_1 = \hat{M}_2 = \cdots = \hat{M}_I = 0, \qquad \hat{C}_J = R_J\,.$$

Case II:

$$\hat{M}_1 = \frac{\alpha_1}{\beta_{11}} = \frac{R_J - \hat{C}_J}{\lambda_{1J}}\,, \qquad \hat{C}_J = D\theta_{1J}^{-1}\,, \qquad \hat{M}_2 = \cdots = \hat{M}_I = 0,$$

all other single-genotype solutions are unstable, and no mixed-genotype solutions exist. [Note that for any two genotypes (G_i and $G_{i'}$), $(\beta_{ii}\beta_{i'i'} - \beta_{ii'}\beta_{ii}) = 0$. Similar results disallow multiple-genotype solutions.] Since it can be shown that only one genotype may persist, we must only compare the I separate single-genotype steady states. If $D > \theta_{1J}R_J > \cdots > \theta_{IJ}R_J$, then Case I (the null solution) is stable, but if $D < \theta_{1J}R_J > \cdots > \theta_{IJ}R_J$, then Case II is stable. The sort of stability analysis described for the two-genotype case leads to the conclusion that the genotype with largest θ_{iJ} (G_1 by construction) will prevail.

We may thus view the θ_{iJ} as a set of fitness parameters, depending both on the organisms (V_{iJ} and K_{iJ}) and on the dilution rate (D). All of these results are known; only the notation is new.

The Multiple-Substrate Model

One Genotype

Consider a single genotype (G_1) and a pair of alternate substrates (S_2 and S_3), either one of which will support growth in the absence of the other. It is convenient to treat this situation in a fashion analogous to that of an enzyme with alternate (competing) substrates (Segel, 1975). This situation is depicted in Fig. 3a, and is described by the dynamic equations

$$\begin{aligned}\dot{M}_1 &= (\mu_{12} + \mu_{13} - D)\, M_1 = \dot{M}_{12} + \dot{M}_{13} \\ &= \left[\frac{V_{12}C_2}{K_{12}\phi_1} + \frac{V_{13}C_3}{K_{13}\phi_1} - D\right] M_1 \\ &= (\mu_{12} - \gamma_{12}D)\, M_1 + (\mu_{13} - \gamma_{13}D)\, M_1 , \\ \dot{C}_j &= (R_j - C_j)\, D - \mu_{1j}\lambda_{1j}M_1 , \qquad j = 2, 3,\end{aligned} \tag{17}$$

where $\phi_1 = [1 + K_{12}^{-1}C_2 + K_{13}^{-1}C_3]$, and where γ_{12} and γ_{13} are the proportional rates of growth on the two substrates. The other parameters have altogether obvious definitions. In this case, one has a pair of "state" equations

$$[\dot{C}_j + \lambda_{1j}\dot{M}_{1j}] = [R_j - C_j - \lambda_{1j}\gamma_{1j}M_1]D \approx 0, \qquad j = 2, 3. \tag{18}$$

Substitution of (18) into (17) yields the logistic analog (5), with ϕ_1 defined as above and

$$\alpha_1 = \sum_j \theta_{1j}R_j - D, \qquad \sum_j \theta_{1j}\Gamma_{1j} = \beta_{11} . \tag{19}$$

The measure $\Gamma_{1j} = \gamma_{1j}\lambda_{1j}$ includes both substrate preference and conversion capabilities of the organism. The extension to multiple substrates is obvious. One obtains (5), with α_1 and β_{11} as defined in (19) and $\phi_1 = [1 + \sum_j K_{ij}^{-1}C_j]$.

If $\sum_j \theta_{1j}R_j < D$ (washout conditions), the system proceeds to the null state ($\hat{M}_1 = 0$, $\hat{C}_j = R_j : j = 2,..., J$); if $\sum_j \theta_{1j}R_j > D$ (growth conditions), the system proceeds to the steady state.

$$\hat{M}_1 = \frac{\alpha_1}{\beta_{11}} = \sum_j \frac{R_j - \hat{C}_j}{\lambda_{1j}} , \qquad \sum_j \theta_{1j}\hat{C}_j = D. \tag{20}$$

Explicit solutions for the $\hat{C}_j$ do not appear to exist, but they may be related by

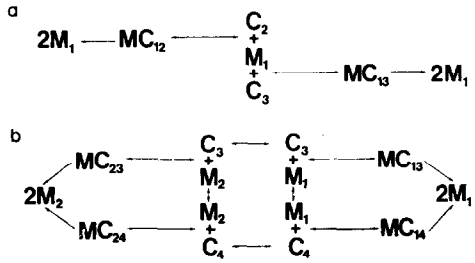

FIG. 3. Autocatalytic growth models for systems limited by the concentrations of two alternate substrates: (a) M_1 = mass of G_1, C_2 = concentration of S_2, C_3 = concentration of S_3; (b) M_i = mass of G_i ($i = 1, 2$), C_j = concentration of S_j ($j = 3, 4$).

the second equation (20). The γ_{1j} measures can be shown to take the equilibrium values

$$\hat{\gamma}_{1j} = \frac{R_j - \hat{C}_j}{\lambda_{1j}} \cdot \frac{\beta_{11}}{\alpha_1} . \tag{21}$$

Two Genotypes

Consider two genotypes (G_1 and G_2) and a pair of substrates (S_3 and S_4). The analog of (17) is given by

$$\begin{aligned} \dot{M}_i &= (\mu_{i3} + \mu_{i4} - D) M_i = \dot{M}_{i3} + \dot{M}_{i4} \\ &= (\mu_{i3} - \gamma_{i3}D) M_i + (\mu_{i4} - \gamma_{i4}D) M_i \\ &= \left[\frac{V_{i3}C_3}{K_{i3}\phi_i} + \frac{V_{i4}C_4}{K_{i4}\phi_i} - D\right] M_i , \qquad i = 1, 2, \\ \dot{C}_j &= (R_j - C_j) D - \mu_{1j}\lambda_{1j}M_1 - \mu_{2j}\lambda_{2j}M_2 , \qquad j = 3, 4. \end{aligned} \tag{22}$$

The definitions of all parameters are obvious. This situation is depicted in Fig. 3b. The two "state" equations now take the form

$$[\dot{C}_j + \lambda_{1j}\dot{M}_{1j} + \lambda_{2j}\dot{M}_{2j}] = [R_j - C_j - \Gamma_{1j}M_1 - \Gamma_{2j}M_2]D \approx 0, \qquad j = 3,4, \tag{23}$$

which reduces (22) to the Lotka–Volterra analog (11), but with the parameters defined by

$$\alpha_i = \sum_j \theta_{ij}R_j - D, \qquad \beta_{ii'} = \sum_j \theta_{ij}\Gamma_{i'j} , \qquad i, i' = 1, 2. \tag{24}$$

The extension to multiple substrates is obvious. One merely extends the substrate list to include (S_j: $j = 3,..., J$). The net result is again the Lotka–Volterra analog (11), but the subscript (j) in (24) now extends to (J). All four steady states may now exist. The details are given below

Case I: Null State

$$\hat{M}_1 = 0 = \hat{M}_2\,, \qquad \hat{C}_j = R_j\,, \qquad j = 3,\ldots, J.$$

Exists: Always.
Stable if:

$$(\alpha_1 < 0 > \alpha_2) \Leftrightarrow \Big(\sum_j \theta_{1j}R_j < D > \sum_j \theta_{2j}R_j\Big).$$

Case II: Competitive Exclusion of G_2

$$\hat{M}_1 = \frac{\alpha_1}{\beta_{11}} = \sum_j \frac{R_j - \hat{C}_j}{\lambda_{1j}}\,, \qquad \hat{M}_2 = 0, \qquad \sum_j \theta_{1j}\hat{C}_j = D.$$

Exists if: $(\alpha_1 > 0) \Leftrightarrow (\sum_j \theta_{1j}R_j > D)$.
Stable if:

$$\frac{\alpha_2}{\beta_{21}} = \frac{\sum_j \theta_{1j}R_j - D}{\sum_j \theta_{2j}\Gamma_{1j}} < \frac{\sum_j \theta_{1j}R_j - D}{\sum_j \theta_{1j}\Gamma_{1j}} = \frac{\alpha_1}{\beta_{11}} > 0.$$

Case III: Competitive Exclusion of G_1

$$\hat{M}_1 = 0, \qquad \hat{M}_2 = \frac{\alpha_2}{\beta_{22}} = \sum_j \frac{R_j - \hat{C}_j}{\lambda_{1j}}\,, \qquad \sum_j \theta_{1j}\hat{C}_j = D.$$

Exists if: $(\alpha_2 > 0) \Leftrightarrow (\sum_j \theta_{2j}R_j > D)$.
Stable if:

$$\frac{\alpha_1}{\beta_{12}} = \frac{\sum_j \theta_{1j}R_j - D}{\sum_j \theta_{1j}\Gamma_{2j}} < \frac{\sum_j \theta_{2j}R_j - D}{\sum_j \theta_{2j}\Gamma_{2j}} = \frac{\alpha_2}{\beta_{22}} > 0.$$

Case IV: Competitive Coexistence of G_1 and G_2

$$\hat{M}_1 = \frac{\alpha_1\beta_{22} - \alpha_2\beta_{12}}{\beta_{11}\beta_{22} - \beta_{12}\beta_{21}} > 0 < \frac{\alpha_2\beta_{11} - \alpha_1\beta_{21}}{\beta_{11}\beta_{22} - \beta_{12}\beta_{21}} = \hat{M}_2\,,$$

$$\sum_j \theta_{1j}\hat{C}_j = D, \qquad \sum_j \theta_{2j}\hat{C}_j = D.$$

Exists if either:

$$\frac{\alpha_1}{\beta_{12}} < \frac{\alpha_2}{\beta_{22}}\,, \qquad \frac{\alpha_1}{\beta_{11}} > \frac{\alpha_2}{\beta_{21}}\,, \qquad \frac{\beta_{11}}{\beta_{12}} < \frac{\beta_{21}}{\beta_{22}}$$

or

$$\frac{\alpha_1}{\beta_{12}} > \frac{\alpha_2}{\beta_{22}}\,, \qquad \frac{\alpha_1}{\beta_{11}} < \frac{\alpha_2}{\beta_{21}}\,, \qquad \frac{\beta_{11}}{\beta_{12}} > \frac{\beta_{21}}{\beta_{22}}\,.$$

Stable if: This latter situation applies.

Contrary to the situation with a single substrate, it *is* possible to have coexistence with two or more substrates. The solution to Case IV may also be

written as $\hat{\mathbf{M}} = \mathbf{B}^{-1}\mathbf{A}$, where $\hat{\mathbf{M}}' = [\hat{M}_1, \hat{M}_2]$, $\mathbf{A}' = [\alpha_1, \alpha_2]$, and $\mathbf{B}$ is the (2×2) matrix of β coefficients. This matrix $\mathbf{B}$ must admit of an inverse, and must therefore be nonsingular. Now $|\mathbf{B}| = (\beta_{11}\beta_{22} - \beta_{12}\beta_{21}) = 0$ for a single substrate, and Case IV cannot be stable, as mentioned above. For two or more substrates, however, the matrix $\mathbf{B}$ may be partitioned into separate matrices, one for each substrate

$$\mathbf{B} = \begin{bmatrix} \beta_{11} & \beta_{12} \\ \beta_{21} & \beta_{22} \end{bmatrix} = \mathbf{B}_3 + \cdots + \mathbf{B}_J, \tag{25}$$

with each such matrix taking the form

$$\mathbf{B}_j = \operatorname{diag}\{\theta_{ij}\} \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix} \operatorname{diag}\{\Gamma_{ij}\}.$$

In general, since each of these submatrices of order $(I = 2)$ is of unit rank, the rank of $\mathbf{B}$ is $H = \min(I = 2, J - I)$. For the case at hand, $H = I = 2 \leqslant J - I$, and $\mathbf{B}$ is of full rank. Hence

$$(\beta_{11}\beta_{22} - \beta_{12}\beta_{21}) = \Big(\sum_j \theta_{1j}\Gamma_{1j}\Big)\Big(\sum_j \theta_{2j}\Gamma_{2j}\Big) - \Big(\sum_j \theta_{1j}\Gamma_{2j}\Big)\Big(\sum_j \theta_{2j}\Gamma_{1j}\Big) \neq 0,$$

and coexistence is possible.

The global behavior of this two-genotype Lotka–Volterra system has been well studied, and may be summarized as follows: (i) Under washout conditions, the system proceeds to the null state, the only existing solution. (ii) Under growth conditions, the system proceeds to one of the steady states descibed by Cases II, III, and IV. (iii) If the mixed equilibrium (Case IV) exists and is stable, neither side solution (Case II or Case III) is stable. (iv) If the mixed equilibrium is unstable, both side solutions are stable, and the system proceeds to one or the other—depending on initial conditions. (v) If one side solution is stable and the other unstable, no mixed solution exists, and the system proceeds to the stable side solution. Which of these situations exists depends on the α and β parameters, which in turn may be specified by the experimental inputs.

Multiple Genotypes

Finally, consider the situation for multiple genotypes $\{G_i: i = 1,..., I\}$ and multiple substrates $\{S_j: j = I + 1,..., J\}$. The dynamic equations are given by

$$\begin{aligned} \dot{M}_i &= \Big(\sum_j \mu_{ij} - D\Big) M_i = \sum_j \dot{M}_{ij} = \sum_j (\mu_{ij} - \gamma_{ij} D) M_i, \\ &= \Big[\sum_j \frac{V_{ij} C_j}{K_{ij} \phi_i} - D\Big] M_i, \qquad i = 1,..., I, \\ \dot{C}_j &= (R_j - C_j) D - \sum_i \mu_{ij} \lambda_{ij} M_i, \qquad j = I + 1,..., J, \end{aligned} \tag{27}$$

with $\phi_i = [1 + \sum_j K_{ij}^{-1} C_j]$. The "state" equations take the form

$$\left[\dot{C}_j + \sum_i \lambda_{ij} \dot{M}_{ij}\right] = \left[R_j - C_j - \sum_i \Gamma_{ij} M_i\right] D \approx 0, \qquad j = I + 1, \ldots, J. \quad (28)$$

The net result is the generalized Lotka–Volterra analog (16), but with α and β parameters as defined in (24)—with suitable changes in the range of summation for i, i', and j. As mentioned earlier, the rank of $\mathbf{B}$ is $H = \min(I, J - I)$, and no more than H genotypes may persist. To ensure that $\mathbf{B}$ is well behaved, assume that $H = I \leqslant J - I$. In this case there are 2^I steady states which may exist. The conditions for existence and the stability properties of these various solutions are complicated. The existence conditions involve only the α and β parameters. The stability conditions also involve the ϕ_i measures. [The one- and two-genotype cases are degenerate, and the ϕ_i cancel from the Liapanov criteria, but for more than two genotypes they must be included.] Under most circumstances, the $\phi_i \sim 1$ at equilibrium, so that no serious difficulty should derive from this complication. The reader interested in a detailed discussion of the multiple-organism problem is referred to Levin (1970), May (1973), and Strobeck (1973). In any event, each of these Lotka–Volterra conditions has its chemostat "kinetic" analog, and the translation is straightforward, albeit elaborate.

Discussion

The model presented is predicated on two ideas. (1) Growth of an organism may be jointly limited by *alternate* substrates. (2) The organism cannot do everything simultaneously and must make a choice between these alternate substrates at any given moment. Experiments in our own lab (Smouse and Kosuda, 1977) and the work of Standing *et al.* (1972) experimentally verify the first contention for the case where the "limiting substrates" are alternate carbon sources. In principle, of course, one should expect alternate nitrogen sources, alternate phosphorous sources, etc., to yield the same result. The model assumes instantaneous adjustment to changing concentrations of these alternate substrates, an assumption which may not be entirely adequate in practice. One is reminded of the diauxie phenomenon seen in batch cultures (Monod, 1942), where alternate carbon sources are utilized sequentially, and the growth curve is biphasic. This same phenomenon exists under continuous culture conditions, but is not so pronounced. Moreover, at low substrate concentrations and/or near equilibrium the organisms utilize alternate substrates simultaneously (Chian and Mateles, 1968; Mateles and Chian, 1969; Standing *et al.*, 1972). Whether substrate choice is instantaneously reversible or sequential, the second contention is empirically verified.

The model is compatible with such substrate choice. The growth of G_1 on S_2

is greater in the absence of S_3 than in its presence, given equal concentrations of S_2 in both cases and the same dilution rate D. This is easily shown as follows.

$$\begin{aligned}\mu_{12}(S_3 \text{ present}) &= \frac{V_{12}C_2}{K_{12}[1 + K_{12}^{-1}C_2 + K_{12}^{-1}C_3]} < \frac{V_{12}C_2}{K_{12}[1 + K_{12}^{-1}C_2]} \\ &= \mu_{12}(S_3 \text{ absent}).\end{aligned} \tag{29}$$

Similarly, $\mu_{13}(S_2 \text{ present}) < \mu_{13}(S_2 \text{ absent})$. The general relationship is seen to be μ_{ij} (multiple substrates) $< \mu_{ij}$ (only S_j present).

In spite of this fact, a very interesting comparison may be made of the equilibrium substrate concentrations. Consider again G_1, S_2, and S_3, and assume that G_1 may grow on either substrate alone (i.e., $\theta_{12}R_2 > D < \theta_{13}R_3$). Now, the single-substrate media yield equilibrium concentrations $\theta_{12}\tilde{C}_2 = D$ and $\theta_{13}\tilde{C}_3 = D$, respectively, and the mixed substrate medium yields $\theta_{12}\hat{C}_2 + \theta_{13}\hat{C}_3 = D$. It follows that

$$[\theta_{12}\hat{C}_2 + \theta_{13}\hat{C}_3 - \theta_{12}\tilde{C}_2] = D - D = [\theta_{12}\hat{C}_2 + \theta_{13}\hat{C}_3 - \theta_{13}\tilde{C}_3]$$

or

$$[\theta_{12}(\hat{C}_2 - \tilde{C}_2) + \theta_{13}\hat{C}_3] = 0 = [\theta_{12}\hat{C}_2 + \theta_{13}(\hat{C}_3 - \tilde{C}_3)]. \tag{30}$$

Since each element of (30) is positive, we must have $\hat{C}_2 < \tilde{C}_2$ and $\tilde{C}_3 > \hat{C}_3$. In general, $\hat{C}_j < \tilde{C}_j$, provided all $\theta_{ij}R_j > D$. Another way to say this is that an organism supported by multiple substrates is in a position to extract a bit more from each one than if it were supported by only that one. It follows that $\hat{M}_1$ is greater for a mix of substrates than the sum of its value on the separate substrates, i.e.,

$$\sum_j \frac{R_j - \hat{C}_j}{\lambda_{1j}} - \sum_j \frac{R_j - \tilde{C}_j}{\lambda_{1j}} = \sum_j \frac{\tilde{C}_j - \hat{C}_j}{\lambda_{1j}} > 0. \tag{31}$$

Having raised the possibility that more than a single substrate may be "limiting," one is inevitably led to ask how any *one* substrate (or *class* of substrates) can be limiting, irrespective of the concentrations of other types of substrates. Rather than simply ignore all but one substrate, consider a model

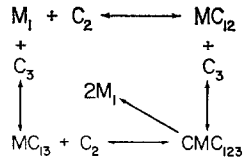

FIG. 4. Autocatalytic growth model for a system jointly limited by the concentrations of two substrates. M_1 = mass of G_1, C_2 = concentrations of S_2, C_3 = concentration of S_3.

where the single genotype G_1 is *jointly* limited by a pair of substrates (S_2 and S_3), e.g., a carbon source and a nitrogen source. Let growth be described as in Fig. 4, which is the analog of a random bisubstrate reaction. The formal growth equation is given by

$$\dot{M}_1 = \left[\frac{V_{123}C_2C_3}{(K_{12} + C_2)(K_{13} + C_3)} - D\right] M_1 . \tag{32}$$

Now, if $C_3 \gg K_{13}$, Eq. (32) takes the approximate form shown as Eq. (1), where $V_{12} = V_{123}C_3/(K_{13} + C_3) \approx V_{123}$. Now, suppose all $(J - 1)$ substrates are *jointly* required. The analog of (32) is

$$\dot{M}_1 = \left[V_{12\cdots J}\prod_{j=2}^{J}\frac{C_j}{K_{1j} + C_j} - D\right] M_1 , \tag{33}$$

which again converges to (1) if $C_j \gg K_{1j}$ $(j = 3,\ldots, J)$. It should be obvious, of course, that since the C_j are changing over time, V_{12} is not really a constant. It is customary to set the reservoir concentrations of all but one substrate very high $(R_j \gg K_{1j})$, and under these conditions, Eq. (1) should be a good approximation. If the pool of "nonlimiting" substrates is seriously depleted in the process of growth, however, such that (33) is no longer a close approximation to (1), then the simple Monod model (or the extensions used here) will fail to adequately describe the behavior of the system. Megee *et al.* (1972) have found similar bisubstrate models useful for describing the behavior of organisms *jointly* limited by a pair of substrates.

The fine detail of the models presented also depends in no small way on the adequacy of the Michaelis–Menten assumptions. A variety of authors (e.g., Herbert, 1958; McGrew and Malette, 1962; Marr *et al.*, 1963; Pirt, 1965, 1972; Droop, 1968, 1974) have shown that one must also consider uptake rates and internal substrate pools for the organisms. Such considerations require theoretical modifications, and the details are given by Droop (1974), Taylor and Williams (1975), and Veldkamp (1977).

I have assumed throughout that growth is limited only by the availability of one or more crucial *substrate(s)*. A voluminous literature (cf. Contois, 1959; Ramkrishna *et al.*, 1967; Jannasch and Mateles, 1974) suggests that the buildup of *metabolic by-products* may limit growth in many situations. Current models are unwieldy, but it seems likely that feedback inhibition analogs might provide a more tractable set of formulations with which to model this situation. The extension to metabolic cross-inhibition and cross-feeding is fairly apparent. A limited amount of work has also been done on the theory of predation in continuous cultures (e.g., Bungay and Cline, 1967; Bungay, 1968; Drake *et al.*, 1966, 1969; Noack, 1968; Tsuchiya *et al.*, 1972; Jost *et al.*, 1973), where the prey are underpinned by a single limiting resource. It is easy to extend the formulations presented here to the case of multiple predators, multiple prey, and

multiple substrates. I shall deal with these and other problems in future communications.

It should be evident that one may devise models of almost any complexity desired, almost at will. The formulation presented here may be viewed as a particularization of a very much more general treatment by Stewart and Levin (1973), who did not specify the manner in which multiple substrates were to be incorporated. An alternative particularization has been used by Peterson (1975) to investigate phytoplankton diversity. Still other formulations can easily be imagined.

What remains is to evaluate the efficacy of these various formulations experimentally. We are currently working on the competition models in my own lab. Whether the models presented are *quantitatively* accurate remains to be seen, but it is already clear that they are *qualitatively* better than the classical models, which simply cannot account for the variety of results obtained under continuous culture conditions. In a purely theoretical vein, these new models provide some additional insights into the likely dynamic behavior of continuous culture microcosms which should be of interest to the population biologist.

Appendix

The purpose of this appendix is to indicate the translation of the stability conditions derived from the chemostat models into the analogous conditions for the Lotka–Volterra transformations. I shall present the results for the one genotype–one substrate and two genotypes–one substrate models in some detail, and shall then indicate the extensions to more genotypes and/or substrates.

One Genotype–One Substrate

The Jacobian ($\mathbf{J}_{MC}$) of the chemostat model, from Eqs. (2), (3), and (7) of the text, is seen to be

$$\mathbf{J}_{MC} = \begin{bmatrix} \frac{\partial \dot{M}_1}{\partial M_1} & \frac{\partial \dot{M}_1}{\partial C_1} \\ \frac{\partial \dot{C}_1}{\partial M_1} & \frac{\partial \dot{C}_2}{\partial C_2} \end{bmatrix} = \begin{bmatrix} (\mu_{12} - D) & M_1 \frac{\partial \mu_{12}}{\partial C_2} \\ -\mu_{12}\lambda_{12} & -D - M_1\lambda_{12} \frac{\partial \mu_{12}}{\partial C_2} \end{bmatrix} \tag{A1}$$

It is convenient to introduce a transformation at this juncture. Capitalizing on the "state" equation (4), we define

$$\mathbf{X} = \begin{bmatrix} X_1 \\ X_2 \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ \lambda_{12} & 1 \end{bmatrix} \cdot \begin{bmatrix} M_1 \\ C_2 \end{bmatrix} = \mathbf{P} \begin{bmatrix} M_1 \\ C_2 \end{bmatrix}. \tag{A2}$$

From the discussion in the text, it is obvious that $X_1 = M_1$ and that $X_2 \rightarrow R_2$

in the vicinity of any steady state. The Jacobian of the transformation is given by

$$\mathbf{J}_X = \mathbf{P}\mathbf{J}_{MC}\mathbf{P}^{-1} = \begin{bmatrix} (\mu_{12} - D) - M_1\lambda_{12}\dfrac{\partial\mu_{12}}{\partial C_2} & M_1\dfrac{\partial\mu_{12}}{\partial C_2} \\ 0 & -D \end{bmatrix}. \tag{A3}$$

The eigenroots (w) of $\mathbf{J}_X$ are the same as those of $\mathbf{J}_{MC}$, but are easier to obtain. In general, such transformations as (A2) reduce the Jacobian to block triangular form, simplifying the analysis.

For the case at hand, the roots (w) are evident by inspection and take the form

$$w_1 = (\mu_{12} - D) - M_1\lambda_{12}\frac{\partial\mu_{12}}{\partial C_2}, \qquad w_2 = -D. \tag{A.4}$$

Since $D > 0$, w_2 is always negative, and we need merely evaluate w_1. Stability of a particular steady state requires that both roots have negative real parts. For Case I ($\hat{M}_1 = 0$, $R_2 = \hat{C}_2$), the second term of w_1 is zero, and stability requires $(\mu_{12} - D) < 0$. Simple algebraic manipulation shows that this condition implies $\theta_{12}R_2 < D$, which may also be written as $\alpha_1 < 0$. For Case II ($\hat{M}_1 > 0$, $R_2 > \hat{C}_2$), the first term of w_1 is zero, and the second takes the form

$$-\hat{M}_1\lambda_{12}\frac{\partial\mu_{12}}{\partial C_2} = -\hat{M}_1\lambda_{12}\left[\frac{V_{12}}{K_{12}\hat{\phi}_1} - \frac{V_{12}\hat{C}_2}{K_{12}^2\hat{\phi}_1^{\,2}}\right] = -\hat{M}_1\lambda_{12}\frac{V_{12}}{K_{12}\hat{\phi}_1^{\,2}}, \tag{A5}$$

which is negative only if $\hat{M}_1 > 0$. This condition, in turn, requires that $\theta_{12}R_2 > D$ ($\alpha_1 > 0$). These conditions are listed in the text.

Two Genotypes–One Substrate

From the text Eqs. (9), the Jacobian can be shown to take the form

$$\mathbf{J}_{MC} = \begin{bmatrix} (\mu_{13} - D) & 0 & M_1\dfrac{\partial\mu_{13}}{\partial C_3} \\ 0 & (\mu_{23} - D) & M_2\dfrac{\partial\mu_{23}}{\partial C_3} \\ -\lambda_{13}\mu_{13} & -\lambda_{23}\mu_{23} & -D - M_1\lambda_{13}\dfrac{\partial\mu_{13}}{\partial C_3} - M_2\lambda_{23}\dfrac{\partial\mu_{23}}{\partial C_3} \end{bmatrix}. \tag{A6}$$

Capitalizing on the state equation (10), one constructs the transform

$$\mathbf{X} = \begin{bmatrix} X_1 \\ X_2 \\ X_3 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ \lambda_{13} & \lambda_{23} & 1 \end{bmatrix} \cdot \begin{bmatrix} M_1 \\ M_2 \\ C_3 \end{bmatrix} = \mathbf{P}\begin{bmatrix} M_1 \\ M_2 \\ C_3 \end{bmatrix}. \tag{A7}$$

Near any steady state, $X_1 = M_1$, $X_2 = M_2$, $X_3 \to R_3$. The Jacobian of the transformation (A7) is given by

$$\mathbf{J}_X = \mathbf{P}\mathbf{J}_{MC}\mathbf{P}^{-1} = \left[\begin{array}{c|c} \mathbf{J}_M & \mathbf{Q} \\ \hline \mathbf{O} & -\mathbf{D} \end{array}\right] \tag{A.8}$$

$$= \left[\begin{array}{cc|c} (\mu_{13} - D) - M_1\lambda_{13}\dfrac{\partial\mu_{13}}{\partial C_3} & -M_1\lambda_{23}\dfrac{\partial\mu_{13}}{\partial C_3} & M_1\dfrac{\partial\mu_{13}}{\partial C_3} \\ -M_2\lambda_{13}\dfrac{\partial\mu_{23}}{\partial C_3} & (\mu_{23} - D) - M_2\lambda_{23}\dfrac{\partial\mu_{23}}{\partial C_3} & M_2\dfrac{\partial\mu_{23}}{\partial C_3} \\ \hline 0 & 0 & -D \end{array}\right].$$

Since $w_3 = -D < 0$, we need only be concerned with the (2×2) matrix $\mathbf{J}_M$. For Case I ($\hat{M}_1 = 0 = \hat{M}_2$, $R_3 = C_3$), most of the terms in $\mathbf{J}_M$ vanish, and we have $w_1 = (\mu_{13} - D)$ and $w_2 = (\mu_{23} - D)$. Both are negative, and the null set is stable, iff $\theta_{13}R_3 < D > \theta_{23}R_3$, which can also be written as $\alpha_1 < 0 > \alpha_2$, the conditions indicated in the text. For Case II ($\hat{M}_1 > 0 = \hat{M}_2$, $R_3 > \hat{C}_3$), the matrix $\mathbf{J}_M$ becomes triangular, and the roots become

$$w_1 = -M_1\lambda_{13}\frac{\partial\mu_{13}}{\partial C_3} = -M_1\lambda_{13}\frac{V_{13}}{K_{13}\hat{\phi}_1{}^2}, \qquad w_2 = (\mu_{23} - D). \tag{A9}$$

Algebraic manipulation shows that both roots are negative only if $\theta_{23}R_3 < \theta_{13}R_3 > D$, which may also be written as $\alpha_2 < \alpha_1 > 0$ or even as

$$\frac{\alpha_2}{\beta_{21}} < \frac{\alpha_2}{\beta_{11}} > 0, \tag{A10}$$

as given in the text. This latter is, of course, the usual Lotka–Volterra condition. By symmetry, Case III ($\hat{M}_1 = 0 < \hat{M}_2$, $R_3 > \hat{C}_3$) yields eigenroots from $\mathbf{J}_M$ which take the form

$$w_1 = (\mu_{13} - D), \qquad w_2 = -M_2\lambda_{23}\frac{\partial\mu_{23}}{\partial C_3} = -M_2\lambda_{23}\frac{V_{23}}{K_{23}\phi_2{}^2}. \tag{A11}$$

Stability requires $\theta_{13}R_3 < \theta_{23}R_3 > D$, which may also be written as $\alpha_1 < \alpha_2 > 0$ or even

$$\frac{\alpha_1}{\beta_{12}} < \frac{\alpha_2}{\beta_{22}} > 0, \tag{A12}$$

as given in the text. Case IV ($\hat{M}_1 > 0 < \hat{M}_2$, $R_3 > \hat{C}_3$) imples that $(\mu_{13} - D) = 0 = (\mu_{23} - D)$, makes $\mathbf{J}_M$ singular, and yields the eigenvalues

$$w_1 = 0, \qquad w_2 = -\hat{M}_1\lambda_{13}\frac{\partial\mu_{13}}{\partial C_3} - \hat{M}_2\lambda_{23}\frac{\partial\mu_{23}}{\partial C_3}. \tag{A13}$$

When one or more roots (w) are zero, this Liapanov treatment cannot be used

to assess stability, but further analysis indicates that no joint (coexistence) steady state exists for the two genotypes–one substrate model. As pointed out in the text, this follows from the fact that $(\beta_{11}\beta_{22} - \beta_{12}\beta_{21}) = 0$, the corresponding Lotka–Volterra statement.

Beginning with the *two genotypes–two substrates* model, it is appropriate to partiton $\dot{M}_1$ into $\dot{M}_{13}$ and $\dot{M}_{14}$ and to partition $\dot{M}_2$ into $\dot{M}_{23}$ and $\dot{M}_{24}$, as in text Eq. (22). This yields a Jacobian of order *six* (rather than *four*). Transformations such as those used above, always derived from the "state" equations, reduce the Jacobian to block-diagonal form. Further matrix manipulations (extremely tedious but nonetheless straightforward) demonstrate that the Lotka–Volterra stability conditions listed in the text are equivalent to the chemostat model statements. Notably, the conditions for a stable mixed equilibrium come down to the requirement that $\hat{M}_2 > 0 < \hat{M}_2$ and that $(\Gamma_{13}\Gamma_{24} - \Gamma_{14}\Gamma_{23}) \times (\theta_{13}\theta_{24} - \theta_{14}\theta_{23}) > 0$. This latter can easily be shown to be $(\beta_{11}\beta_{22} - \beta_{12}\beta_{21}) > 0$, as claimed in the text. As also pointed out above, one requires at least two substrates for this condition to be satisfied.

The extension to more genotypes and/or more substrates is obvious. For any mixed-genotype steady state, it develops that the Jacobian matrix $\mathbf{J}_M$ is inherently singular *unless* there are at least as many substrates as genotypes. The singularity of $\mathbf{J}_M$ means that any such mixed solutions would not be stable. As I pointed out above, the singularity of $\mathbf{J}_M$ is implied by the singularity of $\mathbf{B}$, and no mixed solution set can exist where the corresponding $\mathbf{B}$ matrix is singular. Even in the permissible cases $(J - I \geqslant I)$, the matrix $\mathbf{J}_M$ is nonsingular only if $\mathbf{B}$ is nonsingular. For multiple genotypes and substrates, the nonsingularity of $\mathbf{B}$ is necessary but not sufficient for stability, and the model must also meet certain constraints on the ϕ measures.

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