A Mathematical Theory for Single-Nutrient Competition in Continuous Cultures of Micro-Organisms

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A MATHEMATICAL THEORY FOR SINGLE-NUTRIENT COMPETITION IN CONTINUOUS CULTURES OF MICRO-ORGANISMS*

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Abstract. The continuous culture of micro-organisms using the chemostat is an important research technique in microbiology and population biology. It offers advantages in the form of economical production of micro-organisms for the industrial microbiologist and is a laboratory idealization of nature for population studies. The paper studies a mathematical model, based on Michaelis–Menten kinetics, for one substrate and n competing species. Given the parameters of the system, we answer the basic question as to which species survive and which do not, and determine the limiting behaviors. The primary conclusion is that the species will survive whose Michaelis–Menten constant is smallest in comparison with its intrinsic rate of natural increase.

1. Introduction. The continuous culture of micro-organisms using the chemostat (Novick and Szilard [13]) is an important research technique in microbiology and population biology. It has been used extensively for the isolation and identification of metabolic mutant strains, and it offers advantages in the form of economical production of micro-organisms to the industrial microbiologist (Herbert, Ellsworth, and Telling [4]). It has been used extensively in studies of general properties of population growth and interaction among micro-organisms (Williams [20]; Tsuchiya, Jost, and Fredrickson [19]; Canale, Lustig, Kehrberger, and Salo [1]).

The continuous culture technique, which has extensive description in the literature (for example, see Kubitschek [7]) basically consists of growing a population or several populations of micro-organisms in a culture vessel, into which growth medium is continually added at a fixed rate, and from which medium, cells and by-products are continually removed. The medium supplies all the nutrients or substrates needed for growth of the organisms in excess of demand except for one, which is supplied in limiting amounts. The interest is focused on the several populations competing for the single limiting substrate. The "several" populations may be a deliberate culture of mixed populations, or it may arise as contaminants or mutants of the strains of organisms being cultured (cf. Powell [14]).

The chemostat is perhaps the best laboratory idealization of nature for population studies (Williams [20]). All natural systems are open systems for energy and material substances. The input and removal of nutrients to and from the chemostat represent the continuous turnover of nutrients in nature. The outflow of organisms is formally equivalent to nonspecific death, predation, or emigration, which always occur in nature.

The close parallels in nature are the planktonic communities of unicellular algae in lakes and oceans. The multispecies communities receive nutrient inputs from streams draining eroding watersheds or continental margins, and in lakes from nutrient regeneration during spring and fall overturn (Hutchinson [5, Chaps. 7, 12]). Nonspecific death occurs as cells continually sink out of the well-lit upper layers of water to the unlit bottom of the water column. During the summer months between lake overturns, it is invariably the case that some one nutrient becomes limiting. The nutrient in question may be any one of a variety including phosphorus, nitrogen, silica in the case of diatoms, or even a vitamin such as B_{12}. Moreover, it is generally the case that the same nutrient is limiting to most if not all of the species of planktonic algae at any given time (for example, see Schelske, Rothman, Stoermer, and Santiago [15]). An important fact is that these nutrients are not metabolically substitutable, but rather are metabolic complementary. Growth is therefore limited by the one nutrient in shortest supply, such that the addition of more of other nutrients has no accelerating effect on growth whatsoever. Consequently the chemostat culture with one limiting nutrient is a reasonable model system under these circumstances.

A mathematic model of such systems, featuring the familiar Michaelis–Menten kinetics of the uptake of the limiting substrate, goes back to Monod [11]. The derivation of the model with one substrate and one population is given in various places (for example, see Herbert et al. [4]). The important biological features of this kinetic model are: (a) at low nutrient concentration the rate of uptake and growth is limited by, and proportional to, nutrient concentration, whereas (b) at high concentration the uptake and growth rates saturate and become constant, independent of nutrient concentration. The extension of the basic model (which we use below) to one substrate and several populations appears, for example, in Taylor and Williams [18]. The term nutrient or substrate should be interpreted in a wide sense; as pointed out by Taylor and Williams, it could be an energy source of either organic material or light, a major carbon, nitrogen, or phosphate source, or some trace nutrient (vitamins). With minor modification, the uptake of all these nutrients basically follow Michaelis–Menten kinetics. The analysis presented here is applicable to all such resource-limited systems.

This paper uses the general deterministic model for one substrate and n competing species or strains, and presents a rigorous mathematical analysis of the asymptotic behavior of this system. In particular, given the parameters of the system—growth rates, Michaelis–Menten constants, input concentration of the limiting nutrient, and dilution and death rates—we answer the question of which species survive and which do not, and determine the limiting values. Although some partial results exist in the literature on this problem, we believe that this paper represents the most complete treatment of the system yet available. In particular, it generalizes the work of Powell [14], makes his conclusions mathematically rigorous, and gives a mathematical explanation to some observations of Taylor and Williams [18] in their numerical experiments.

2. The model. The general continuous flow culture is described briefly in the Introduction and in detail in the references cited. We specifically assume that the

input concentration, $S^{(0)}$, and the dilution rate, D, are constant, the only competition between species is for the nutrient (no toxins are produced, for example), and that the mixing in the vessel is perfect. Further, it is assumed that growth rates adjust instantaneously to changes in the nutrient concentration, i.e., there are no time lags in the system. With these assumptions, the model is given by (Taylor and Williams [18])

$$S'(t) = (S^{(0)} - S(t))D - \sum_{i=1}^{n} \frac{m_i}{y_i} \frac{x_i(t)S(t)}{a_i + S(t)},$$

$$x_i'(t) = \frac{m_i x_i(t)S(t)}{a_i + S(t)} - Dx_i(t),$$

$$(2.1)_n$$

$$S(0) = S_0 > 0,$$

$$x_i(0) = x_{i0} > 0, \qquad\qquad i = 1, 2, \cdots, n, \quad n \geqq 1,$$

where

$$' = \frac{d}{dt}$$

$t = $ time

$x_i(t) = $ concentration of ith organism at time t

$S(t) = $ concentration of substrate at time t

$m_i = $ maximum specific growth rate for the ith organism

$y_i = $ cell growth yield for the ith organism

$a_i = $ Michaelis–Menten constant for the ith organism.

We analyze the behavior of solutions of this system of ordinary differential equations.

3. Statement of results. In this section we state the principal results of the paper. The proofs and certain technical lemmas are deferred to the next section. The first lemma is a statement that the system $(2.1)_n$ is as "well-behaved" as one intuits from the biological problem.

LEMMA 3.1. *The solutions $S(t)$, $x_i(t)$, $i = 1, \cdots, n$, of $(2.1)_n$ are positive and bounded.*

The first theorem provides conditions under which the organism cannot survive given the fixed dilution rate and the fixed input rate of nutrient.

THEOREM 3.2. *Let $b_i = m_i/D$, $i = 1, \cdots, n$. If*

(i) $b_i \leqq 1$,

or

(ii) $\dfrac{a_i}{b_i - 1} > S^{(0)}$ *if $b_i > 1$,*

then $\lim_{t \to \infty} x_i(t) = 0$.

This theorem states that if the maximum growth rate m_i of the ith organism is less than the dilution rate or if the parameter $a_i/(b_i - 1) > S^{(0)}$, the organism will

die out as time becomes large. Note that the resulting behavior is competition-independent.

Our basic hypothesis is

$$0 < \frac{a_1}{b_1 - 1} < \frac{a_2}{b_2 - 1} \leqq \cdots \leqq \frac{a_n}{b_n - 1},$$

(H$_n$)

$$\frac{a_1}{b_1 - 1} < S^{(0)}, \qquad b_i > 1.$$

The equations may be relabeled without loss of generality, so that the parameters $\lambda_i = a_i/(b_i - 1)$ are nondecreasing in i. (H$_n$) excludes equality of this parameter for the first species.

THEOREM 3.3. *Let* (H$_n$) *hold. The solutions of* (2.1)$_n$ *satisfy*

$$\lim_{t \to \infty} S(t) = \frac{a_1}{b_1 - 1},$$

$$\lim_{t \to \infty} x_1(t) = y_1 \left(S^{(0)} - \frac{a_1}{b_1 - 1} \right),$$

$$\lim_{t \to \infty} x_i(t) = 0, \qquad 2 \leqq i \leqq n.$$

This theorem states that under the hypothesis (H$_n$) only one species survives, the one with the lowest value of λ_i, and gives the limiting concentrations. For a given species, the parameter λ_i depends on two measured quantities, the growth rate and the Michaelis–Menten constant. It is biologically reasonable to assume that for two distinct species, the corresponding parameters will be different. Hence (H$_n$) (with all strict inequalities) is a biologically reasonable assumption.

If $a_1/(b_1 - 1) = S^{(0)}$, then $\lim_{t \to \infty} x_i(t) = 0, i = 2, \cdots, n$ by Theorem 3.2. In this case, however, the lowest species also dies out.

THEOREM 3.4. *Let* (H$_n$) *hold except that* $a_1/(b_1 - 1) = S^{(0)}$. *Then*

$$\lim_{t \to \infty} S(t) = S^{(0)},$$

$$\lim_{t \to \infty} x_i(t) = 0, \qquad \qquad i = 1, \cdots, n.$$

The following theorem considers the case of equal λ's.

THEOREM 3.5. *Let*

$$0 < S^* = \frac{a_1}{b_1 - 1} = \frac{a_2}{b_2 - 1} = \cdots = \frac{a_n}{b_n - 1},$$

where $b_1 > 1$. *If* $S^* < S^{(0)}$, *then*

$$\lim_{t \to \infty} S(t) = S^{(0)},$$

and

$$\lim_{t\to\infty} x_i(t) = x_i^* > 0, \qquad\qquad i = 1, \cdots, n,$$

where

$$S^* + \sum_{i=1}^n \frac{x_i^*}{y_i} = S^{(0)}.$$

If $S^* = S^{(0)}$, then

$$\lim_{t\to\infty} S(t) = S^{*\prime}$$

and

$$\lim_{t\to\infty} x_i(t) = 0, \qquad\qquad i = 1, 2, \cdots, n.$$

4. Proofs.

Proof of Lemma 3.1. Since $x_i(0) = x_{i0} > 0$, then by the representation

(4.1) $$x_i(t) = x_{i0} \exp\left(\int_0^t \left(\frac{m_i S(\xi)}{a_i + S(\xi)} - D\right) d\xi\right),$$

we have $x_i(t) > 0$ provided $S(\xi) > -a_i$ for $0 \leq \xi \leq t$. Suppose that $S(t)$ is not positive for all $t \geq 0$. Since $S(0) = S_0 > 0$ then there exists a point T_0 with $S(T_0) = 0$ and $S(t) > 0$ for $0 \leq t < T_0$. For $0 \leq t \leq T_0$,

$$S'(t) > -S(t)D - \sum_{i=1}^n \frac{m_i}{y_i} \frac{x_i(t)S(t)}{a_i + S(t)}$$

or

$$\frac{S'(t)}{S(t)} > -D - \sum_{i=1}^n \frac{m_i}{y_i} \frac{x_i(t)}{a_i + S(t)}.$$

Integrating from 0 to T_0 and taking the exponential of both sides, it follows that

$$S(T_0) > S_0 \exp\left(\int_0^{T_0} \left\{-D - \sum_{i=1}^n \frac{m_i}{y_i} \frac{x_i(\xi)}{a_i + S(\xi)}\right\} d\xi\right) > 0.$$

This is a contradiction and hence $S(t), x_i(t)$ are positive for all $t \geq 0$.

Multiplying the equation for x_i in $(2.1)_n$ by $1/y_i$ and adding yield

(4.2) $$S'(t) + \sum_{i=1}^n \frac{x_i'(t)}{y_i} = S^{(0)}D - D\left(S(t) + \sum_{i=1}^n \frac{x_i(t)}{y_i}\right)$$

which is a linear equation with constant coefficient in the variable $S(t) + \sum_{i=1}^n (x_i(t)/y_i)$. Solving this equation yields

(4.3) $$S(t) + \sum_{i=1}^n \frac{x_i(t)}{y_i} = A_n e^{-Dt} + S^{(0)},$$

where $A_n = (S_0 + \sum_{i=1}^n x_{i0}/y_i) - S^{(0)}$. As $t \to \infty$, $A_n e^{-Dt} \to 0$. The sum on the left side is bounded, and since each term is positive, each term is bounded. In particular, for $\varepsilon > 0$, there exists t_0, such that if $t \geq t_0$, $S(t) \leq S^{(0)} + \varepsilon$.

Proof of Theorem 3.2. A rearrangement of (4.1) yields

$$x_i(t) = x_{i0} \exp \int_0^t \left(\frac{(m_i - D)S(\xi) - a_i D}{a_i + S(\xi)} \right) d\xi.$$

If $b_i \leq 1$, then

$$x_i(t) \leq x_{i0} \exp \int_0^t \left(\frac{-a_i D}{a_i + S(\xi)} \right) d\xi$$

$$\leq C x_{i0} \exp \left(\frac{-a_i D}{a_i + S^{(0)} + 1} \right)(t - t_0),$$

where t_0 is chosen so that for $t \geq t_0$, $S(t) \leq S^0 + 1$ and $C = \exp \int_{t_0} (-a_i D/(a_i + S(\xi)))\, d\xi$. Since the exponent is negative and $x_i(t) > 0$, $\lim_{t \to \infty} x_i(t) = 0$. Rearranging (4.1) yields

(4.1')
$$x_i(t) = x_{i0} \exp \int_0^t \left(\frac{m_i - D}{a_i + S(\xi)} \right)\left(S(\xi) - \frac{a_i}{b_i - 1} \right) d\xi.$$

If $b_i > 1$, then the first factor of the integrand is positive. Let $0 < \varepsilon < (a_i/(b_i - 1)) - S^{(0)}$, and choose $t_0 > 0$ such that $S(t) \leq S^{(0)} + \varepsilon$ for $t \geq t_0$. Then for an appropriate constant C, it follows that

$$x_i(t) \leq C x_{i0} \exp \left\{ \left(\frac{m_i - D}{a_i + S^{(0)} + 1} \right)\left(S^{(0)} + \varepsilon - \frac{a_i}{b_i - 1} \right)(t - t_0) \right\}.$$

The first factor in the exponent is positive, the second is negative, so $\lim_{t \to \infty} x_i(t) = 0$. Hereafter we always assume $b_1 > 1$.

We collect now some elementary facts which follow directly from (H_n) and the form of equation, and which will be used in proofs that follow.

(F-1)
$$\text{If } S(t) > \frac{a_i}{b_i - 1}, \quad x_i'(t) > 0.$$

$$\text{If } S(t) = \frac{a_i}{b_i - 1}, \quad x_i'(t) = 0.$$

$$\text{If } S(t) < \frac{a_i}{b_i - 1}, \quad x_i'(t) < 0.$$

(F-2) $\qquad\qquad x_i''(t)$ exists and (using Lemma 3.1) is bounded.

We note now the following lemma ([9, p. 345]).

LEMMA 4.1. *Let* $\omega(t) \in C^2[t_0, \infty)$, $\omega(t) \geq 0$, $K > 0$.
  (i) *If* $\omega'(t) \geq 0$, $\omega(t)$ *is bounded and* $\omega''(t) < K$ *for all* $t \geq t_0$, *then* $\omega'(t) \to 0$ *as* $t \to \infty$.
  (ii) *If* $\omega'(t) \leq 0$ *and* $\omega''(t) \geq -K > -\infty$ *for all* $t \geq t_0$, *then* $\omega'(t) \to 0$ *as* $t \to \infty$.
This lemma and (F-2) combine to yield

(F-3)   If $x_i(t)$ is monotone and $\lim_{t \to \infty} x_i(t) = x_i^* > 0$,

then $\lim_{t \to \infty} S(t) = \dfrac{a_i}{b_i - 1}$.

LEMMA 4.2. *Suppose* (H_n) *holds. For some* t_0, *if*

$$S(t) < \frac{a_1}{b_1 - 1} \quad \text{for } t \geq t_0$$

or if

$$S(t) > \frac{a_1}{b_1 - 1} \quad \text{for } t \geq t_0,$$

then

$$\lim_{t \to \infty} S(t) = S^* = \frac{a_1}{b_1 - 1},$$

$$\lim_{t \to \infty} x_i(t) = x_1^* = y_1(S^{(0)} - S^*),$$

$$\lim_{t \to \infty} x_1(t) = 0, \qquad\qquad\qquad i = 2, \cdots, n.$$

Proof. Suppose first that $S(t) < a_1/(b_1 - 1)$ for $t \geq t_0$. Then by (F-1), $x_i'(t) < 0$, $t \geq t_0$, $i = 1, \cdots$, and $\lim_{t \to \infty} x_i(t) = x_i^*$ exists. By (F-3), $x_i^* > 0$ for any $i \neq 1$ would contradict the assumption $S(t) < a_1/(b_1 - 1)$, $t \geq t_0$. If $x_1^* = 0$ as well, $\lim_{t \to \infty} S(t) = S^{(0)}$ by (4.3), but $S^{(0)} > a_1/(b_1 - 1)$ by (H_n), which for sufficiently large t would contradict the fact $S(t) < a_1/(b_1 - 1)$. Hence $x_1^* > 0$ and $x_i^* = 0$, $i = 2, \cdots, n$. From (F-3), it follows that $\lim_{t \to \infty} S(t) = S^* = a_1/(b_1 - 1)$.

If there is a point t_0 such that $S(t) > a_1/(b_1 - 1)$ for $t \geq t_0$, then $x_1'(t) > 0$. Since $x_1(t)$ is bounded, $\lim_{t \to \infty} x_1(t) = x_1^* > 0$ exists. From (F-3), it follows that $\lim_{t \to \infty} S(t) = a_1/(b_1 - 1)$. Since $a_i/(b_i - 1) > a_1/(b_1 - 1)$, $i = 2, \cdots, n$, $x_i'(t) < 0$, $i \neq 1$ for $T \geq T$, some T, so $\lim_{t \to \infty} x_i(t) = x_i^*$ exists. From (F-3), it follows that $x_i^* = 0$, $i \neq 1$.

As a consequence of this lemma, if $\lim_{t \to \infty} x_1(t)$ does not exist, $S(t)$ must be above and below $\lambda_1 = a_1/(b_1 - 1)$ for arbitrarily large values of t.

LEMMA 4.3. *Let*

$$0 < \frac{a_1}{b_1 - 1} \leq \frac{a_i}{b_i - 1} < \frac{a_k}{b_k - 1}.$$

If

    (i) $a_k \leq a_i$ *or*
    (ii) $a_k > a_i$ *and* $b_k \leq b_i$,
then $\lim_{t \to \infty} x_k(t) = 0$.

Proof. If there exists t_0 such that $S(t) \geq a_k/(b_k - 1)$ or $S(t) \leq a_i/(b_i - 1)$ for $t \geq t_0$, then, from (F-3), $\lim_{t \to \infty} x_k(t) = 0$. Hence we may assume there exists a point t_0 such that $a_i(b_i - 1) < S(t_0) < a_k/(b_k - 1)$. Let $\xi > 0$. Then

$$(4.4) \qquad \xi \frac{x_k'(t)}{Dx_k(t)} - \frac{x_i'(t)}{Dx_i(t)} = \frac{1}{(a_i + S(t))(a_k + S(t))} P_\xi(S(t)),$$

where

(4.5)
$$P_\xi(z) = z^2[\xi(b_k - 1) - (b_i - 1)] + z[\xi\{a_i(b_k - 1) - a_k\}$$
$$-\{a_k(b_i - 1) - a_i\}] - a_k a_i(\xi - 1),$$

(4.6)
$$P_\xi(S(t_0)) < 0, \quad \text{if } \xi > 0.$$

The lemma will be proved by obtaining a representation of $x_k(t)/x_i(t)$. To analyze this representation, information is needed about the quadratic $P_\xi(z)$. The technical arguments in the proof involve selection of a proper value of ξ To do this we first analyze this quadratic in some detail. Several proofs in the sequel make use of this type of argument.

The discriminant $D(\xi)$ of $P_\xi(z)$ is given by

(4.7)
$$D(\xi) = \xi^2[a_i(b_k - 1) + a_k]^2 - 2E\xi + [a_k(b_i - 1) + a_i]^2,$$

where

(4.8)
$$E = [a_i(b_k - 1) + a_k] \cdot [a_k(b_i - 1) + a_i] + 2(a_k - a_i) \cdot [a_i(b_k - 1) - a_k(b_i - 1)].$$

If $a_k < a_i$ and $a_i/(b_i - 1) < a_k/(b_k - 1)$, then $(a_k - a_i) \cdot [a_i(b_k - 1) - a_k(b_k - 1)] > 0$. Using this fact in the computation of the discriminant D^* of $D(\xi)$, we have that

(4.9)
$$D^* = E^2 - [a_i(b_k - 1) + a_k]^2[a_k(b_i - 1) + a_i]^2 > 0.$$

It follows that $D(\xi) = 0$ has two real roots ξ_1, ξ_2. Furthermore, ξ_1, ξ_2 are positive since $D(0) > 0$ and $D(\eta) > 0$ for all $\eta < 0$.

If $\xi^* > 0$ is chosen between ξ_1, ξ_2, then $D(\xi^*) < 0$. Hence $P_{\xi^*}(z) = 0$ has no real roots and by (4.6), $P_{\xi^*}(z) < 0$ for all z. Put $\xi = \xi^*$ in (4.4). It follows that

(4.10)
$$\xi^* \frac{x_k'(t)}{Dx_k(t)} - \frac{x_i'(t)}{Dx_i(t)} = \frac{1}{(a_i + S(t))(a_k + S(t))} P_{\xi^*}(S(t))$$
$$\leq \frac{1}{(a_i + S_{\max})(a_k + S_{\max})} \cdot \max_{0 \leq z \leq S_{\max}} P_{\xi^*}(z)$$
$$= \zeta < 0,$$

where $S_{\max} = \sup_{0 \leq t < \infty} S(t)$. Integrating from 0 to t and taking exponentials on both sides of (4.10) yields

$$\left(\frac{x_k(t)}{x_{k0}}\right)^{\xi^*/D} \leq \left(\frac{x_i(t)}{x_{i0}}\right)^{1/D} e^{\zeta t} \leq M e^{\zeta t}.$$

It follows that $\lim_{t \to \infty} x_k(t) = 0$, since $x_i(t)$ is positive and bounded and ζ is negative.

If $a_k = a_i$ and $a_i/(b_i - 1) < a_k/b_k - 1$, then $b_k < b_i$, and

$$x_k(t) = x_{k0} \left(\frac{x_i(t)}{x_{i0}}\right)^{m_k/m_i} \exp\left(m_k \cdot \left(\frac{1}{b_i} - \frac{1}{b_k}\right)\right).$$

Again we have $\lim_{t \to \infty} x_k(t) = 0$ and the proof for (i) is complete.

For case (ii) we have $a_k > a_i$, $b_k \leqq b_i$, and $a_i/(b_i - 1) < a_k/(b_k - 1)$. First suppose that $b_k < b_i$ and choose $\xi^* = (b_i - 1)/(b_k - 1) > 1$. Using ξ^* in (4.5) yields

$$P_{\xi^*}(z) = [\xi^*\{a_i(b_k - 1) - a_k\} - \{a_k(b_i - 1) - a_i\}]z - a_k a_i(\xi^* - 1).$$

Then

$$\xi^*\{a_i(b_k - 1) - a_k\} - \{a_k(b_i - 1) - a_i\}$$

$$= \frac{1}{b_k - 1}[(b_k - 1)(b_i - 1)(a_i - a_k) + (a_i(b_k - 1) - a_k(b_i - 1))]$$

$$< 0.$$

Equation (4.4) in this case gives

$$\xi^* \frac{x'_k(t)}{Dx_k(t)} - \frac{x'_i(t)}{Dx_i(t)} \leqq \frac{P_{\xi^*}(0)}{(a_i + S_{max})(a_k + S_{max})} < 0.$$

As in the proof of (i), it follows that $\lim_{t \to \infty} x_k(t) = 0$.

For the case $b_k = b_i$ in (4.8), (4.9), then

$$D^* = -4a_i a_k(a_i - a_k)^2(b_k - 1)b_k^2 < 0.$$

Hence $D(\xi) > 0$ for all ξ. Rewrite (4.5) for this case as

(4.11) $$P_\xi(z) = \xi[(b_k - 1)z - a_k][z + a_i] - [(b_k - 1)z - a_i][z + a_k].$$

Let $z_0 > \max[S_{max}, a_k/(b_k - 1)]$ and choose

$$\xi^* = \frac{[(b_k - 1)z_0 - a_i][z_0 + a_k]}{[(b_k - 1)z_0 - a_k][z_0 + a_i]}.$$

Since $a_k > a_i$, $\xi^* > 1$, and hence from (4.5), $P_{\xi^*}(z)$ has one positive and one negative root. However, $P_{\xi^*}(z_0) = 0$ and $P(a_k/(b_k - 1)) = -(a_k - a_i)(a_k b_k/(b_k - 1)) < 0$, so $P_{\xi^*}(S(t)) < 0$ on $0 < S(t) \leqq S_{max}$. The argument is completed as before using (4.4).

LEMMA 4.4. *There exists t_0 and $\gamma > 0$ such that $S(t) \geqq \gamma$ for $t \geqq t_0$.*

Proof. By (4.3), we choose t_0 such that

$$S(t) + \sum_{i=1}^{n} \frac{x_i(t)}{y_i} \leqq S^{(0)} + 1 \quad \text{for } t \geqq t_0,$$

and thereby obtain the estimate

$$\sum_{i=1}^{n} x_i(t) \leqq \left(\max_{1 \leqq i \leqq n} y_i\right)(S^{(0)} + 1).$$

By $(2.1)_n$, we have

$$S'(t) + \left(D + \sum_{i=1}^{n} \frac{m_i}{y_i} \cdot \frac{x_i(t)}{a_i + S(t)}\right)S(t) = S^{(0)}D,$$

or

$$S'(t) + \left(D + \left(\max_{1 \leqq i \leqq n} \frac{m_i}{a_i y_i}\right)\left(\sum_{i=1}^{n} x_i(t)\right)\right)S(t) \geqq S^{(0)}D,$$

or

$$S'(t)+\left(D+\left(\max_{1\leq i\leq n}\frac{m_i}{a_iy_i}\right)\left(\max_{1\leq i\leq n}y_i\right)(S^{(0)}+1)\right)S(t)\geq S^{(0)}D.$$

Let $A=D+(\max_{1\leq i\leq n}m_i/a_iy_i)(\max_{1\leq i\leq n}y_i)(S^{(0)}+1)$. The last inequality may be written as

$$S'(t)+AS(t)\geq S^{(0)}D.$$

Using the comparison equation

$$x'(t)+Ax(t)=S^{(0)}D,$$
$$x(t_0)=S(t_0),$$

it follows that

$$S(t)\geq S(t_0)\,e^{-A(t-t_0)}+\frac{S^{(0)}D}{A},\qquad t\geq t_0,$$

or, dropping the first term which is nonnegative,

$$S(t)\geq\frac{S^{(0)}D}{A}=\gamma,\qquad t\geq t_0.$$

LEMMA 4.5. *Let $S^{(0)}>a_k/(b_k-1)>0$. Then there exists $\varepsilon>0$ such that*

(i) $$\alpha_{k1}(\varepsilon)=\frac{[S^{(0)}(b_k-1)-\varepsilon b_k+a_k]+\sqrt{D_1(\varepsilon)}}{2(b_k-1)}<S^{(0)},$$

$$\alpha_{k2}(\varepsilon)=\frac{[S^{(0)}(b_k-1)-\varepsilon b_k+a_k]-\sqrt{D_1(\varepsilon)}}{2(b_k-1)}>\frac{a_k}{b_k-1},$$

where

$$D_1(\varepsilon)=[S^{(0)}(b_k-1)-\varepsilon b_k+a_k]^2-4(b_k-1)a_kS^{(0)}>0.$$

(ii) *The solution $z(t)$, $t\geq t_0$ of the differential equation*

$$z'(t)=(S^{(0)}-z(t))D-\frac{m_kz(t)}{a_k+z(t.}(S^{(0)}-z(t)-\varepsilon),$$

$$z(t_0)>0$$

is positive. If $z(t_0)<\alpha_{k2}(\varepsilon)<\alpha_{k1}(\varepsilon)$, then $z(t)$ is strictly increasing and $z(t)\leq\alpha_{k2}(\varepsilon)$ for $t\geq t_0$.

Proof. Since

$$D_1(0)=[S^{(0)}(b_k-1)-a_k]^2>0,$$
$$\alpha_{k1}(0)=S^{(0)},$$
$$\alpha_{k2}(0)=\frac{a_k}{b_k-1}$$

and

$$\frac{d}{d\varepsilon}\alpha_{k1}(0)=\frac{1}{2(b_k-1)}\left[-b_k-\frac{b_k(S^{(0)}(b_k-1)+a_k)}{S^{(0)}(b_k-1)-a_k}\right]<0,$$

$$\frac{d}{d\varepsilon}\alpha_{k2}(0)=\frac{b_k}{2(b_k-1)}\cdot\left[\frac{2a_k}{S^{(0)}(b_k-1)-a_k}\right]>0,$$

for $\epsilon>0$ and small, (i) follows.

The equation

$$z'(t)=(S^{(0)}-z(t))D-\frac{m_k z(t)}{a_k+z(t)}(S^{(0)}-z(t)-\varepsilon)$$

may be factored as

$$z'(t)=\frac{(m_k-D)}{a_k+z(t)}\cdot[z(t)-\alpha_{k1}(\varepsilon)][z(t)-\alpha_{k2}(\varepsilon)].$$

Then $z'(t)$ is positive to the right of t_0 if $0<z(t_0)<\alpha_{k2}(\varepsilon)$. $z(t)$ cannot cross the line $z=\alpha_{k2}(\varepsilon)$ with a positive slope and (ii) follows.

Before stating the next lemma we observe

(F-4)   If $0<\dfrac{a_i}{b_i-1}<\dfrac{a_k}{b_k-1}$, $a_k>a_i$, $b_k>b_i$,

then

(i) $\dfrac{a_i}{b_i-1}<\dfrac{a_k}{b_k-1}<\dfrac{a_k b_i-a_i b_k}{b_k-b_i}.$

(ii)   If $0<S<\dfrac{a_k b_i-a_i b_k}{b_k-b_i}$,   then $\dfrac{m_k}{a_k+S}<\dfrac{m_i}{a_i+S}.$

(F-4) follows from simple algebraic computations.

LEMMA 4.6. *Let* (H_n) *hold. Then* $\lim_{t\to\infty}x_i(t)=0$, $i=2,\cdots,n$.

Proof. The proof will follow the ideas of Lemma 4.3 with the main technical problem being the selection of an appropriate ξ^*. Suppose $\limsup_{t\to\infty}x_j(t)>0$ for some $j\geqq2$. From Lemma 4.2 there exists a sequence $\{t_n\}$, $\lim_{t\leftarrow\infty}t_n=\infty$, such that $S(t_n)=a_1/(b_1-1)$, $S'(t_n)<0$. From (4.3) we have

(4.12) $$S(t)+\sum_{i\in I}\frac{x_i(t)}{y_i}=\left(A_n e^{-Dt}-\sum_{i\notin I}\frac{x_i(t)}{y_i}\right)+S^{(0)},$$

where $I=\{j|1\leqq j\leqq n,\ \lim_{t\to\infty}x_j(t)\neq0\}$. Let $k=\max I$, and note that $k\neq1$. In view of Theorem 3.2, we know $a_k/(b_k-1)\leqq S^{(0)}$.

First we assume $a_k/(b_k-1)<S^{(0)}$. Let $0<\varepsilon<\min_{j\in I}\varepsilon_{j2}$, where ε_j corresponds to $a_j/(b_j-1)$ in Lemma 4.5. In (4.12) the bracketed quantity tends to zero as t approaches infinity so there exists $T_\varepsilon>0$ such that

(4.13) $$S^{(0)}-S(t)-\varepsilon<\sum_{i\in I}\frac{x_i(t)}{y_i}<S^{(0)}-S(t)+\varepsilon,\qquad t\geqq T_\varepsilon.$$

Hence

$$S'(t) = (S^{(0)} - S(t))D - \sum_{i=1}^{n} \frac{x_i(t)}{y_i} \frac{m_i S(t)}{a_i + S(t)}$$

$$\leqq (S^{(0)} - S(t))D - \left\{ \min_{j \in I} \frac{m_j S(t)}{a_j + S(t)} \right\} \sum_{j \in I} \frac{x_j(t)}{y_j}$$

$$\leqq (S^{(0)} - S(t))D - \left\{ \min_{j \in I} \frac{m_j S(t)}{a_j + S(t)} \right\} (S^{(0)} - S(t) - \varepsilon)$$

for $t \geqq T_\varepsilon$. Recall that $S(t_n) = a_1/(b_1 - 1)$ for every n. Comparing solutions of the above inequality with solutions of

$$z'(t) = (S^{(0)} - z(t))D - \left\{ \min_{j \in I} \frac{m_j z(t)}{a_j + z(t)} \right\} (S^{(0)} - z(t) - \varepsilon),$$

(4.14)

$$z(t_n) = S(t_n),$$

it follows that $S(t) \leqq z(t)$, $t \geqq t_n$. Let

$$I_1 = \left\{ j \in I \,\middle|\, \frac{a_j}{b_j - 1} = \frac{a_k}{b_k - 1} \right\},$$

$$I_2 = \left\{ j \in I \,\middle|\, \frac{a_j}{b_j - 1} < \frac{a_k}{b_j - 1} \right\}.$$

In view of Lemma 4.3 we may assume for each $i \in I_1$, $j \in I_2$, that $a_i > a_j$, $b_i < b_j$. As observed in (F-4),

$$\frac{a_j}{b_j - 1} < \frac{a_i}{b_i - 1} < \frac{a_i b_j - a_j b_i}{b_i - b_j},$$

and from (F-4)(ii), if

$$0 < z(t) < \min_{\substack{i \in I_1 \\ j \in I_2}} \left\{ \frac{a_i b_j - a_j b_i}{b_i - b_j} \right\},$$

then

$$z'(t) = (S^{(0)} - z(t))D - \left\{ \min_{j \in I_1} \frac{m_j z(t)}{a_j + z(t)} \right\} (S^{(0)} - z(t) - \varepsilon).$$

Choose ε so small that

$$\max_{i \in I_1} \alpha_{i2}(\varepsilon) < \min_{\substack{i \in I_1 \\ j \in I_2}} \left\{ \frac{a_i b_j - a_j b_i}{b_i - b_j} \right\},$$

where $\alpha_{i2}(\varepsilon)$ corresponds to $a_i/(b_i - 1)$ in Lemma 4.5. Then the solution $z(t)$ of

(4.14) satisfies

$$z(t) \leq \min_{\substack{i \in I_1 \\ j \in I_2}} \left\{ \frac{a_i b_j - a_j b_i}{b_i - b_j} \right\}.$$

Hence $S(t) < (a_k b_1 - a_1 b_k)/(b_k - b_1) = \beta$. By Lemma 4.4 we have $S(t) \geq \gamma$, $t \geq t_n$, n sufficiently large.

We seek now to choose ξ^* such that $P_{\xi^*}(z) < 0$ for $\gamma \leq z \leq \beta$. Using the factored form, (4.11), of $P_\xi(z)$, we note that $(d/d\xi)[P_\xi(z)] = [(b_k - 1)z - a_k][z + a_1]$. Thus if $0 < z < a_k/(b_k - 1)$, $(d/d\xi)P_\xi(z) < 0$, and if $z > a_k/(b_k - 1)$, $(d/d\xi)P_\xi(z) > 0$. Thus if $0 < \xi < 1$,

$$P_\xi(z) > P_1(z), \qquad 0 < z < \frac{a_k}{b_k - 1},$$

$$P_\xi(z) < P_1(z), \qquad z > \frac{a_k}{b_k - 1}.$$

$P_1(z) = 0$ at $z = 0$ and $z = (a_k b_1 - a_1 b_k)(b_k - b_1)$, as may be seen from (4.5). Thus for any $0 < \xi < 1$, $P_\xi(\beta) < 0$. Further, for ξ sufficiently close to one, $P_\xi(\gamma) < 0$, since $P_1(\gamma) < 0$. Thus there exists a $\xi^* < 1$ such that $P_{\xi^*}(z) < 0$ for $\gamma \leq z \leq \beta$.

It now follows that

$$\xi^* \frac{x_k'(t)}{x_k(t)} - \frac{x_1'(t)}{x_1(t)} = \frac{P_{\xi^*}(S(t))}{(a_1 + S(t))(a_k + S(t))}$$

$$< \max_{\gamma \leq z \leq \beta} \frac{P_{\xi^*}(z)}{(a_1 + S_{\max})(a_k + S_{\max})} = \zeta < 0.$$

Integrating both sides from t_n to t, taking exponentials, and letting t tend to infinity, yields

$$\lim_{t \to \infty} x_k(t) = 0,$$

which is the desired contradiction.

We show that $a_k/(b_k - 1) = S^{(0)}$ cannot occur. Since $S(t_0) = a_1/(b_1 - 1)$, and

(4.15) $$\left. \frac{dS}{dt} \right|_{S = S_0} < 0,$$

$S(t)$ cannot cross $S = a_k/(b_k - 1) = S^{(0)}$. Thus $S(t) < a_k/(b_k - 1)$, $t \geq t_0$, and $\lim_{t \to \infty} x_k(t)$ exists. If this limit is positive, by (F-3), $\lim_{t \to \infty} S(t) = a_k/(b_k - 1) = S^{(0)}$. But then, by (F-3) and Lemma 4.2, $\lim_{t \to \infty} S(t) = a_1/(b_1 - 1) < a_k/(b_k - 1)$, which is a contradiction.

LEMMA 4.7. *Let* (H_n) *hold. Then the critical point* $(S^*, x_1^*, 0, \cdots, 0)$ *of* $(2.1)_n$ *is asymptotically stable, where* $S^* = a_1/(b_1 - 1)$, $x_1^* = y_1(S^{(0)} - S^*)$.

Proof. The eigenvalues of the coefficient matrix of the variational equation at the critical point are negative. To see this, note that the coefficient matrix is

$$
\begin{bmatrix}
-D - \dfrac{a_1 m_1 x_1^*}{y_1(a_1+S^*)^2}, & \dfrac{m_1 S^*}{y_1(a_1+S^*)}, & \cdots, & \dfrac{m_n S^*}{y_n(a_n+S^*)} \\[2ex]
\dfrac{a_1 m_1 x_1^*}{y_1(a_1+S^*)^2}, & 0, & 0, & \cdots \\[2ex]
0, & 0, & \dfrac{m_2 S^*}{a_2+S^*} - D, & 0 \\[2ex]
 & & & \ddots \\[1ex]
 & \cdots & & \dfrac{m_n S^*}{a_n+S^*} - D
\end{bmatrix}
$$

Let $K_i = D((b_i-1)S^* - a_i)/(a_i + S^*)$, $i = 2, \cdots, n$. The eigenvalues of the above matrix satisfy

$$
(\lambda - K_2) \cdots (\lambda - K_n)\left(\lambda^2 + \left(D + \frac{a_1 m_1 x_1^*}{y_1(a_1+S^*)^2}\right)\lambda + \frac{a_1 m_1^2 x_1^* S^*}{y_1(a_1+S^*)^3}\right),
$$

$K_1 < 0$, $i = 2, \cdots, n$, by (H_n). The quadratic has positive coefficients and hence its roots have negative real parts. This establishes the asymptotic stability of the critical point.

Proof of Theorem 3.3. From Lemma 4.6 it follows that $\lim_{t\to\infty} x_i(t) = 0$, $i = 2, \cdots, n$. If also $\lim_{t\to\infty} x_1(t) = 0$, then from (4.3) it follows that $\lim_{t\to\infty} S(t) = S^{(0)}$. This makes the exponent in (4.1') positive for sufficiently large t, and contradicts the above limits being zero. If $\lim_{t\to\infty} S(t)$ exists, then the theorem is proved by (4.3) and (F-3). If this limit does not exist, denote the omega limit set [12] of a trajectory of $(2.1)_n$, $(S(t), x_1(t), \cdots, x_n(t))$, by Ω.

Recall that if $\lim_{t\to\infty} S(t)$ does not exist, there is a sequence $\{t_j\}$, $\lim_{j\to\infty} t_j = \infty$, such that $S(t_j) = a_1/(b_1-1) = S^*$. Thus by (4.3), Ω must contain the closure of the set

$$
\left\{ S^*, \left[S^{(0)} - S^* + \left\{ \left(S_0 + \sum_{i=1}^{n} \frac{x_{i0}}{y_i}\right) - S^{(0)} \right\} e^{-Dt_j} - \sum_{i=2}^{n} \frac{x_i(t_j)}{y_i} \right] y_1, x_2(t_j), \cdots, x_n(t_j) \right\}
$$

or $(S^*, x_1^*, 0, \cdots, 0) \in \Omega$. But this critical point is asymptitically stable by Lemma 4.7, that is, $\Omega = (S^*, x_1^*, 0, \cdots, 0)$. The theorem follows since a trajectory is asymptotic to its omega limit set.

Proof of Theorem 3.4. From Theoerem 3.2, $\lim_{t\to\infty} x_i(t) = 0$, $i = 2, \cdots, n$. Since $S^{(0)} = a_1/(b_1-1)$, by (4.15) there exists t_0 such that $S(t)$ cannot cross $S = a_1/(b_1-1) = S^{(0)}$ from below for $t \geq t_0$. From (F-3) and (4.3), $\lim_{t\to\infty} x_1(t) = 0$ and $\lim_{t\to\infty} S(t) = a_1/(b_1-1)$.

Proof of Theorem 3.5. Let $S^* < S^{(0)}$. If $A_n \leq 0$, then, differentiating (4.3), it follows that

$$
S'(t) + \sum_{i=1}^{n} \frac{x_i'(t)}{y_i} \geq 0, \qquad t \geq 0.
$$

From this we know that either there exists a t_0 such that $S(t) \geq S^*$ for all $t \geq t_0$ or $S(t) < S^*$ for $t > 0$. If $S(t) < S^*$, then $\lim_{t \to \infty} x_i(t) = x_i^*$ for $i = 1, 2, \cdots, n$ by (F-1). If $x_i^* = 0$ for all $i = 1, 2, \cdots, n$, then (4.3) would contradict $S(t) < S^* < S^{(0)}$, so for some k, $\lim_{t \to \infty} x_k(t) = x_k^* > 0$. Using this in (4.1) yields

$$\int_0^\infty \frac{S(\xi) - S^*}{a_k + S(\xi)} \, d\xi > -\infty.$$

Furthermore, since $S(t)$ is bounded,

$$\int_0^\infty (S(\xi) - S^*) \, d\xi > -\infty.$$

For $i \neq k$,

$$\int_0^\infty \frac{S(\xi) - S^*}{a_i + S(\xi)} \, d\xi > \frac{1}{a_i} \int_0^\infty (S(\xi) - S^*) \, d\xi$$

$$> -\infty.$$

Using this in (5.1) yields

$$\lim_{t \to \infty} x_i(t) = x_i^* > 0, \qquad i \neq k.$$

If $S(t) \geq S^*$, $t \geq t_0$, then $x_i'(t) \geq 0$, and necessarily $\lim_{t \to \infty} x_i(t) = x_i^* > 0$. In either case, (F-3) and (4.3) finish the proof.

If $A_n > 0$, then

$$S'(t) + \sum_{i=1}^n \frac{x_i'(t)}{y_i} < 0$$

for all t. Thus either $S(t) > S^*$ for $t \geq 0$ or there is a t_0 such that $S(t) < S^*$ for $t > t_0$. In either case one of the above arguments will apply.

If $S^* = S^{(0)}$, one may argue as above to obtain $\lim_{t \to \infty} x_i(t) = x_i^*$ and $\lim_{t \to \infty} S(t) = S^* = S^{(0)}$. From (4.3) it follows that $x_i^* = 0$, $i = 1, \cdots, n$.

5. Discussion. Most of the analysis centers on the parameter $\lambda_i = a_i / (b_i - 1)$, where a_i is the Michaelis–Menten constant and $b_i = m_i / D$, where m_i is the maximum specific growth rate and D is the constant dilution rate. For any species i whose parameter is too large (λ_i greater than the input concentration, $S^{(0)}$), survival is not posssible even in the absence of competition from other micro-organisms for the nutrient. Under this condition, the concentration of the species tends to zero as $t \to \infty$. For those species where λ_i is not too large, competition determines survival. For different species it is biologically reasonable to assume that the corresponding λ's are not precisely identical (the λ's are, after all, measured quantities). With this assumption—hypothesis (H_n) of the preceding section—we give a complete answer: only the species having the smallest λ survives, and its limiting value is determined. This is the principal result of the paper (Theorem 3.3).

Powell's result [14] is a special case of our result. In fact, our result is a more rigorous proof even in Powell's special case. Powell made the simplifying assump-

tion that the culture had achieved an equilibrium of one micro-organism before the appearance of the second competing organism, contaminant, or mutant. We do not require this assumption. Moreover, the paper provides a mathematical proof of the observation of Taylor and Williams' numerical experiment [18] which concluded that "only a single species will survive if growth is limited by a single substrate." This conclusion was also reached by Stewart and Levin [17] although a mathematically rigorous proof was not given. This conclusion applies in the case for which nutrient is input at a constant rate. It is of some importance that the outcome of competition is independent of the initial number of competitors. Survival, although not the limiting value, is independent of the yield constant, y_i.

We note that the analysis here is global; at no point do we assume that the initial conditions are in the neighborhood of a critical point, an assumption which is implicit, though not always stated, if a linear stability analysis is performed.

It is of interest to relate these findings to general questions of competition and the coexistence of species in nature. λ_i can be related to the population growth parameter, r_i, the intrinsic rate of natural increase of the ith species, as: $\lambda_i = (a_i/r_i)D$. In Equation $(2.1)_n$, r_i is formally equivalent to the quantity $(m_i - D)$, where m_i is interpreted as the maximal "birth" rate under resource-unlimited conditions and D is the "death" rate. Note the simplicity of the result in these terms; *the species whose Michaelis–Menten constant is smallest in comparison with its intrinsic rate of increase will win* (note that all species experience the same death rate due to washout by dilution). If the intrinsic rates of increase for a series of competing micro-organisms are all roughly equivalent, then the result is even more elegant: *the species whose Michaelis–Menten constant is smallest (r's equal) for the limiting nutrient will win*. Recall that this constant is the nutrient concentration for which rate of uptake is half maximal. It is irrelevant how abundant the competitors are at the start, or how efficiently the species convert the nutrient into cell growth (yield).

In the Introduction we mentioned the applicability of results of our model system to the planktonic communities of unicellular algae in lakes and oceans. Dugdale's paper [2] was one of the earliest to discuss algal growth rates under conditions of nutrient limitation in terms of Michaelis–Menten kinetics. He suggested the significance of nutrient limitation theory to the study of phytoplankton competition and succession, but not many workers have attacked these problems directly or indirectly. A few people, however, have made very explicit hypotheses based on Michaelis–Menten kinetics, as this quote from Eppley and Coatsworth [3] demonstrates:

> Our hypothesis is that [Michaelis–Menten] values for solute uptake provide a quantitative comparison of the abilities of different species to utilize low levels of nutrients. As such, [considering nitrogen uptake], values for the uptake of NO_3^-, NO_2^-, and NH_4^+ for a series of species of phytoplankton, involved sequentially in a seasonal succession, should follow in order of declining nutrient concentrations. Each succeeding species should show a lower [Michaelis–Menten] constant than the preceding one *if* declining level of the nutrient in question is indeed significant in succession.

As we have seen from the mathematical analysis, this was a very prophetic remark. Kilham [6] made a similar suggestion in discussing the Michaelis–Menten

constants for a silica uptake in diatoms, and predicted that which diatom was dominant during seasonal succession would be determined by the species with the lowest Michaelis–Menten constant still capable of growing at the given ambient level of silica. Because of the nonsteady-state condition of nutrient flow into lakes during seasonal succession, Kilham's prediction seems very likely, provided that the diatom species have similar intrinsic rates of increase and sinking rates. The dominant species would be expected to exclude the remaining species if nutrient conditions were to stabilize.

Much has been made of the so-called "paradox of the plankton"—the seemingly paradoxical coexistence of many species of planktonic algae in a well-mixed body of water with usually one or at least few limiting nutrients. How is such coexistence possible? Our analysis suggests that for an indefinite number of species to survive together they must have equal ratios of Michaelis–Menten constants to intrinsic rates of increase. If the ratios are very close, the rate of competitive exclusion will proceed at a very slow pace. The pace may be slow enough that the species at a disadvantage can persist until some random flush of nutrients results in regeneration. This is contrary to conventional ecological wisdom which says that stable coexistence is impossible for two or more species making a living in identical ways. However, May [8] reached a similar conclusion, arguing from a modified set of classical Lotka–Volterra competition equations, that his deterministic model set no limit to the number of coexisting, identical species. See also the example of McGehee and Armstrong [9] and the analysis of Smith, Shugart, O'Neill, Booth, and McNaught [16] of zooplankton feeding on phytoplankton.

The conclusions of this paper apply to pure exploitative competition with no direct interference between rivals. All species or strains have access to the limiting nutrient and compete only by lowering the common nutrient pool. Under this case it should be noted that it is possible to predict the outcome of competition from the dynamics of nutrient uptake and growth of each species or strain grown alone. This contrasts with the classical Lotka–Volterra-type equations, which cannot predict outcomes until the species are actually grown together to measure interaction coefficients.

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