



Predicting stability of mixed microbial cultures from single species experiments: 1. Phenomenological model

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Available online 8 December 2004

Abstract

The growth of mixed microbial cultures on mixtures of substrates is a fundamental problem of both theoretical and practical interest. On the one hand, the literature is abundant with experimental studies of mixed-substrate phenomena [T. Egli, The ecological and physiological significance of the growth of heterotrophic microorganisms with mixtures of substrates, *Adv. Microbiol. Ecol.* 14 (1995) 305–386]. On the other hand, a number of mathematical models of mixed-substrate growth have been analyzed in the last three decades. These models typically assume specific kinetic expressions for substrate uptake and biomass growth rates and their predictions are formulated in terms of parameters of the model. In this work, we formulate and analyze a general mathematical model of mixed microbial growth on mixtures of substitutable substrates. Using this model, we study the effect of mutual inhibition of substrate uptake rates on the stability of the equilibria of the model. Specifically, we address the following question: *How much of the dynamics exhibited by two competing species can be inferred from single species data?* We provide geometric criteria for stability of various types of equilibria corresponding to non-competitive exclusion, competitive exclusion, and coexistence of two competing species in terms of growth isoclines and consumption curves. A growth isocline is a curve in the plane of substrate concentrations corresponding to the zero net growth of a given species. In [G.T. Reeves, A. Narang, S.S. Pilyugin, Growth of mixed cultures on mixtures of substitutable substrates: The operating diagram for a structured model, *J. Theor. Biol.* 226 (2004) 143–157], we introduced consumption curves as sets of all possible combinations of substrate concentrations

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corresponding to balanced growth of a single microbial species. Both types of curves can be obtained in single species experiments.

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Keywords: Microbial growth; Phenomenological model; Mixed cultures; Substitutable substrates; Mutual uptake inhibition; Coexistence; Growth isocline; Consumption curve

1. Introduction

The coexistence of competing species is a problem that has attracted the attention of many researchers. The earliest mathematical formulation of the problem is attributed to the works of Lotka [1] and Volterra [2] in the 1920s. In the Lotka–Volterra model, two species compete for two non-replenishable resources whose availability decreases linearly with the abundance of each competing species. The model focussed entirely on the dynamics of the competing species, and did not describe the dynamics and/or the possible interactions between the resources. The development of resource-based models began with Monod's experimental studies which established a quantitative relation between the growth of microbial populations and the availability of resources [3]. Specifically, Monod demonstrated that when the growth of microbes is limited by a single resource (or substrate), the specific growth rate is a saturable function of the substrate concentration, and the specific substrate uptake rate is proportional to the specific growth rate. Powell generalized Monod's model to the growth of two microbial species limited by a single substrate [4]. Over the last three decades, the mathematical theory of microbial competition has been a subject of interest for many researchers. A book of Smith and Waltman [5] provides an excellent account of this theory and contains extensive bibliography on this topic. The cornerstone of the theory is the *principle of competitive exclusion* which states that at most one species can survive in the well-stirred chemostat limited by a constant supply of a single growth-limiting substrate. This principle has been validated both theoretically and experimentally [4,6,8,7,9–11].

In the presence of multiple growth-limiting substrates, it becomes important to specify the nutritional requirements satisfied by the substrates. Two growth-limiting substrates are *substitutable* if they satisfy identical nutritional requirements, so that a species can grow on either one of the substrates. The two substrates are *complementary* or *essential* if they satisfy distinct nutritional requirements, so that growth is impossible in the absence of either one of the substrates. Over the last several decades, a series of models have been introduced that recognize not only the existence of the resources for which the species compete, but also the nature of the nutrient requirements satisfied by the resources [12,13,15,16]. In this paper, we focus on the growth of two microbial species in a chemostat fed with a mixture of two substitutable substrates, and obtain a series of results that improve upon the findings of previous studies.

Leon and Tumpson assumed that substrate uptakes in substitutable mixtures are *non-interacting*, i.e., the uptake rate of a substrate is unaffected by the presence of the other substrate [12]. It was shown that under these conditions, two species coexist at a stable equilibrium if and only if each species consumes more of the substrate which influences their growth rate more strongly. This is a generalization of the Gilpin–Justice stability criterion derived from the Lotka–Volterra model for competing species [23]. However, the experimental literature provides sufficient evi-

dence that the substrate uptake rates are not independent. In Section 2.1, we demonstrate that for microbial cultures growing on mixtures of substitutable substrates, the interaction between the substrates is, in general, mutually inhibitory. Although the nature of such inhibitory interaction remains unclear, the experimental evidence shows that each substrate inhibits the rate of uptake of the other substrate.

An extensive analysis of a mathematical model in which two substitutable substrates may inhibit each others uptake was conducted by Ballyk and Wolkowicz [16]. They analyzed the existence and uniqueness of equilibria of the model and obtained important results regarding the stability properties of these equilibria. Specifically, they obtained necessary and sufficient conditions for the uniform persistence for single- and mixed species cultures, proved the global asymptotic stability of the single species equilibria, and provided a complete classification of dynamic outcomes in the case of non-interacting substrates. They also showed that the growth of a single species may exhibit multiple equilibria and that in some cases the presence of the second competing species enables a given species to survive in the chemostat, a phenomenon referred to as the competitor-mediated coexistence. In [17], Ballyk and Wolkowicz studied the effects of ‘enrichment’ of single species cultures by varying the feed concentrations and demonstrated that for intermediate dilution rates an increase in the feed concentration of one of the substrates may result in the extinction of the microbial culture. In both articles [16] and [17], Ballyk and Wolkowicz made an additional assumption regarding the specific growth rate of each microbial species which effectively restricted the geometry of growth isoclines to the class of monotone curves even though the functions modeling the specific growth rates may be non-monotone. In this paper, we extend their analysis to the case of non-monotone growth isoclines and show that most of their conclusions remain valid. We also provide a necessary and sufficient condition for stability of single species equilibria and extend their analysis of hysteresis-like switching behavior of single species growing on a mixture of two (substitutable substrates) to a more general class of uptake and growth rates. Finally, we present a constructive proof for the existence of Hopf bifurcations, and provide a sufficient condition for this bifurcation. The possibility of Hopf bifurcations was mentioned in [16].

In their work, Ballyk and Wolkowicz followed an analytical approach. In contrast, Tilman developed a graphical theory of two-species growth on arbitrary mixtures of substrates [13,14]. He showed that the existence of the non-trivial steady state at any given dilution rate and feed concentrations could be predicted from single-species data. Specifically, a coexistence steady state exists if and only if the dilution rate is such that the two growth isoclines intersect at positive substrate concentrations, and the vector of feed concentrations lies in the positive cone generated by the consumption vectors at the intersection of the growth isoclines (Fig. 1). Tilman calculated the growth isoclines and consumption vectors by appealing to kinetic models that assume specific functional forms for the growth and substrate uptake rates. We show below that in microbial cultures, the growth isoclines and consumption vectors can be determined directly from the experimental data without any recourse to models. Tilman also developed a graphical interpretation of the generalized Gilpin–Justice criterion: A coexistence steady state is asymptotically stable if and only if the two pairs of vectors, namely, the slopes of the two growth isoclines and the consumption vectors for the two species, have the same orientation. In this paper, we show that when the interaction between the substrates is mutually inhibitory, the generalized Gilpin–Justice criterion is necessary, but not sufficient for the stability of coexistence equilibria. The feed concentrations can be varied in such a way that the coexistence steady state undergoes a Hopf bifurcation even

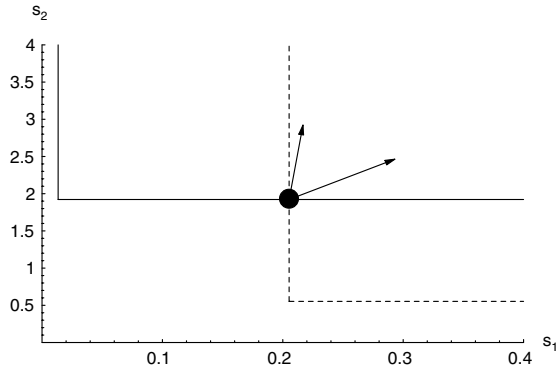


Fig. 1. Application of Tilman's criterion for existence of non-trivial steady states to growth of *A. formosa* (species 1) and *C. meneghianiana* (species 2) on a mixture of phosphate (s_1) and silicate (s_2) [13,14]. The full and dashed lines show the *growth isoclines* for species 1 and 2 at the dilution rate, $D = 0.251/\text{day}$. These curves are given by the equation $r_i^s(s_1, s_2) = D$, where r_i^s is the specific growth rate of the i th species on s_1 and s_2 . The intersection point corresponds to the substrate concentrations at the coexistence steady state. The arrows show the *consumption vectors*, (r_{11}^s, r_{12}^s) and (r_{21}^s, r_{22}^s) , at the intersection point, where r_{ij}^s is the specific uptake rate of the j th substrate by the i th species. Tilman showed that the coexistence steady state exists if and only if the feed concentrations lie in the positive cone of the consumption vectors. To calculate the growth isoclines and consumption vectors, he assumed that $r_i^s \equiv \min\{r_{i1}^s(s_1), r_{i2}^s(s_2)\}$ and $r_{ij}^s = r_i^s/Y_{ij}$, where $r_i^s(s_j)$ and Y_{ij} denote the specific growth rate and yield of the i th species on s_j alone.

though the Gilpin–Justice condition is satisfied. This method may also be applied to the case of complementary substrates where the Hopf bifurcations were reported previously [15].

In this paper, we show that the conditions for existence and stability of equilibria can be expressed in simple graphical terms. More importantly, the validity of the graphical criteria can be ascertained by inspection of the single-species data. To this end, we define the notion of the consumption curve for a given species. We show that such curves are completely determined by the single-species data. The stability of the semitrivial steady state is completely determined by the manner in which the growth isocline and the consumption curve intersect. This result enables us to give a graphical interpretation of the multiple steady states that can occur in single-species growth. More generally, we show that consideration of the consumption curves together with the growth isoclines clarifies the analogy with the Lotka–Volterra model for competing species.

The rest of this paper is organized as follows. In Section 2, we describe a mathematical model of microbial competition that includes the mutually inhibitory interaction between the substrates. We term this model *phenomenological*¹ because it does not include a specific mechanism for such inhibitory interaction. In Section 2.1, we provide the experimental basis for our modeling assumptions. In Sections 2.2 and 3, we analyze the consumption curves and the growth isoclines and derive the criteria for the existence and stability of equilibria of the model. We revisit the notion of

¹ The present report lays out the foundation for the study of a *physiological* model in which we describe a specific mechanism of substrate interaction by including the dynamics of transport enzymes that catalyze the uptake of the substrates. There, we use the insights from the analysis of the phenomenological model to study the existence and stability of the equilibria of the physiological model.

substrate preference in Section 4. We illustrate possible applications of our results in 5. The summary of the results is presented in Section 6.

2. The model

We consider the model

$$\dot{s}_j = D(s_j^f - s_j) - c_1 r_{1j}(s_1, s_2) - c_2 r_{2j}(s_1, s_2), \quad (1)$$

$$\dot{c}_i = c_i(r_i^g(s_1, s_2) - D), \quad (2)$$

where c_i , $i = 1, 2$ denote microbial concentrations and s_j , $j = 1, 2$ denote the substrate concentrations. The function r_{ij} represents the specific uptake rate of the j th substrate by the i th species, and r_i^g denotes the specific growth rate of the i th species. The dilution rate D and the substrate feed concentrations s_j^f are the operating parameters and can be adjusted in the course of an experiment.

We make the following assumptions regarding the specific uptake and growth rates.

1. The specific uptake rate r_{ij} , $i = 1, 2$ of substrate s_1 increases monotonically with s_1 but is inhibited by an increase of substrate s_2 (and vice versa). We refer to this phenomenon as the *mutual inhibition of substrate uptake*. Mathematically, we postulate that for all $i, j = 1, 2$ and for all $s_1, s_2 > 0$, $r_{ij}(s_1, s_2) > 0$ with $r_{i1}(0, s_2) = 0$ and $r_{i2}(s_1, 0) = 0$ so that neither substrate can be consumed in its absence, and

$$\frac{\partial r_{i1}}{\partial s_1}(s_1, s_2) > 0, \quad \frac{\partial r_{i2}}{\partial s_2}(s_1, s_2) > 0, \quad s_j > 0, \quad (3)$$

$$\frac{\partial r_{i1}}{\partial s_2}(s_1, s_2) < 0, \quad \frac{\partial r_{i2}}{\partial s_1}(s_1, s_2) < 0, \quad s_j > 0 \quad (4)$$

for $i, j = 1, 2$.

2. The specific growth rate of the i th species has the form

$$r_i^g(s_1, s_2) = Y_{i1}r_{i1}(s_1, s_2) + Y_{i2}r_{i2}(s_1, s_2), \quad (5)$$

where Y_{ij} denotes the yield of species c_i when it grows on substrate s_j alone. In the microbiological literature, the yields are said to be *additive* whenever the specific growth rate admits of such a representation. Note that if the mutual inhibition is weak (or non-existent as in the model of Leon and Tumpson [12]), $r_i^g(s_1, s_2)$ is an increasing function of both substrate concentrations.

We show below that these assumptions are supported by the single-species data for mixed-substrate growth in both batch and continuous cultures.

Remark. Although Eqs. (1) and (2) and the assumptions (3) and (4) are the same as in [16], there is an important difference. Ballyk and Wolkowicz make an additional assumption that the specific growth rate r_i^g of the i th species is strictly increasing in s_1 and that there exists a critical

concentration s_1^c such that $\frac{\partial r_i^E}{\partial s_2} > 0$ for $s_1 < s_1^c$ and $\frac{\partial r_i^E}{\partial s_2} < 0$ for $s_1 > s_1^c$ for all $s_2 > 0$. They distinguish between the substrates based on the maximal growth rates that would result if each substrate was supplied alone, so that one substrate (s_1) supports a higher maximal growth rate and the other substrate (s_2) supports a lower maximal growth rate. In their model, when both substrates are in short supply, increasing the concentration of either one is beneficial. At the critical concentration $s_1 = s_1^c$, the growth rate on s_1 alone equals the maximal growth rate on s_2 alone. Once this critical concentration is exceeded ($s_1 > s_1^c$), then increasing the concentration of s_2 inhibits growth, but not below the lower maximal growth rate. This assumption effectively restricts the growth isoclines (the indifference curves using the terminology in [16]) to the case of monotone curves. We do not make such assumption in this paper.

2.1. Experimental basis for model assumptions

The data obtained in batch cultures show that the specific uptake rate of a substrate is never improved by the addition of another substitutable substrate to the medium. Table 1 illustrates this point with experimental data for *E. coli* K12. The third column of the table shows the change in the specific uptake rate of s_1 when s_2 is added to a culture of *E. coli* K12 that is already growing on s_1 . The fourth column of the table shows the change in the specific uptake rate of s_2 when s_1 is added to a culture of *E. coli* K12 growing on s_2 . The first four rows provide clear evidence of mutual inhibition: Each substrate inhibits the uptake of the other substrate. The last two rows show the striking effect of a mutual inhibition that is highly asymmetric. Glucose inhibits the uptake of fumarate and succinate so strongly that there is no consumption of these organic acids. Only glucose is consumed until it is almost completely exhausted.

The substrate concentrations used in batch cultures are typically quite large (0.1–1 g/l). Analysis of the data obtained from continuous cultures confirms the existence of a mutually inhibitory interaction at the lower substrate concentrations (~ 0.01 g/l). Before describing the experimental evidence, we give a brief overview of the experiments. In the literature, steady state continuous culture data have been reported for two types of experiments.

Table 1
Evidence of mutually inhibitory interaction in batch cultures of *E. coli* K12 (from [24])

Substrate pair		% change in specific uptake rate		% change in specific growth rate	
s_1	s_2	s_1	s_2	s_1	s_2
Pyruvate	Fumarate	–12%	–33%	79%	22%
L-Lactate	Fumarate	–20%	–35%	72%	22%
Pyruvate	Oxoglutarate	–20%	–39%	61%	61%
L-Lactate	Oxoglutarate	–37%	–23%	72%	67%
Glucose	Fumarate	0%	–100%	0%	80%
Glucose	Succinate	0%	–100%	0%	68%

The first and second columns show the pair of substitutable substrates, s_1 and s_2 , with which the experiment was performed. The third column (resp., fourth) shows the percent change in the specific uptake rate of s_1 (resp., s_2) when s_2 (resp., s_1) is added to the medium. The fifth (resp., sixth) column shows the percent change in the specific growth rate when s_2 (resp., s_1) is added to a culture growing on s_1 (resp., s_2).

1. The dilution rate is varied, while the feed concentrations are held fixed. Fig. 2(a) shows the data obtained when such experiments were performed with *H. polymorpha* as the pure species, and glucose plus methanol as the two growth-limiting substrates. Both substrates are consumed at low dilution rates, only glucose is consumed at intermediate dilution rates, and neither substrate is consumed at large dilution rates. Such pattern of substrate consumption has been observed in many different systems, and we shall provide a mechanistic model for this pattern in the sequel to this paper.
2. The composition of the feed is varied, while the dilution rate and the *total* feed concentration are held fixed. Fig. 2(c) shows the data obtained when such experiments were performed with *E. coli* ML308, as the pure species, and glucose plus galactose as the two growth-limiting substrates. The data shows that the concentration of a substrate increases with the proportion of the substrate in the feed.

The experiments of the first type can be performed at various fixed feed concentrations. Egli and coworkers obtained such data for the growth of *H. polymorpha* on a mixture of glucose and methanol (Fig. 3(a) and (b)). Given any residual methanol concentration, this data

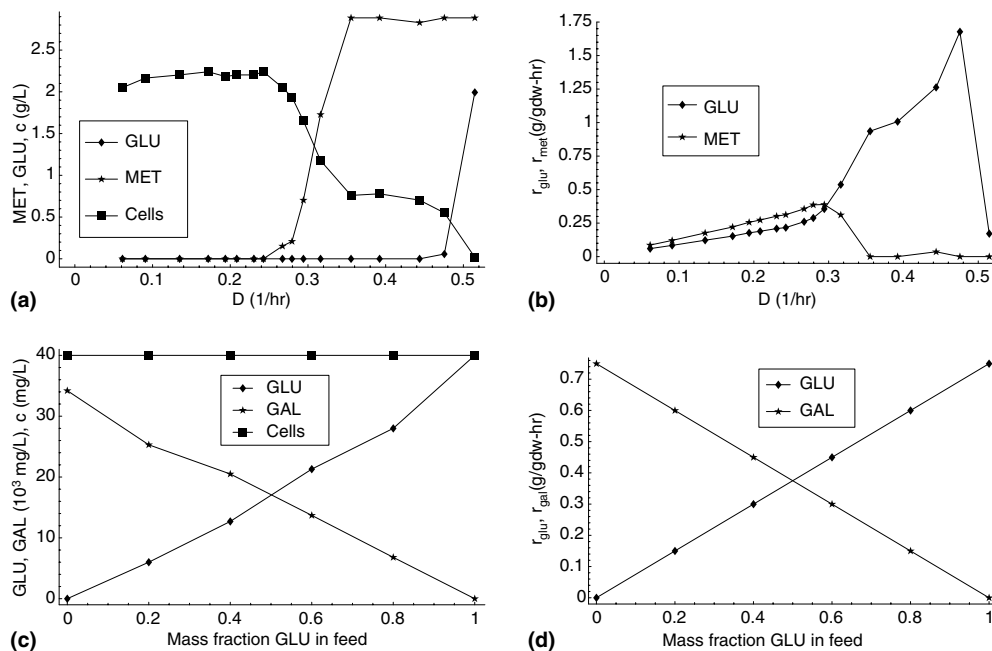


Fig. 2. Variation of the steady state concentrations and specific substrate uptake rates in typical single-species experiments. *Upper panel*: Experimental data for growth of *H. polymorpha* at varying dilution rates, and fixed feed concentrations of 2 g/l of glucose and 3 g/l of methanol (from [18,19]). (a) The cell density and residual concentrations of glucose and methanol at various dilution rates. (b) The corresponding specific uptake rates of glucose and methanol. *Lower panel*: Experimental data for growth of *E. coli* ML308 growing on a mixture of glucose and galactose at varying feed compositions, and fixed dilution rate (0.31/h) and total substrate concentration (100 mg/l) [20]. (c) The cell density and residual concentrations of glucose and galactose at various feed compositions. (d) The corresponding specific uptake rates of glucose and galactose.

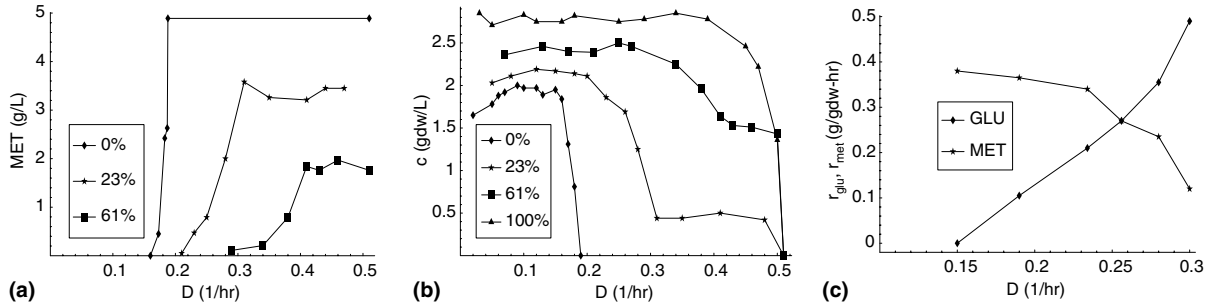


Fig. 3. Evidence of mutually inhibitory interaction in continuous cultures (from [19]) (a) The steady state substrate concentrations during growth of *H. polymorpha* on various mixtures of glucose and methanol. Each data set was obtained at a fixed feed concentration. The total feed concentration of glucose plus methanol was always 5 g/l. The feed concentrations were varied by changing the weight percent glucose in the feed (see legend). (b) The corresponding cell densities. (c) The specific substrate uptake rates of glucose and methanol when the concentration of residual methanol is 0.01 g/l. The specific uptake rates were calculated from the data in (a) and (b).

immediately yield the corresponding set of dilution rates and specific substrate uptake rates. Fig. 3(c) shows the specific uptake rates of glucose and methanol as a function of the dilution rate when the residual methanol concentration is 0.01 g/l. Note that the specific uptake rate of methanol decreases with D . The glucose concentrations under these conditions were below the detection limit of 2 mg/l. However, since the specific uptake rate of glucose increases with D , so must the glucose concentration. Thus, the data imply that glucose inhibits the uptake of methanol even if the concentration of methanol is small.

The data in columns 5 and 6 of Table 1 suggest that despite the mutual inhibition of substrate uptakes, the addition of a second substrate always stimulates the specific growth rate, so that $r_i^g(s_1, s_2)$ is an increasing function of s_1 and s_2 . This is true in many cases, and in the sequel to this paper, we describe a physiological mechanism for this behavior. However, there are instances in which the addition of a second substrate depresses the specific growth rate. When xylose or glycerol is added to a batch culture of *H. polymorpha* growing on glucose, the specific growth rate *decreases* from 0.611/h to 0.471/h and 0.521/h, respectively [25]. Likewise, when 3-phenylpropionic acid is added to a batch culture of *E. coli* ML308 growing on glucose, the specific growth rate decreases from 0.911/h to 0.701/h [26]. Thus, in general, $r_i^g(s_1, s_2)$ is a non-monotone function.

2.2. Consumption curves and growth isoclines

In what follows, we shall constantly appeal to the notions of consumption curves and growth isoclines. Here, we define these curves and show that they are completely determined by single-species data.

Definition. We define the *consumption curve* Φ_i of the i th species as the locus of all pairs (s_1, s_2) such that

$$\frac{s_1^f - s_1}{r_{i1}(s_1, s_2)} = \frac{s_2^f - s_2}{r_{i2}(s_1, s_2)} \geq 0,$$

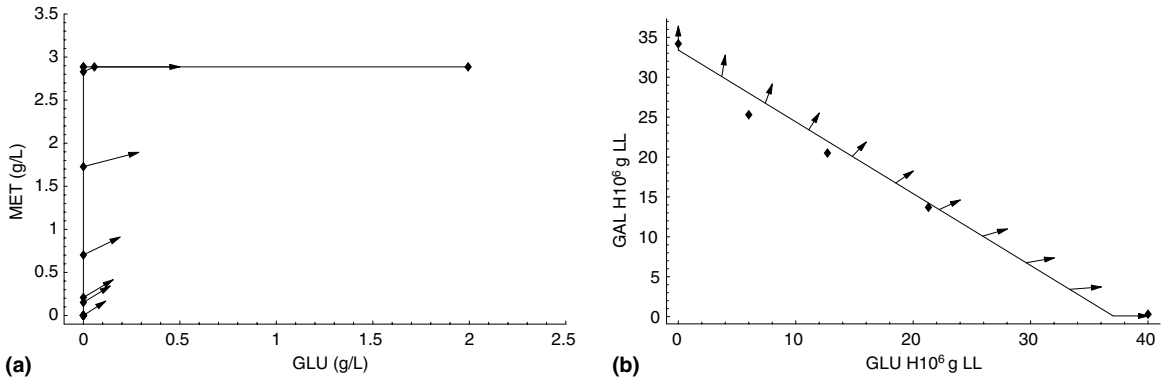


Fig. 4. (a) The consumption curve for *H. polymorpha* growing on a mixture of 2 g/l of glucose and 3 g/l of methanol. The curve was derived from the data Fig. 2(a). (b) The growth isocline for *E. coli* ML308 growing on a mixture of glucose and galactose at a dilution rate of 0.31/h. The curve was derived from the data in Fig. 2(b).

with $0 \leq s_j \leq s_j^f$. We emphasize that the consumption curves for both species are independent of the dilution rate D .

The consumption curve Φ_i represents the set of all possible substrate concentrations (s_1, s_2) obtained at equilibrium where the i th species alone is cultivated at fixed feed concentrations, (s_1^f, s_2^f) . Indeed, consider the culture inoculated with species c_1 alone, so that $c_2 = 0$. At an equilibrium, $\dot{s}_1 = \dot{s}_2 = 0$ and thus according to (1),

$$\frac{c_1}{D} = \frac{s_1^f - s_1}{r_{11}(s_1, s_2)} = \frac{s_2^f - s_2}{r_{12}(s_1, s_2)}. \tag{6}$$

Therefore, the (s_1, s_2) coordinates of any single species equilibrium with $c_2 = 0$ satisfy the relation defining Φ_1 . Similarly, the (s_1, s_2) coordinates of any single species equilibrium with $c_1 = 0$ satisfy the relation defining Φ_2 .

The consumption curve for a given species can be constructed from the single-species data obtained at fixed feed concentrations. Fig. 4(a) shows the consumption curve for *H. polymorpha* constructed from the data shown in Fig. 2(a). Superimposed on the consumption curve are the corresponding consumption vectors, (r_{i1}, r_{i2}) , derived from Fig. 2(b). As expected from (6), the consumption vectors point toward the feed point (s_1^f, s_2^f) . The data show that the consumption curve is the graph of an increasing function. In the following Lemma, we argue that this property is a consequence of the mutually inhibitory interaction between the substrates.

Lemma 1. *The consumption curve Φ_i of the i th species is a graph of a smooth function $s_2 = \eta_i(s_1^f, s_2^f, s_1)$ which is monotonically increasing in s_1 and s_2^f and monotonically decreasing in s_1^f . Moreover, $s_2 = \eta_i(s_1^f, s_2^f, s_1)$ is defined for all $0 \leq s_1 \leq s_1^f$ and*

$$\lim_{s_1 \rightarrow 0} \eta_i(s_1^f, s_2^f, s_1) = 0, \quad \lim_{s_1 \rightarrow s_1^f} \eta_i(s_1^f, s_2^f, s_1) = s_2^f.$$

Proof. The consumption curve Φ_i is implicitly defined as the set of all solutions of the equation

$$F_i(s_1, s_2, s_1^f, s_2^f) = (s_1^f - s_1)r_{i2}(s_1, s_2) - (s_2^f - s_2)r_{i1}(s_1, s_2) = 0.$$

For $0 < s_j < s_j^f$,

$$\frac{\partial F_i}{\partial s_1} = -r_{i2} + (s_1^f - s_1) \frac{\partial r_{i2}}{\partial s_1} - (s_2^f - s_2) \frac{\partial r_{i1}}{\partial s_1} < 0,$$

$$\frac{\partial F_i}{\partial s_2} = r_{i1} + (s_1^f - s_1) \frac{\partial r_{i2}}{\partial s_2} - (s_2^f - s_2) \frac{\partial r_{i1}}{\partial s_2} > 0,$$

due to assumptions (3) and (4). Consequently, equation $F_i = 0$ defines a smooth function $s_2 = \eta_i(s_1^f, s_2^f, s_1)$ which is monotonically increasing in s_1 . Since

$$F_i(0, 0, s_1^f, s_2^f) = F_i(s_1^f, s_2^f, s_1^f, s_2^f) = 0,$$

it follows that $\eta_i(s_1^f, s_2^f, 0) = 0$ and $\eta_i(s_1^f, s_2^f, s_1^f) = s_2^f$.

To show that η_i is a decreasing function of s_1^f , we differentiate equation $F_i(s_1, \eta_i(s_1^f, s_2^f, s_1), s_1^f, s_2^f) = 0$ with respect to s_1^f to obtain

$$\begin{aligned} r_{i2}(s_1, \eta_i(s_1^f, s_2^f, s_1)) + (s_1^f - s_1) \frac{\partial r_{i2}}{\partial s_2}(s_1, \eta_i(s_1^f, s_2^f, s_1)) \frac{\partial \eta_i}{\partial s_1^f}(s_1^f, s_2^f, s_1) \\ - (s_2^f - s_2) \frac{\partial r_{i1}}{\partial s_2}(s_1, \eta_i(s_1^f, s_2^f, s_1)) \frac{\partial \eta_i}{\partial s_1^f}(s_1^f, s_2^f, s_1) = 0. \end{aligned}$$

Since $\frac{\partial r_{i2}}{\partial s_2} > 0$ and $\frac{\partial r_{i1}}{\partial s_2} < 0$, we find that $\frac{\partial \eta_i}{\partial s_1^f}(s_1^f, s_2^f, s_1) < 0$. A similar argument shows that $\frac{\partial \eta_i}{\partial s_2^f}(s_1^f, s_2^f, s_1) > 0$. \square

Definition. We define the *growth isocline* G_i of the i th species as the locus of all pairs (s_1, s_2) such that $r_i^g(s_1, s_2) = D$ with $s_j \geq 0$. We note that G_i is independent of the feed concentrations s_j^f .

The growth isocline for a given species can be constructed from the single-species data obtained at a fixed dilution rate. Fig. 4(b) shows the growth isocline for *E. coli* ML308 constructed from the data shown in Fig. 2(c). Superimposed on the growth isocline are the corresponding consumption vectors $(r_{\text{glu}}^s, r_{\text{gal}}^s)$ obtained from the data shown in Fig. 2(d). Thus, all the information required to apply Tilman's criterion for existence of a non-trivial steady state can be completely determined from single-species data without appealing to specific kinetic models.

If $r_i^g(s_1, s_2)$ is an increasing function of both substrates, the growth isocline is a graph of a monotone decreasing function in the (s_1, s_2) plane. However, as noted above, there are instances where $r_i^g(s_1, s_2)$ is non-monotone. It is therefore possible that for some range of dilution rates, the growth isocline may be a graph of a non-monotone function or it may consist of several disconnected curves (i.e. not be graph of any function).

3. Equilibria and their stability

The model equations (1) and (2) yield three types of equilibria:

1. The trivial equilibrium, denoted ϕ_{00} , at which neither species is present the chemostat ($c_1 = c_2 = 0$);
2. The semitrivial equilibria at which only one of the two species is present in the chemostat: ϕ_{10} ($c_1 > 0, c_2 = 0$), and ϕ_{01} ($c_2 > 0, c_1 = 0$);
3. The non-trivial equilibrium, denoted ϕ_{11} , at which the both species are present in the chemostat ($c_1, c_2 > 0$).

In this section, we discuss the existence and stability of all three types of steady states.

3.1. The trivial equilibrium ϕ_{00}

Lemma 2. *The trivial equilibrium ϕ_{00} : $(s_1, s_2, c_1, c_2) = (s_1^f, s_2^f, 0, 0)$ of (1) and (2) always exists. It is asymptotically stable if $r_1^g(s_1^f, s_2^f) < D$ and $r_2^g(s_1^f, s_2^f) < D$ and unstable if one of these inequalities is reversed.*

Proof. Existence and uniqueness of ϕ_{00} is straightforward. The variational matrix of (1) and (2) at ϕ_{00} is given by

$$J(\phi_{00}) = \begin{pmatrix} -D & 0 & -r_{11} & -r_{21} \\ 0 & -D & -r_{12} & -r_{22} \\ 0 & 0 & r_1^g - D & 0 \\ 0 & 0 & 0 & r_2^g - D \end{pmatrix}, \quad (7)$$

where $r_{ij} = r_{ij}(s_1^f, s_2^f)$ and $r_i^g = r_i^g(s_1^f, s_2^f)$. The eigenvalues of (7) are given by

$$\lambda_1 = \lambda_2 = -D, \quad \lambda_3 = r_1^g - D, \quad \lambda_4 = r_2^g - D.$$

It follows that ϕ_{00} is stable if and only if $r_i^g < D$ for $i = 1, 2$. \square

3.2. The semitrivial equilibria ϕ_{10} and ϕ_{01}

Any semitrivial equilibrium ϕ_{10} of (1) and (2) must satisfy $c_1 > 0, c_2 = 0$. Eqs. (1) and (2) imply that the projection of such an equilibrium onto the (s_1, s_2) plane must be an intersection of the consumption curve and the growth isocline of the 1st species. Now, if the mutual inhibition is weak, $\partial r_i^g / \partial s_1, \partial r_i^g / \partial s_2 > 0$, and the growth isocline is monotone. In this case, the monotonicity of the consumption curve implies that the specific growth rate increases monotonically along the consumption curve. Since $r_i^g(0, 0) = 0 < D$, a unique semitrivial equilibrium exists if and only if $r_i^g(s_1^f, s_2^f) > D$. However, if the mutual inhibition is strong, a growth isocline may be non-monotone. It can then intersect the consumption curve several times, thus generating multiple semitrivial equilibria. In general, it is difficult to provide necessary and sufficient conditions for

the existence of multiple semitrivial equilibria without making specific assumptions about the nature of the function $r_i^g(s_1, s_2)$. Since there is little experimental data to support such assumptions, we will assume the existence of such semitrivial equilibria, and provide a geometric criterion for their stability. The following notion is crucial for this purpose.

Definition. Let (s_1, s_2) be a point of intersection of the consumption curve Φ_i and the growth isocline G_i of the i th species. Let $F^i(s_1, s_2, s_1^f, s_2^f)$ be the tangent vector to the consumption curve Φ_i oriented in the direction of increasing s_j and let $\nabla r_i^g(s_1, s_2)$ be the gradient of the specific growth rate. We say that the substrates s_1 and s_2 are

- locally *synergistic* for the i th species if $\nabla r_i^g(s_1, s_2) \cdot F^i(s_1, s_2, s_1^f, s_2^f) > 0$;
- locally *antagonistic* for the i th species if $\nabla r_i^g(s_1, s_2) \cdot F^i(s_1, s_2, s_1^f, s_2^f) < 0$.

Both types of substrate interactions are illustrated in Fig. 5(a). Biologically, the substrates are synergistic if increasing both substrate levels along the consumption curve results in the increase of the overall growth rate and antagonistic otherwise.

Remark. In general, the slope of the consumption curve through a given point (s_1, s_2) depends on both feed concentrations s_j^f . Therefore, the same substrates may behave synergistically or antagonistically at different feed concentrations. Nevertheless, if $\nabla r_i^g(s_1, s_2) \in R_+^2$ then the substrates are synergistic for all feed concentrations, and if $\nabla r_i^g(s_1, s_2) \in R_-^2$ then the substrates are antagonistic for all feed concentrations.

The following Theorem elucidates the relation between the particular type of substrate interaction and the stability of the semitrivial equilibrium of (1) and (2).

Theorem 3. *The semitrivial equilibrium $\phi_{10} = (s_1, s_2, c_1, 0)$ is asymptotically stable if and only if $r_2^g(s_1, s_2) < D$ and the substrates s_1 and s_2 are locally synergistic at ϕ_{10} . If $r_2^g(s_1, s_2) > D$ and the*

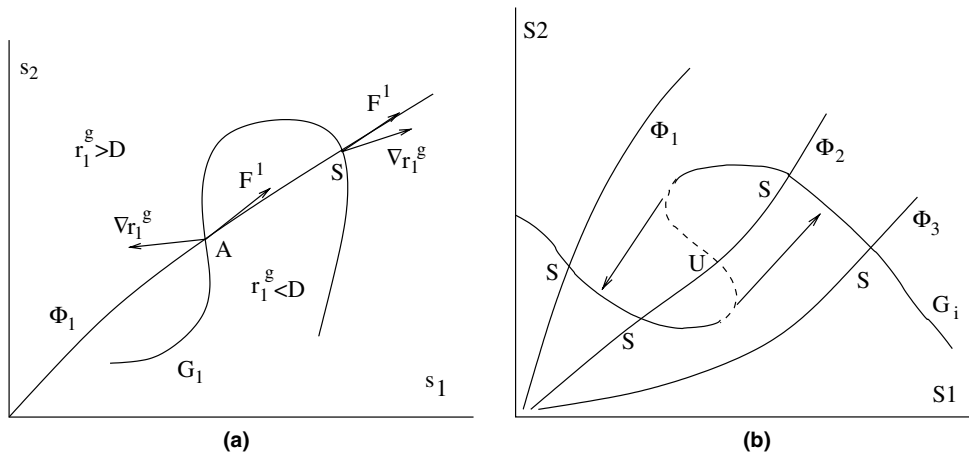


Fig. 5. Stability of the semitrivial equilibria. (a) Two different types of interaction between substrates s_1 and s_2 . At the intersection A , the substrates are antagonistic because $\nabla r_1^g \cdot F^1 < 0$. At the intersection S , the substrates are synergistic because $\nabla r_1^g \cdot F^1 > 0$. (b) Possible hysteresis in the single-species case. The growth isocline of the i th species is given by G_i . The stable and unstable equilibria are represented by S and U , respectively.

substrates s_1 and s_2 are locally synergistic at ϕ_{10} , then ϕ_{10} is unstable with one positive eigenvalue. If $r_2^g(s_1, s_2) > D$ and the substrates s_1 and s_2 are locally antagonistic at ϕ_{10} , then ϕ_{10} is unstable with two positive eigenvalues. In each of the above cases, the variational matrix of (1) and (2) at ϕ_{10} admits only real eigenvalues.

Proof. The variational matrix of (1) and (2) at ϕ_{10} has the form

$$J(\phi_{10}) = \begin{pmatrix} -D - c_1 \frac{\partial r_{11}}{\partial s_1} & -c_1 \frac{\partial r_{11}}{\partial s_2} & -r_{11} & -r_{21} \\ -c_1 \frac{\partial r_{12}}{\partial s_1} & -D - c_1 \frac{\partial r_{12}}{\partial s_2} & -r_{12} & -r_{22} \\ c_1 \frac{\partial r_1^g}{\partial s_1} & c_1 \frac{\partial r_1^g}{\partial s_2} & 0 & 0 \\ 0 & 0 & 0 & r_2^g - D \end{pmatrix}, \tag{8}$$

where $r_{ij} = r_{ij}(s_1, s_2)$, $\frac{\partial r_{ij}}{\partial s_k} = \frac{\partial r_{ij}}{\partial s_k}(s_1, s_2)$, $\frac{\partial r_1^g}{\partial s_j} = Y_{11} \frac{\partial r_{11}}{\partial s_j} + Y_{12} \frac{\partial r_{12}}{\partial s_j}$, and $r_i^g = r_i^g(s_1, s_2)$.

Clearly, one eigenvalue of (8) is $\lambda_4 = r_2^g - D$. The remaining three eigenvalues of (8) are the eigenvalues of the submatrix

$$A = \begin{pmatrix} -D - c_1 \frac{\partial r_{11}}{\partial s_1} & -c_1 \frac{\partial r_{11}}{\partial s_2} & -r_{11} \\ -c_1 \frac{\partial r_{12}}{\partial s_1} & -D - c_1 \frac{\partial r_{12}}{\partial s_2} & -r_{12} \\ c_1 \frac{\partial r_1^g}{\partial s_1} & c_1 \frac{\partial r_1^g}{\partial s_2} & 0 \end{pmatrix}. \tag{9}$$

The determinant of A equals

$$\det A = c_1 \frac{\partial r_1^g}{\partial s_1} \left(c_1 r_{12} \frac{\partial r_{11}}{\partial s_2} - D r_{11} - c_1 r_{11} \frac{\partial r_{12}}{\partial s_2} \right) - c_1 \frac{\partial r_1^g}{\partial s_2} \left(c_1 r_{12} \frac{\partial r_{11}}{\partial s_1} + D r_{12} - c_1 r_{11} \frac{\partial r_{12}}{\partial s_1} \right). \tag{10}$$

Observe that the following relations must hold at ϕ_{10} :

$$c_1 r_{11} = D(s_1^f - s_1), \quad c_1 r_{12} = D(s_2^f - s_2), \quad Y_{11} r_{11} + Y_{12} r_{12} = D. \tag{11}$$

Substituting the first two relations of (11) into (10), we can rewrite the determinant of A as

$$\det A = c_1 D \frac{\partial r_1^g}{\partial s_1} \left((s_2^f - s_2) \frac{\partial r_{11}}{\partial s_2} - r_{11} - (s_1^f - s_1) \frac{\partial r_{12}}{\partial s_2} \right) - c_1 D \frac{\partial r_1^g}{\partial s_2} \left((s_2^f - s_2) \frac{\partial r_{11}}{\partial s_1} + r_{12} - (s_1^f - s_1) \frac{\partial r_{12}}{\partial s_1} \right),$$

or simply as

$$\det A = -c_1 D \frac{\partial r_1^g}{\partial s_1} \frac{\partial F_1}{\partial s_2} + c_1 D \frac{\partial r_1^g}{\partial s_2} \frac{\partial F_1}{\partial s_1} = -c_1 D \nabla r_1^g \cdot F^1, \tag{12}$$

where $F^1 = \left(\frac{\partial F_1}{\partial s_2}, -\frac{\partial F_1}{\partial s_1} \right)$ is the tangent vector to the consumption curve Φ_1 oriented in the direction of increasing s_j . Since $c_1 D > 0$, we conclude that $\det A < 0$ if the substrates are locally synergistic and $\det A > 0$ if the substrates are locally antagonistic.

One eigenvalue of A is given by $\lambda_3 = -D$. Indeed, a direct calculation shows that $(Y_{11}, Y_{12}, 1) \cdot (A + DI) = 0$ due to the third relation in (11). The trace of A is given by

$$\text{tr} A = -2D - c_1 \frac{\partial r_{11}}{\partial s_1} - c_1 \frac{\partial r_{12}}{\partial s_2}.$$

Since $\lambda_3 = -D$, the sum of the two remaining eigenvalues λ_1 and λ_2 must be strictly negative

$$\lambda_1 + \lambda_2 = -D - c_1 \frac{\partial r_{11}}{\partial s_1} - c_1 \frac{\partial r_{12}}{\partial s_2} < 0.$$

Moreover, the product $\lambda_1 \lambda_2$ equals $-\frac{\det A}{D}$ because $\lambda_3 = -D$. If $\det A < 0$ (synergistic substrates), then $\lambda_1 \lambda_2 > 0$ which implies that $\Re \lambda_{1,2} < 0$. If $\det A > 0$ (antagonistic substrates), then $\lambda_1 \lambda_2 < 0$ which implies that $\lambda_2 < 0 < \lambda_1$. This concludes the proof of Theorem 3. \square

Remarks

1. If the model (1) and (2) does not include the mutual inhibition of uptake rates (e.g., in the model of Leon and Tumpson [12]), then the substrates are synergistic for all feed concentrations. In this case, the variational matrix (8) has three real negative eigenvalues and the fourth eigenvalue $\lambda_4 = r_2^g - D$ may be positive or negative.
2. The positivity of $\lambda_4 = r_2^g - D$ is traditionally interpreted as the invasion criterion. Specifically, $\lambda_4 = r_2^g - D > 0$ if and only if the equilibrium ϕ_{10} can be successfully invaded by the second species. The instability of ϕ_{10} resulting from antagonistic interaction between substrates can be associated with the sub-optimal consumption regime of the first species. Indeed, if r_1^g is locally decreasing along Φ_1 at ϕ_{10} , then there exists a semitrivial equilibrium ϕ'_{10} with lower values of s_1 and s_2 where the substrates are synergistic.
3. If ϕ_{10} is unique, it implies that the substrates are locally synergistic. Moreover, it implies that $r_1^g(s_1^f, s_2^f) > D$ so that ϕ_{00} is unstable. In this case, our conclusions are the same as in [16].
4. The stability analysis of Theorem 3 provides a plausible explanation of ‘hysteresis’ effects observed in single-species growth. For instance, if the substrate feed composition is changed continuously, a single species culture may exhibit an abrupt switch from one consumption regime to another [21]. This phenomenon cannot be observed when the growth isocline is a monotonically decreasing curve. But if the growth isocline is a non-monotone curve such as shown in Fig. 5(b), then it is possible to vary the feed concentrations to generate the sequence of the consumption curves such as Φ_1 , Φ_2 , and Φ_3 . When $\Phi = \Phi_1$ or $\Phi = \Phi_3$, there is a unique stable equilibrium. When $\Phi = \Phi_2$, there are two stable and one unstable semitrivial equilibria. If the consumption curve varies as $\Phi_1 \rightarrow \Phi_2 \rightarrow \Phi_3 \rightarrow \Phi_2 \rightarrow \Phi_1$ the observed (stable) equilibrium will follow a hysteresis curve with the jumps indicated by the arrows in Fig. 5(b). In [17], Ballyk and Wolkowicz analyzed the hysteretic switching between the trivial and semitrivial equilibria, whereas in general the switches may occur between two semitrivial equilibria as shown in Fig. 5(b).

The next result concerns the global convergence of solutions in the single-species case. The original proof was presented in [16] for the case when the semitrivial equilibrium ϕ_{10} is either unique or non-existent. Theorem 4 simply extends the proof to the case of multiple semitrivial equilibria.

Theorem 4. *If $c_1 = 0$ or $c_2 = 0$, then any solution of (1) and (2) converges to an equilibrium. If ϕ_{10} is unique, then all solutions of (1) and (2) with $c_1 > 0$ and $c_2 = 0$ converge to ϕ_{10} . Similarly, if ϕ_{01} is unique, then all solutions of (1) and (2) with $c_1 = 0$ and $c_2 > 0$ converge to ϕ_{01} .*

Proof. We will prove the theorem in case $c_2 = 0$ (the case $c_1 = 0$ is analogous). If $c_2 \equiv 0$, then the system (1) and (2) reduces to

$$\begin{aligned}\dot{s}_1 &= D(s_1^f - s_1) - c_1 r_{11}(s_1, s_2), \\ \dot{s}_2 &= D(s_2^f - s_2) - c_1 r_{12}(s_1, s_2), \\ \dot{c}_1 &= c_1(Y_{11}r_{11}(s_1, s_2) + Y_{12}r_{12}(s_1, s_2) - D).\end{aligned}$$

Multiplying the first equation by Y_{11} , the second equation by Y_{12} and then adding both to the third equation, we find that

$$\frac{d}{dt}(Y_{11}s_1 + Y_{12}s_2 + c_1) = DY_{11}s_1^f + DY_{12}s_2^f - D(Y_{11}s_1 + Y_{12}s_2 + c_1).$$

Consequently, the affine set

$$L = \{c_1 = Y_{11}(s_1^f - s_1) + Y_{12}(s_2^f - s_2)\}$$

is an attracting invariant set of (1) and (2). It is clear that $\lambda_3 = -D$ describes the exponential rate of convergence to this set. The dynamics of (1) and (2) on L is determined by the limiting system,

$$\dot{s}_1 = D(s_1^f - s_1) - \{Y_{11}(s_1^f - s_1) + Y_{12}(s_2^f - s_2)\}r_{11}(s_1, s_2) = \Upsilon_1(s_1, s_2), \quad (13)$$

$$\dot{s}_2 = D(s_2^f - s_2) - \{Y_{11}(s_1^f - s_1) + Y_{12}(s_2^f - s_2)\}r_{12}(s_1, s_2) = \Upsilon_2(s_1, s_2). \quad (14)$$

The system (13) and (14) is strictly cooperative for $0 < s_j \leq s_j^f$. Indeed, since $\frac{\partial r_{11}}{\partial s_2} \leq 0$ and $\frac{\partial r_{12}}{\partial s_1} \leq 0$, we have that

$$\frac{\partial \Upsilon_1}{\partial s_2} = -\{Y_{11}(s_1^f - s_1) + Y_{12}(s_2^f - s_2)\} \frac{\partial r_{11}}{\partial s_2} + Y_{12}r_{11} > 0,$$

and

$$\frac{\partial \Upsilon_2}{\partial s_1} = -\{Y_{11}(s_1^f - s_1) + Y_{12}(s_2^f - s_2)\} \frac{\partial r_{12}}{\partial s_1} + DY_{11}r_{12} > 0.$$

Specifically, this implies that any solution of (13) and (14) converges to an equilibrium. Using the theory of asymptotically autonomous systems [22], we conclude that any solution of the full system converges to an equilibrium. As we remarked earlier, whenever ϕ_{10} is unique, it is the only stable equilibrium with $c_2 = 0$. Consequently, all solutions of (1) and (2) with $c_1 > 0$ and $c_2 = 0$ converge to ϕ_{10} . \square

3.3. The non-trivial equilibrium ϕ_{11}

The non-trivial equilibrium ϕ_{11} is characterized by the relations

$$r_1^g = r_2^g = D, \quad D(s_j^f - s_j) = c_1 r_{1j} + c_2 r_{2j}, \quad j = 1, 2.$$

Specifically, the projection of ϕ_{11} onto the (s_1, s_2) plane must be an intersection of the growth isoclines G_1 and G_2 .

Definition. We define the *envelope of coexistence* as the set of all points in the (s_1, s_2) plane that lie between the two consumption curves Φ_1 and Φ_2 . The following theorem shows that only those intersections of G_1 and G_2 that occur within the envelope of coexistence correspond to the coexistence equilibrium ϕ_{11} .

Theorem 5. *An intersection of two growth isoclines is a projection of a coexistence equilibrium ϕ_{11} onto the (s_1, s_2) plane if and only if belongs to the envelope of coexistence.*

Proof. Any intersection of G_1 and G_2 corresponds to a point (s_1, s_2) such that $r_1^g = r_2^g = D$. Setting $\dot{s}_j = 0$ in (1) and solving for c_1 and c_2 , we obtain the following expressions

$$c_1 = D \frac{r_{22}(s_1^f - s_1) - r_{21}(s_2^f - s_2)}{r_{11}r_{22} - r_{12}r_{21}}, \tag{15}$$

$$c_2 = D \frac{r_{11}(s_2^f - s_2) - r_{12}(s_1^f - s_1)}{r_{11}r_{22} - r_{12}r_{21}}. \tag{16}$$

The point of intersection (s_1, s_2) corresponds to ϕ_{11} if and only if $c_1, c_2 > 0$. Therefore a necessary condition for coexistence is that $s_1 < s_1^f, s_2 < s_2^f$ and the numerators in (15) and (16) have the same sign.

Consider Eq. (16). The sign of its numerator $r_{11}(s_2^f - s_2) - r_{12}(s_1^f - s_1)$ is opposite to the sign of $F_1(s_1, s_2, s_1^f, s_2^f)$. Consequently, the sign of this expression is constant both above and below the consumption curve Φ_1 . It is clear that

$$F_1(s_1, 0, s_1^f, s_2^f) = -s_2^f r_{11}(s_1, 0) < 0$$

for all $0 < s_1 < s_1^f$. Therefore, the numerator of (16) is positive if the point (s_1, s_2) is below Φ_1 and negative if the point (s_1, s_2) is above Φ_1 . A similar argument shows that the numerator of (15) is positive if the point (s_1, s_2) is above Φ_2 and negative if the point (s_1, s_2) is below Φ_2 . Therefore, a necessary condition for coexistence is that the growth isoclines intersect within the envelope of coexistence as shown in Fig. 6.

To complete the proof, we need to show that whenever the growth isoclines intersect within the envelope of coexistence, the signs of the numerators and denominators of (15) and (16) agree as well. We denote an intersection of G_1 and G_2 by $+/+$ if both numerators in (15) and (16) are positive and by $-/-$ if both numerators in (15) and (16) are negative. Consider a point (\hat{s}_1, \hat{s}_2) which is a $+/+$ intersection of G_1 and G_2 . As we argued above, (\hat{s}_1, \hat{s}_2) must be such that $0 < \hat{s}_j < s_j^f$ and

$$\begin{aligned} r_{11}(\hat{s}_1, \hat{s}_2)(s_2^f - \hat{s}_2) - r_{12}(\hat{s}_1, \hat{s}_2)(s_1^f - \hat{s}_1) &> 0, \\ r_{21}(\hat{s}_1, \hat{s}_2)(s_2^f - \hat{s}_2) - r_{22}(\hat{s}_1, \hat{s}_2)(s_1^f - \hat{s}_1) &< 0. \end{aligned}$$

These inequalities imply that

$$\frac{r_{12}(\hat{s}_1, \hat{s}_2)}{r_{11}(\hat{s}_1, \hat{s}_2)} < \frac{s_2^f - \hat{s}_2}{s_1^f - \hat{s}_1} < \frac{r_{22}(\hat{s}_1, \hat{s}_2)}{r_{21}(\hat{s}_1, \hat{s}_2)}.$$

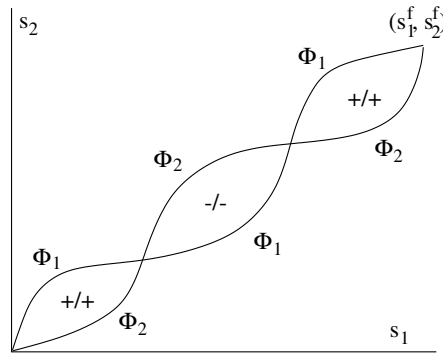


Fig. 6. The envelope of coexistence. The signs of both numerators of (15) and (16) are shown as +/+ or -/-.

Since $r_{ij}(\hat{s}_1, \hat{s}_2) > 0$, we conclude that

$$r_{11}(\hat{s}_1, \hat{s}_2)r_{22}(\hat{s}_1, \hat{s}_2) - r_{12}(\hat{s}_1, \hat{s}_2)r_{21}(\hat{s}_1, \hat{s}_2) > 0,$$

and thus (\hat{s}_1, \hat{s}_2) is indeed a projection of ϕ_{11} onto the (s_1, s_2) plane. A similar argument applies to any intersection of type -/-. \square

Remarks

1. Since the geometry of the growth isoclines can be quite complicated, a non-trivial equilibrium may exist in the absence of one or both semitrivial equilibria. For instance, it is possible that the growth isoclines intersect within the envelope of coexistence but neither isocline intersects the corresponding consumption curve. In such cases, neither species can survive in the chemostat in the absence of its competitor. Furthermore, multiple equilibria ϕ_{11} can exist.
2. The projection (\hat{s}_1, \hat{s}_2) of ϕ_{11} onto the (s_1, s_2) plane is determined exclusively by the dilution rate. If the dilution rate is fixed, the concentrations of both species c_1 and c_2 are determined by the feed concentrations s_1^f and s_2^f . Moreover, c_1 and c_2 are linear functions of s_1^f and s_2^f .

The local stability of ϕ_{11} is determined by the variational matrix of (1) and (2) which has the form

$$J(\phi_{11}) = \begin{pmatrix} -D - c_1 \frac{\partial r_{11}}{\partial s_1} - c_2 \frac{\partial r_{21}}{\partial s_1} & -c_1 \frac{\partial r_{11}}{\partial s_2} - c_2 \frac{\partial r_{21}}{\partial s_2} & -r_{11} & -r_{21} \\ -c_1 \frac{\partial r_{12}}{\partial s_1} - c_2 \frac{\partial r_{22}}{\partial s_1} & -D - c_1 \frac{\partial r_{12}}{\partial s_2} - c_2 \frac{\partial r_{22}}{\partial s_2} & -r_{12} & -r_{22} \\ c_1 \frac{\partial r_1^g}{\partial s_1} & c_1 \frac{\partial r_1^g}{\partial s_2} & 0 & 0 \\ c_2 \frac{\partial r_2^g}{\partial s_1} & c_2 \frac{\partial r_2^g}{\partial s_2} & 0 & 0 \end{pmatrix}, \tag{17}$$

where $r_{ij} = r_{ij}(\hat{s}_1, \hat{s}_2)$, $\frac{\partial r_{ij}}{\partial s_k} = \frac{\partial r_{ij}}{\partial s_k}(\hat{s}_1, \hat{s}_2)$, and $\frac{\partial r_i^g}{\partial s_j} = Y_{i1} \frac{\partial r_{i1}}{\partial s_j} + Y_{i2} \frac{\partial r_{i2}}{\partial s_j}$, for $i, j = 1, 2$.

Lemma 6. *The condition*

$$\det \begin{pmatrix} \frac{\partial r_1^g}{\partial s_1} & \frac{\partial r_1^g}{\partial s_2} \\ \frac{\partial r_2^g}{\partial s_1} & \frac{\partial r_2^g}{\partial s_2} \end{pmatrix} \cdot \det \begin{pmatrix} r_{11} & r_{21} \\ r_{12} & r_{22} \end{pmatrix} > 0 \quad (18)$$

is a necessary condition for asymptotic stability of ϕ_{11} .

Proof. The determinant of (17) is given by

$$\det J(\phi_{11}) = c_1 c_2 \det \begin{pmatrix} \frac{\partial r_1^g}{\partial s_1} & \frac{\partial r_1^g}{\partial s_2} \\ \frac{\partial r_2^g}{\partial s_1} & \frac{\partial r_2^g}{\partial s_2} \end{pmatrix} \cdot \det \begin{pmatrix} r_{11} & r_{21} \\ r_{12} & r_{22} \end{pmatrix}.$$

At ϕ_{11} , $c_1 > 0$ and $c_2 > 0$. If ϕ_{11} is asymptotically stable, then $J(\phi_{11})$ has all four eigenvalues with negative real parts and thus $\det J(\phi_{11})$ must be positive. \square

Corollary. *If*

$$\det \begin{pmatrix} \frac{\partial r_1^g}{\partial s_1} & \frac{\partial r_1^g}{\partial s_2} \\ \frac{\partial r_2^g}{\partial s_1} & \frac{\partial r_2^g}{\partial s_2} \end{pmatrix} \cdot \det \begin{pmatrix} r_{11} & r_{21} \\ r_{12} & r_{22} \end{pmatrix} < 0,$$

then ϕ_{11} is unstable for all combinations of feed concentrations s_1^f and s_2^f such that $c_1 > 0$ and $c_2 > 0$.

The necessary condition (18), is a generalization of the Gilpin–Justice condition [23] which states that *each competitor must have a higher preference for the substrate that is more beneficial to its own growth.*

If the substrates are completely independent (no inhibition), the sufficiency of the criterion was demonstrated in [16,23]. In the next section, we show that in the presence of mutual inhibition, the condition (18) alone is not sufficient to conclude the stability of ϕ_{11} . Specifically, we show that if the substrates s_1 and s_2 are locally antagonistic for one species and locally synergistic for the other species, then one can vary the feed concentrations so that ϕ_{11} undergoes a Hopf bifurcation. The possibility of Hopf bifurcations was mentioned in [16].

3.4. Existence of Hopf bifurcations

Theorem 7. *Suppose that the coexistence equilibrium ϕ_{11} is such that*

$$\det \begin{pmatrix} \frac{\partial r_1^g}{\partial s_1} & \frac{\partial r_1^g}{\partial s_2} \\ \frac{\partial r_2^g}{\partial s_1} & \frac{\partial r_2^g}{\partial s_2} \end{pmatrix} > 0, \quad \det \begin{pmatrix} r_{11} & r_{21} \\ r_{12} & r_{22} \end{pmatrix} > 0,$$

with $\nabla r_1^g \in R_-^2$ and $\nabla r_2^g \in R_+^2$. Then it is possible to vary each of the feed concentrations s_j^f in such a way that two eigenvalues of $J(\phi_{11})$ enter the positive half-plane.

Proof. By Theorem 5, the inequality

$$\det \begin{pmatrix} r_{11} & r_{21} \\ r_{12} & r_{22} \end{pmatrix} > 0$$

implies that the projection (\hat{s}_1, \hat{s}_2) of the coexistence equilibrium ϕ_{11} is located below Φ_1 and above Φ_2 , that is,

$$\eta_2(s_1^f, s_2^f, \hat{s}_1) < \hat{s}_2 < \eta_1(s_1^f, s_2^f, \hat{s}_1).$$

Now let us keep s_1^f fixed and vary the value $z = s_2^f$. We denote the resulting point by $\phi_{11}(z)$. Due to Lemma 1, both functions $\eta_i(s_1^f, z, \hat{s}_1)$ are strictly increasing in z . Consequently, there exist values $z_- < s_2^f < z_+$ such that $\hat{s}_2 = \eta_1(s_1^f, z_-, \hat{s}_1) = \eta_2(s_1^f, z_+, \hat{s}_1)$, and

$$\eta_2(s_1^f, z, \hat{s}_1) < \hat{s}_2 < \eta_1(s_1^f, z, \hat{s}_1)$$

for all $z_- < z < z_+$. It follows that $\phi_{11}(z)$ is a coexistence equilibrium for all z such that $z_- < z < z_+$. Moreover, our assumptions and Lemma 6 imply that $\det J(\phi_{11}(z)) > 0$ for all $z_- < z < z_+$.

When $z = z_-$, the point (\hat{s}_1, \hat{s}_2) is an intersection of both growth isoclines G_1 and G_2 and the consumption curve Φ_1 , i.e. it is a semitrivial equilibrium ϕ_{10} . Since $\nabla r_1^g \in R_-^2$, the substrates are locally antagonistic for species 1. Using the results of Theorem 3, we conclude that $J(\phi_{11}(z_-))$ has two negative eigenvalues $\lambda_1, \lambda_2 < 0$, one positive eigenvalue $\lambda_3 > 0$, and one zero eigenvalue $\lambda_4 = 0$ (due to the fact that $r_2^g(\hat{s}_1, \hat{s}_2) = D$). Now consider the limiting case $z \downarrow z_-$. On the one hand, $\det J(\phi_{11}(z)) > 0$. On the other hand three eigenvalues of $J(\phi_{11}(z))$ must converge to λ_1, λ_2 , and λ_3 as $z \downarrow z_-$. We conclude that the fourth eigenvalue of $J(\phi_{11}(z))$ must be strictly positive as $z \downarrow z_-$.

When $z = z_+$, the point (\hat{s}_1, \hat{s}_2) is an intersection of both growth isoclines G_1 and G_2 and the consumption curve Φ_2 , i.e. a semitrivial equilibrium ϕ_{01} . Since $\nabla r_2^g \in R_+^2$, the substrates are locally synergistic for species 2. Using the results of Theorem 3, we conclude that $J(\phi_{11}(z_+))$ has three negative eigenvalues $\lambda_1, \lambda_2, \lambda_3 < 0$, and one zero eigenvalue $\lambda_4 = 0$ (due to the fact that $r_1^g(\hat{s}_1, \hat{s}_2) = D$). Now consider the limiting case $z \uparrow z_+$. On the one hand, $\det J(\phi_{11}(z)) > 0$. On the other hand three eigenvalues of $J(\phi_{11}(z))$ must converge to λ_1, λ_2 , and λ_3 as $z \uparrow z_+$. We conclude that the fourth eigenvalue of $J(\phi_{11}(z))$ must be strictly negative as $z \uparrow z_+$.

To summarize, $\det J(\phi_{11}(z))$ remains strictly positive for all $z_- < z < z_+$, the eigenvalues of $J(\phi_{11}(z))$ are continuous functions of z and no eigenvalue of $J(\phi_{11}(z))$ becomes zero for $z_- < z < z_+$. Furthermore, $J(\phi_{11}(z))$ has four negative eigenvalues as $z \uparrow z_+$ and $J(\phi_{11}(z))$ has two positive and two negative eigenvalues as $z \downarrow z_-$. We conclude that there must exist three values of $z_-^h < z_0^h < z_+^h$ such that two of the eigenvalues of $J(\phi_{11}(z))$ become complex conjugate with a positive real part at $z = z_-^h$, this pair of eigenvalues crosses the imaginary axis at $z = z_0^h$ (which is a necessary condition for the Hopf bifurcation), and become a pair of real negative eigenvalues at $z = z_+^h$.

This argument shows that one can vary s_2^f so that a pair of complex eigenvalues of $J(\phi_{11}(z))$ enter the positive half-plane. A similar result can be attained by varying s_1^f . \square

Remarks

1. Due to the computational complexity, we did not verify the sufficient (non-linear) condition for the Hopf bifurcation. Therefore, we can only argue that the Hopf bifurcation will occur for a generic case of (1) and (2). In all of the numerical examples (not shown here), the Hopf bifurcation was always supercritical.

2. It remains an open question whether the condition that the eigenvalues of $J(\phi_{11}(z))$ have the same signs as $z \downarrow z_-$ and $z \uparrow z_+$ would be sufficient to eliminate the possibility of a Hopf bifurcation.

4. The notion of substrate preference

Suppose that the mixed culture has current substrate concentrations (s_1, s_2) . This means that the amount of substrate s_j at this point is reduced by the quantity $s_j^f - s_j$. We define the *utilization ratio* as

$$v = \frac{s_1^f - s_1}{s_2^f - s_2}.$$

For given substrate concentrations (s_1, s_2) , the rate at which c_i consumes s_j is given by r_{ij} . We define the *uptake preference ratio* of the species c_i as

$$\rho_i = \frac{r_{i1}}{r_{i2}}.$$

We say that c_i prefers to consume more of s_1 than of s_2 if $\rho_i > 1$. Similarly, c_i prefers to consume more of s_2 than of s_1 if $\rho_i < 1$. The quantity ρ_i provides a quantitative measurement of the magnitude of such consumption preference of species c_i .

The consumption curve Φ_i can be expressed as the set of all substrate concentrations (s_1, s_2) where the utilization ratio and the uptake preference ratio of c_i are equal, that is, $\rho_i = v$. The inequality $\rho_i < v$ implies that consumption preference of species c_i is lower than the current utilization ratio, and therefore c_i prefers to utilize more of s_2 than it is currently utilizing and less of s_1 than it is currently utilizing. Such single species culture will evolve towards the lower values of v . Similarly, the single species culture with $\rho_i > v$ will evolve towards the higher values of v . In this context, the consumption curve Φ_i represents that state of balance between consumption and utilization.

In Theorem 5, we proved that c_1 and c_2 can coexist at an equilibrium provided that (s_1, s_2) belongs to the envelope of coexistence. In fact, Eqs. (15) and (16) imply that c_1 and c_2 can coexist, that is,

$$\text{sign } c_1 = \text{sign } \frac{v - \rho_2}{\rho_1 - \rho_2} > 0, \quad \text{sign } c_2 = \text{sign } \frac{\rho_1 - v}{\rho_1 - \rho_2} > 0,$$

if either $\rho_1 < v < \rho_2$ or $\rho_2 < v < \rho_1$. Therefore, the envelope of coexistence can be interpreted as the set of all substrate concentrations where $v \in (\rho_1, \rho_2)$, that is, the utilization ratio is intermediate to the uptake preference ratios. In fact, the following argument shows that in the long term all coexistence solutions must persist within the envelope of coexistence. Assuming that $c_1, c_2 > 0$ and $0 < s_j < s_j^f$, we find that the time derivative of v can be expressed as

$$\dot{v} = \frac{c_1 r_{12}}{s_2^f - s_2} (\rho_1 - v) + \frac{c_2 r_{22}}{s_2^f - s_2} (\rho_2 - v).$$

The terms $c_1 r_{12}$, $c_2 r_{22}$ and $s_2^f - s_2$ are strictly positive. Therefore, outside of the envelope of coexistence, v is a monotone function which is increasing if $v < \min(\rho_1, \rho_2)$ and decreasing if $v > \max(\rho_1, \rho_2)$.

5. Application

Our results allow one to infer the stability of competition equilibria from the single species data by superimposing the consumption curves and the growth isoclines for each of the competing species on the same (s_1, s_2) plot. The experimental procedures for obtaining these curves were described in detail in section 2.1. Here we discuss how our method would perform in several hypothetical situations.² We also discuss the limitations of the method.

Let us begin by considering two simplest *hypothetical* experimental measurements in which two species c_1 and c_2 were individually grown on the mixture of two substrates s_1 and s_2 . Suppose that we measured the consumption curves and the growth isoclines and superimposed them on the same (s_1, s_2) plot and as a result obtained one of the cases shown in Fig. 7.

We begin by marking appropriate intersections of the consumption curves and the growth isoclines. For instance, ϕ_{10} is the intersection of Φ_1 and G_1 and it corresponds to the semitrivial equilibrium where c_1 is the resident species. Similarly, ϕ_{01} is the intersection of Φ_2 and G_2 and it corresponds to the semitrivial equilibrium where c_2 is the resident species. We mark the intersection of G_1 and G_2 as ϕ_{11} because in both cases it occurs within the envelope of coexistence and thus corresponds to a coexistence equilibrium. Note that in both cases shown in Fig. 7, the mutual inhibition of substrate uptake rates must be sufficiently weak because both growth isoclines are graphs of monotonically decreasing functions. Each growth isocline G_i divides the (s_1, s_2) plane into regions where $r_i^g < D$ and $r_i^g > D$. Furthermore, the region that includes the origin always corresponds to the former case since $r_i^g(0,0) < D$. It is now geometrically obvious that $r_i^g(s_1^f, s_2^f) > D, i = 1, 2$ in Fig. 7 and thus the trivial equilibrium is unstable. In addition, the gradient vectors ∇r_i^g are orthogonal to the growth isoclines G_i and these vectors must point into the region where $r_i^g > D$. Therefore, we conclude that both vectors ∇r_i^g are strictly positive at any point along the respective growth isocline G_i . Among other things, this means that the substrates s_1 and s_2 are locally synergistic both at ϕ_{10} and ϕ_{01} . This is not really surprising because otherwise these semitrivial equilibria would be unstable and thus experimentally unattainable.

What are the differences between (a) and (b) in Fig. 7? We observe that in case (a), ϕ_{10} belongs to the region where $r_2^g > D$ and ϕ_{01} belongs to the region where $r_1^g > D$. This means that both of these single species equilibria are unstable towards the invasion by the other species. In case (b), both single species equilibria are stable towards the invasion by the other species. Finally, how much can we say about stability of the coexistence equilibrium ϕ_{11} ? In both cases, ϕ_{11} lies below Φ_1 and above Φ_2 . Therefore, the determinant

$$\det \begin{pmatrix} r_{11} & r_{21} \\ r_{12} & r_{22} \end{pmatrix}$$

is positive in both cases. It is also clear that in case (a), the vector ∇r_2^g has a greater slope than the vector ∇r_1^g . Hence the vector pair $(\nabla r_1^g, \nabla r_2^g)$ has a positive orientation, e.g. the determinant

² Although the experimental literature is abundant with data on single species growth, we could not find two separate measurements obtained with the same combination of substitutable substrates. This illustrates the lack of systematic approach to studying mixed microbial cultures.

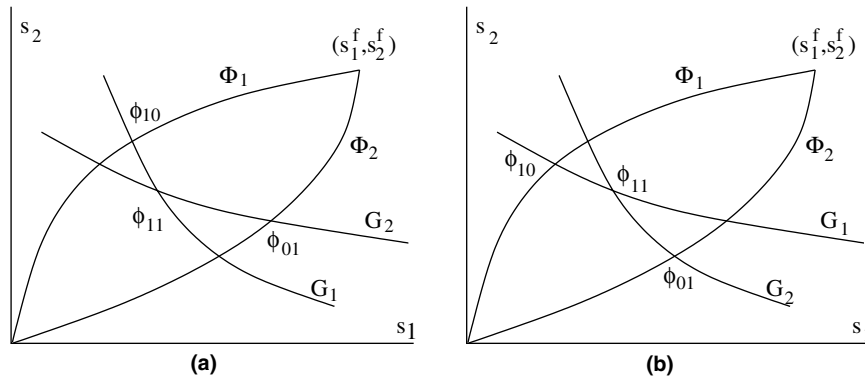


Fig. 7. Two possible plots of the consumption curves and the growth isoclines obtained in a hypothetical experiment. The consumption curves are shown as Φ_i and the growth isoclines are shown as G_i , $i = 1, 2$.

$$\det \frac{\partial(r_1^g, r_2^g)}{\partial(s_1, s_2)}$$

is also positive. Thus, in case (a), the coexistence equilibrium ϕ_{11} satisfies the necessary condition for stability. In case (b), the orientation of $(\nabla r_1^g, \nabla r_2^g)$ is negative and thus the coexistence equilibrium ϕ_{11} is unstable.

Due to its graphical nature, our method allows one to depict all essential information regarding the growth and consumption properties of both species in the same simple plot. But the method is limited because only stable equilibria can be observed in a single species experiment. It is therefore feasible that in some cases only parts of a given consumption curve and/or growth isocline can be obtained in a single species experiment. For instance, the dashed part of the growth isocline in Fig. 5(b) corresponds to an unstable single species equilibrium, hence we will be unable to obtain it in the experiment. Instead, by varying the ratio of the substrate feed concentrations we are going to observe the hysteretic loop of single species equilibria as shown in Fig. 5(b). Interestingly, we might be able to observe a part of this unstable growth isocline by adding a second microbial species and varying the feed concentrations to stabilize the coexistence equilibrium as shown in Theorem 7. In other words, there may be instances when we can obtain some single species data from the mixed culture experiments that we would not be able to obtain otherwise.

6. Conclusions

In this paper, we analyzed the existence and stability properties of equilibria exhibited by a mixed continuous culture in which two microbial species compete for two perfectly substitutable substrates. Most importantly, we wanted to understand the interplay between the mutual inhibition of the specific substrate uptake rates and the dynamic outcomes of microbial competition in

the mixed cultures. In addition, this work laid out the foundation for studying the stability of mixed microbial cultures in the context of more complex physiological models.

We took a non-parametric approach to the problem that does not require any specific assumptions regarding the particular functions that model the rates of substrate uptake and microbial growth. We derived a set of general predictions based on the relative geometries of two types of curves, namely, the consumption curves Φ_i and the growth isoclines G_i that are defined for each of the microbial species. Both of these curves can be conveniently plotted on the (s_1, s_2) plane of substrate concentrations.

For a single species case, we demonstrated that all semitrivial equilibria are represented by intersections of the corresponding consumption curve and the growth isocline. We have also proved that the stability of such equilibria is determined exclusively by a particular type of local substrate interaction: synergistic or antagonistic. The substrates are synergistic if increasing both substrate levels along the consumption curve Φ results in the increase of the specific growth rate r^g of the resident species, and they are antagonistic otherwise. Using this terminology, we showed that a semitrivial equilibrium is stable if and only if the substrates are synergistic. In addition, we proved that all single species solutions must converge to an equilibrium. Finally, we demonstrated that a semitrivial equilibrium is locally unstable towards the invasion by the second microbial species if and only if the specific growth rate r^g of the invading species exceeds the specific growth rate of the resident species (which is equal to the dilution rate D).

In the case when both species are present in the reactor, we demonstrated that an intersection of the growth isoclines G_1 and G_2 corresponds to a non-trivial (coexistence) equilibrium if and only if this intersection occurs within the envelope of coexistence. We also showed that the Gilpin–Justice condition [23]

$$\det \frac{\partial(r_1^g, r_2^g)}{\partial(s_1, s_2)} (\rho_1 - \rho_2) > 0$$

(which is equivalent to (18)) is necessary but not sufficient for stable coexistence. In particular, we showed that under special circumstances the coexistence equilibrium may change its stability via a Hopf bifurcation when the substrate feed concentrations are varied. In the most general setting, we were unable to provide a sufficient condition for the stability of the coexistence equilibrium, or equivalently, a condition that would rule out the possibility of a Hopf bifurcation.

We believe that this work provides several significant contributions to the theory of mixed microbial cultures. First of all, we generalized the graphical approach of Tilman [13,14] and extended the analytical approach of Ballyk and Wolkowicz [16,17] to a broader class of uptake functions and non-monotone growth isoclines. This mixed approach will serve as a foundation for studying more complex physiological models of mixed microbial growth. Secondly, we were able to add several new results to the nearly complete analysis in [16]. In particular, we obtained the necessary and sufficient conditions for the stability of semitrivial equilibria. We also provided a constructive proof for the existence of Hopf bifurcations. Finally, we extended the analysis of hysteresis in single-species cultures presented in [17] and provided a plausible explanation of switching behavior in single-species cultures [21].

Acknowledgments

This work was partially completed when S.S.P. was visiting the Mathematical Biosciences Institute at the Ohio State University. The authors are grateful to the anonymous referees and the handling editor whose valuable comments were helpful in improving the quality of this manuscript.

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