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Predicting stability of mixed microbial cultures from single species experiments: 2. Physiological model

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Abstract

In this paper, we study the equilibria of a physiological model describing the continuous culture in which two microbial populations compete for two substitutable resources. This work is an extension of the stability analysis of the phenomenological model of mixed microbial growth [M.M. Ballyk, G.S.K. Wolkowicz, Exploitative competition in the chemostat for two perfectly substitutable resources, Math. Biosci. 118 (1993) 127–180; S.S. Pilyugin, G.T. Reeves, A. Narang, Predicting stability of mixed microbial cultures from single species experiments: 2. Phenomenological model]. Here, we investigate the influence of the *peripheral enzymes* that catabolize the substrate uptake on the stability of the mixed culture. We show that, under steady state conditions, an increase in the concentration of one substrate inhibits the uptake of the other substrate(s). We present the criteria for existence, uniqueness, and stability of various types of equilibria. We formulate these criteria in terms of growth isoclines and consumption curves for each of the competing species. Since both types of curves can be obtained from a single species experiment, our approach provides a direct connection between theory and experiment and allows one to infer the dynamics of mixed cultures from the dynamics of single species cultures. By expressing the stability criteria in terms of intracellular properties, the model establishes a link between ecology and molecular biology.

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1. Introduction

The problem of competitive coexistence in mixed microbial cultures has attracted the attention of many researchers over the past decades. Several models of competition for substitutable resources have been published [1–4]. Assuming that the main metabolic pathway of an organism has a finite capacity, an increase in the metabolism of one substrate should downregulate the uptake of other substrates. Ballyk and Wolkowicz generalized the existing models by explicitly including the mutual inhibition of substrate uptake rates [5]. In our previous work [6], we extended their analysis by relaxing one of their assumptions. However, all the above papers including [5] and [6] are phenomenological in the sense that they simply assume the existence of mutual inhibition without providing a specific mechanism for it. The aim of the present report is to extend the predictions obtained from the phenomenological model [6] to a physiological model that properly accounts for such a mechanism.

Our physiological model introduces the *peripheral enzymes* as new variables. These enzymes catalyze the transport and peripheral catabolism of a substrate, i.e., they move the substrate from the extracellular space into the interior of the cell, and break it down into one or more molecules that can be released into the so-called *central metabolic pathways* for further catabolism. In general, microbes have a specific and unique set of peripheral enzymes for each substrate. In the absence of a substrate, the corresponding peripheral enzymes are synthesized at vanishingly small basal rates. However, as soon as the substrate appears in the environment, the synthesis rate of these enzymes increases dramatically. The motivation for introducing the peripheral enzymes as variables is the following. In [6], we presented experimental data showing that during both batch and continuous growth on a mixture of two substrates, the interaction between the two substrates is mutually inhibitory. As we show below, these inhibitory effects are exerted through the peripheral enzymes [7].

When microbes are grown in a batch reactor containing a surplus of two substitutable substrates, one of the substrates is typically exhausted before the other, leading to the appearance of two successive exponential growth phases. During the first exponential growth phase, when both substrates are present in the medium, the cells consume both or only one of the substrates. For instance, when *E. coli* K12 is grown on a mixture of fumarate and pyruvate, both substrates are consumed during the first phase (Fig. 2(a)). This is called *simultaneous* substrate utilization. The specific substrate uptake rates of fumarate and pyruvate during mixed-substrate growth are less than the specific uptake rates observed during growth on the individual substrates. As noted in [6], this implies that the interaction between the substrates is mutually inhibitory. When the mutual inhibition is highly asymmetric, it results in the utilization of only one of the substrates, further accentuating the role of the peripheral enzymes in this interaction. An example of this occurs when *E. coli* K12 is grown on a mixture of fumarate and glucose, consumption of fumarate is almost completely inhibited, so that glucose alone is consumed during the first phase (Fig. 2(b)). This is called *diauxic* or *preferential* substrate utilization, and the substrates consumed during the first and second phases are referred to as the 'preferred' and 'less preferred' substrates, respectively. Studies in molecular biology have shown that diauxic growth occurs because

in the presence of 'preferred substrate' (glucose), the synthesis of peripheral enzymes for the 'less preferred substrate' (fumarate) is completely abolished [7]. The very same phenomenon also occurs in continuous cultures. In [6], we showed that when *Hansenula polymorpha* is grown on a mixture of glucose and methanol, there is a range of dilution rates ($0.35 \leqslant D \leqslant 0.5$ l/h) in which glucose is consumed, whereas methanol passes through the reactor without any consumption at all (see Figure 2 of [6]). Fig. 2(c) shows that methanol uptake is abolished in this range of dilution rates because the activity of alcohol oxidase, a peripheral enzyme for methanol, is negligibly small. Thus, the mutually inhibitory interactions observed during single-species growth on a mixture of substitutable substrates are exerted through the peripheral enzymes associated with the substrates.

In phenomenological models of mixed-culture growth, the mutual substrate inhibition is captured by assuming that the single-species substrate uptake rates are such that each substrate inhibits the uptake of the other substrate. This approach is formally correct, but offers no insight into the molecular basis of the mutual inhibition. Given our knowledge of the molecular mechanism, it seems appropriate to formulate models of mixed-culture growth that treat the peripheral enzymes as additional state variables. In earlier work, we formulated a model of single-species growth on mixtures of substitutable substrates that took due account of the peripheral enzymes [8,10,11], and we showed that the model captured and explained all the properties of single-species, mixed-substrate growth summarized in the experimental literature [12]. We then extended this physiological model to include two species competing for two substitutable substrates, and conducted an extensive numerical study of the steady states [13]. The goal of this paper is to rigorously analyze the model. To this end, we use the insights obtained from the analysis of the phenomenological model in [6], and arrive at a series of results concerning the existence, uniqueness, and stability of equilibria of the two-species physiological model. We find that under specific assumptions regarding the kinetics of enzyme synthesis, the stability criteria developed in [6] are preserved despite the additional variables contained in the physiological model. Specifically, we show that

- The substrate concentrations uniquely determine the physiological steady state, that is, the steady state levels of all intracellular entities such as the transport enzymes and the inducer molecules.
- The transport enzyme for substrate S_1 is downregulated when the concentration of S_2 is increased, and vice versa. This is the molecular mechanism underlying the mutual inhibition.
- The mutual inhibition is relatively weak. Thus, even though an increase in the concentration of a substrate inhibits the specific uptake rate of the other substrate, it stimulates the specific growth rate. Using the terminology developed in [6], the substrates S_1 and S_2 always act synergistically.
- Each growth isocline is a graph of a decreasing function, and each consumption curve is a graph of an increasing function in terms of the substrate concentrations.

For a detailed review of concepts of the consumption curve, and synergistic/antagonistic interaction between substitutable substrates, we refer the reader to [6].

The paper is organized as follows. In Section 2, we present a detailed derivation of the structured model and show that it exhibits the mutual substrate uptake inhibition under steady state conditions. In Section 3, we study how the extracellular processes determine the dynamics of transport enzymes and analyze two limiting cases when the enzymes are fast and slow. Specifically, we show that if the enzymes are fast, the dynamics of the physiological model is closely

approximated by the dynamics of the phenomenological model. In Sections 4 and 5, we study existence, uniqueness, and stability of equilibria of the physiological model. Section 6 contains the discussion and it concludes the paper.

2. The model

The kinetic scheme of our model is shown in Fig. 1. As a notational convention for the rest of the paper, the index i will denote the species number, and the index j will denote the substrate number. Thus, C_i denotes the ith species, S_j denotes the jth substrate, E_{ij} denotes the 'lumped' system of inducible enzymes catalyzing the uptake and peripheral catabolism of S_j by C_i, X_{ij} denotes the inducer for E_{ij}, and C_i^- denotes all intracellular constituents in the ith species, except E_{ij} and X_{ij}. The concentrations of these entities are denoted by the lower-case letters c_i, s_j, e_{ij}, x_{ij}, and c_i^-. Here, c_i and s_j are based on the volume of the chemostat, and expressed in the units gdw/l and g/l, respectively; the remaining variables, e_{ij}, x_{ij} and c_i^- are mass-fractions of cellular biomass, and expressed in the units, g/gdw. [1]

When formulating a structured growth model that involves both extra- and intracellular entities, one has to properly formulate the mass-balance equations [14]. Since there is often a confusion about the mass-balance, we provide a brief description of mass-balance laws here. Let V be the volume of the reactor, F be the volumetric flow rate, z be the mass-fraction of an intracellular entity Z, and c be the cell density. If the entity Z is synthesized at a specific rate α and removed at a specific rate β, then the mass of Z in the reactor is zcV, and it changes according to the mass-balance law

$$\frac{\mathrm{d}(zcV)}{\mathrm{d}t} = \alpha cV - \beta cV - zcF.$$

Dividing out the constant volume V in the above equation, and introducing the dilution rate $D = F/V$, we obtain

$$c\frac{\mathrm{d}z}{\mathrm{d}t} = \alpha c - \beta c - Dzc - z\frac{\mathrm{d}c}{\mathrm{d}t}.$$

Dividing out the cell concentration $c > 0$, we finally obtain

$$\frac{\mathrm{d}z}{\mathrm{d}t} = \alpha - \beta - \left(D + \frac{1}{c}\frac{\mathrm{d}c}{\mathrm{d}t}\right)z.$$

As we show below, the last term accounts for the dilution of Z due to growth.

Regarding the kinetics of the metabolic pathway of C_i, we assume that

1. The specific uptake rate r_{ij}^{s} of S_j by C_i satisfies

$$r_{ij}^{\mathrm{s}} \equiv V_{ij}^{\mathrm{s}} e_{ij} \frac{s_j}{K_{ij}^{\mathrm{s}} + s_j}.$$

[1] It is conventional to measure cell content in grams of dry weight (gdw). The cell concentrations are measured in gdw/l and the mass-fractions of intracellular variables are measured in g/gdw.

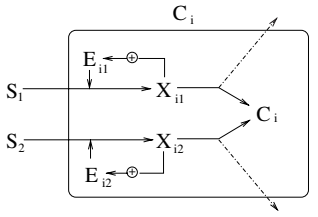

Fig. 1. Kinetic scheme of the metabolic pathway of C_i. E_{ij} denotes the transport enzyme of C_i catalyzing the uptake of substrate S_j. X_{ij} denotes the inducer for E_{ij}, and C_i^- denotes all intracellular components of C_i other than E_{ij} and X_{ij}.

2. The specific rate of breakdown of X_{ij} into energy and free biomass C_i^-, denoted r_{ij}^x, is given by $r_{ij}^x \equiv k_{ij}^x x_{ij}$.
3. The rate of production of free biomass C_i^- is a fixed fraction $Y_{ij} r_{ij}^x$ of r_{ij}^x. [2] The fraction $(1 - Y_{ij}) r_{ij}^x$ corresponds to overflow metabolism, energy spillage, and maintenance.
4. The specific rate of *inducible* enzyme synthesis, denoted r_{ij}^e, is given by
$$r_{ij}^e \equiv V_{ij}^e \frac{x_{ij}}{K_{ij}^e + x_{ij}}.$$
5. The specific rate of *constitutive* enzyme synthesis, denoted r_{ij}^*, is constant $r_{ij}^* \equiv k_{ij}^*$.
6. The specific rate of enzyme degradation, denoted r_{ij}^d, is proportional to e_{ij}, that is, $r_{ij}^d \equiv k_{ij}^d e_{ij}$.

Writing the mass-balance equations for e_{ij}, x_{ij}, and c_i^-, we obtain

$$\frac{ds_j}{dt} = D\left(s_j^f - s_j\right) - r_{1j}^s c_1 - r_{2j}^s c_2, \tag{1}$$

$$\frac{de_{ij}}{dt} = r_{ij}^e + r_{ij}^* - r_{ij}^d - \left(D + \frac{1}{c_i}\frac{dc_i}{dt}\right) e_{ij}, \tag{2}$$

$$\frac{dx_{ij}}{dt} = r_{ij}^s - r_{ij}^x - \left(D + \frac{1}{c_i}\frac{dc_i}{dt}\right) x_{ij}, \tag{3}$$

$$\frac{dc_i^-}{dt} = \sum_{j=1}^{2} \left(Y_{ij} r_{ij}^x + r_{ij}^d - r_{ij}^e - r_{ij}^*\right) - \left(D + \frac{1}{c_i}\frac{dc_i}{dt}\right) c_i^-, \tag{4}$$

where s_j^f denotes the concentration of S_j in the feed, and D is the dilution rate. Adding Eqs. (2)–(4) and observing that the sum of mass-fractions of all the intracellular components is unity,

$$\sum_{j=1}^{2} (x_{ij} + e_{ij}) + c_i^- = 1, \quad i = 1,2,$$

[2] The stoichiometric constant $0 \leqslant Y_{ij} \leqslant 1$ is traditionally referred to as the yield coefficient.

we find that

$$\frac{dc_i}{dt} = (r_i^g - D)c_i, \quad r_i^g \equiv Y_{i1}r_{i1}^x + Y_{i2}r_{i2}^x.$$

where r_i^g denotes the specific growth rate of the ith species. Evidently, $D + (1/c_i)(dc_i/dt) = r_i^g$, so that the last term in Eqs. (2)–(4) represents the dilution of the corresponding intracellular variable due to growth.

We make an additional assumption that the kinetics of inducer X_{ij} occurs on a much faster time scale than the time scale of the reactor and introduce the quasi steady state approximation $r_{ij}^x \doteq r_{ij}^s$, so that

$$x_{ij} = \frac{r_{ij}^s}{k_{ij}^x}, \quad \frac{dc_i}{dt} = (r_i^g - D)c_i, \quad \text{and} \quad r_i^g \doteq Y_{i1}r_{i1}^s + Y_{i2}r_{i2}^s, \qquad i,j = 1,2.$$

Under this quasisteady state approximation, we rewrite Eqs. (1)–(4) as

$$\frac{ds_j}{dt} = D\left(s_j^f - s_j\right) - r_{1j}^s c_1 - r_{2j}^s c_2, \quad j = 1,2, \tag{5}$$

$$\frac{de_{ij}}{dt} = V_{ij}^e \frac{e_{ij}\sigma_{ij}}{\bar{K}_{ij}^e + e_{ij}\sigma_{ij}} + k_{ij}^* - k_{ij}^d e_{ij} - r_i^g e_{ij}, \quad i,j = 1,2, \tag{6}$$

$$\frac{dc_i}{dt} = (r_i^g - D)c_i, \quad i = 1,2, \tag{7}$$

where

$$\bar{K}_{ij}^e = \frac{K_{ij}^e k_{ij}^x}{V_{ij}^s}, \quad \sigma_{ij} = \frac{s_j}{K_{ij}^s + s_j}, \qquad i,j = 1,2,$$

and r_i^g, the specific growth rate of c_i, is given by

$$r_i^g = Y_{i1}r_{i1}^s + Y_{i2}r_{i2}^s = Y_{i1}V_{i1}^s e_{i1}\sigma_{i1} + Y_{i2}V_{i2}^s e_{i2}\sigma_{i2}, \quad i = 1,2. \tag{8}$$

The above model was introduced in our earlier report [13], where we presented an extensive numerical study of this model. In this paper, we analyze a generalization of the model (5)–(8). Before doing so, we give an intuitive explanation of the model to illustrate the fact that the predictions are indeed consistent with the dynamics observed in single-species, mixed-substrate experiments.

We begin by observing that the entire transient of single-species batch growth can be obtained by integrating the five equations obtained by fixing i and letting $D = 0$ in Eqs. (5)–(7). However, the evolution of the peripheral enzyme levels during the finite time interval corresponding to the *first* exponential growth phase is described by only two equations. To see this, it suffices to observe that during the first exponential growth phase, the concentrations of both substrates are at supersaturating levels, i.e., $s_j \gg K_{ij}^s$. Under these substrate-excess conditions, constitutive enzyme synthesis is negligibly small ($k_{ij}^* \ll r_{ij}^e$), and the degradation rates of the peripheral enzymes are negligible compared to their dilution rates ($k_{ij}^d \ll r_i^g$). Hence, Eq. (6) can be approximated by

$$\dot{e}_{ij} \approx V_{ij}^e \frac{e_{ij}\sigma_{ij}}{\bar{K}_{ij}^e + e_{ij}\sigma_{ij}} - \left(\sum_{k=1}^{2} Y_k V_{ik}^s e_{ik}\right) e_{ij}, \quad j = 1,2. \tag{9}$$

These equations, which describe the evolution of the peripheral enzyme levels during the first exponential growth phase, exhibit the dynamics similar to that of the Lotka-Volterra model for competing species

$$\dot{N}_j = a_j N_j - \left(\sum_{k=1}^{2} b_{jk} N_k\right) N_j, \quad j = 1,2, \tag{10}$$

where a_j denotes the unrestricted specific growth rate of the jth species, and b_{jk} are parameters that characterize the intensity of intraspecific and interspecific competition. Now, for certain values of the parameters, a_j and b_{jk}, the Lotka-Volterra equations yield global dynamics corresponding to extinction of one of the species or coexistence of both species. It is therefore not surprising that Eq. (9) yield similar dynamics for suitable parameter values. The first case (Fig. 2(c)), in which both peripheral enzymes 'coexist', mirrors the dynamics of simultaneous substrate utilization. The second case (Fig. 2(d)), which shows 'extinction' of E_1 during the first exponential growth phase, reflects the preferential utilization of S_2.

In summary, the model states that the dynamics observed in substrate-excess batch cultures are the outcome of 'competitive interactions' between the peripheral enzymes of the substrates. Indeed,

- Each peripheral enzyme promotes its own synthesis because production of these enzymes is autocatalytic. This is intuitively evident from Fig. 1. The higher the level of E_{ij}, the larger the synthesis rate of X_{ij} and E_{ij}.
- Each peripheral enzyme inhibits the synthesis of the peripheral enzyme for the other substrate by increasing its dilution rate.

The preferential growth pattern occurs because the 'preferred' substrate increases the dilution rate of the peripheral enzyme for the 'less preferred' substrate to such an extent that this enzyme becomes 'extinct'. The model provides a simple explanation for certain empirical generalizations [12]. Substrates that support high growth rates tend to strongly dilute the enzymes for other substrates, and thus are likely to be the 'preferred' substrates. However, if their capacity for supporting high growth rates is diminished by decreasing their initial concentration in the experiment, they are unable to dilute the enzymes for the other substrates, resulting in simultaneous substrate utilization.

In continuous cultures, the uptake of 'less preferred substrate' becomes greatly diminished at high dilution rates. This phenomenon becomes more transparent if we let $k_{ij}^{*} = 0$. Fig. 3 (lower panel) shows that the model predicts the steady state profiles observed when the dilution rate is changed at fixed feed concentrations. As the dilution rate approaches a threshold value, the ability of the cells to consume the 'less preferred substrate' is significantly reduced. Equivalently, the residual concentration of the 'less preferred substrate' converges to the corresponding feed concentration which clearly indicates the lack of consumption. Such threshold value of the dilution rate corresponds to a transcritical bifurcation at which the peripheral enzyme level for the 'less preferred substrate' becomes zero. Indeed, at steady state, Eq. (6), the mass balance for peripheral enzymes, reads

$$0 \approx V_{ij}^{e} \frac{e_{ij}\sigma_{ij}}{\bar{K}_{ij}^{e} + e_{ij}\sigma_{ij}} - \left(D + k_{ij}^{d}\right) e_{ij} \Rightarrow V_{ij}^{e} \frac{\sigma_{ij}}{\bar{K}_{ij}^{e} + e_{ij}\sigma_{ij}} = D + k_{ij}^{d} \quad \text{or} \quad e_{ij} = 0.$$

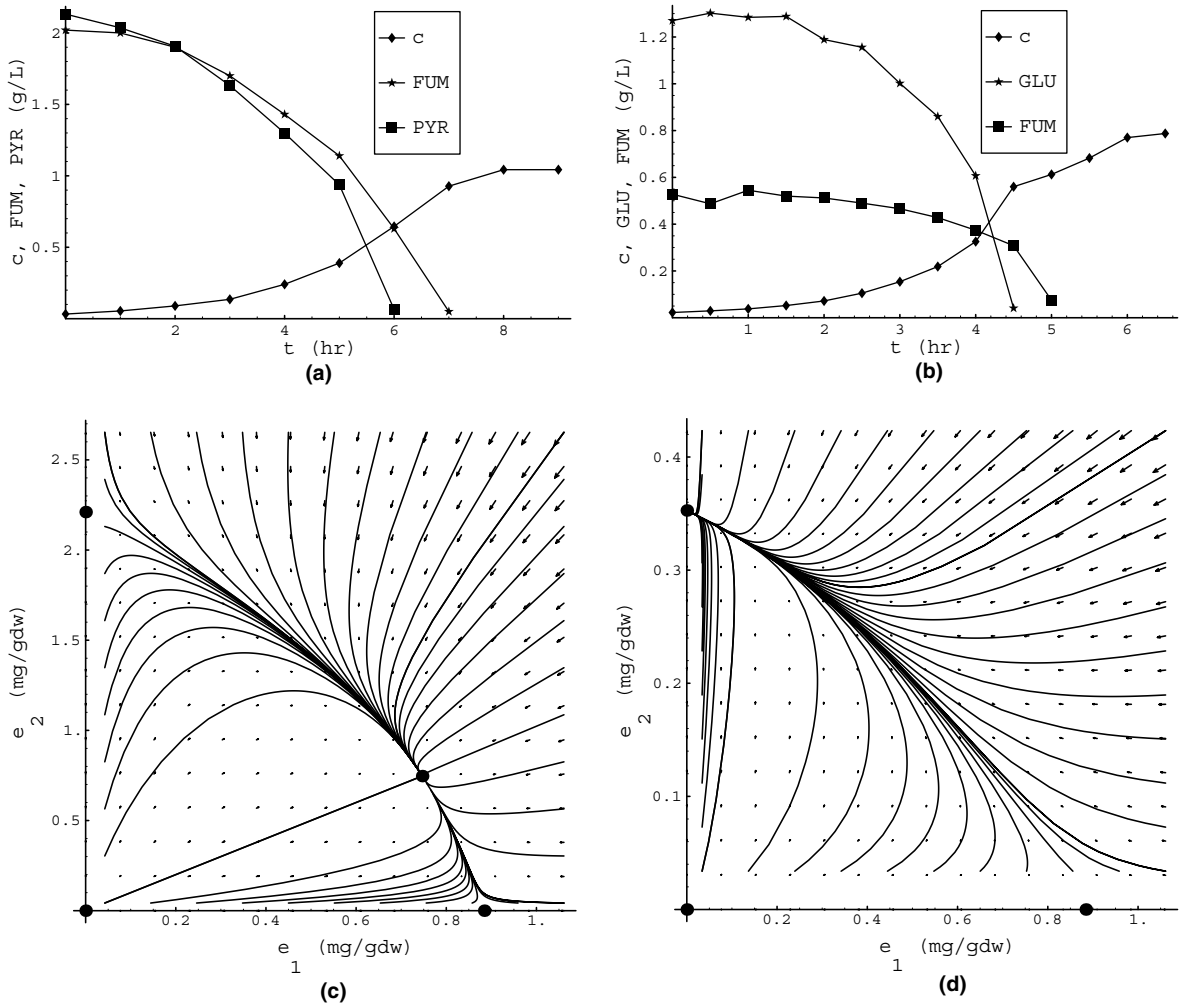

Fig. 2. Substrate utilization patterns observed in batch growth of *E. coli* K12 on mixtures of two carbon sources (from [9]). *Upper panel*: Experimental data showing (a) simultaneous utilization of fumarate and pyruvate, (b) sequential utilization of glucose and fumarate. *Lower panel*: Model simulations from [10] showing the initial dynamics of the peripheral enzymes corresponding to (c) simultaneous substrate utilization, (d) sequential substrate utilization.

At sufficiently high D, the first relation cannot be satisfied, and the cells switch to the steady state $e_{ij} = 0$.

Having given intuitive explanations of the model in the special case of single-species growth, we now consider the following generalization of the multiple-species model (5)–(8)

$$\frac{\mathrm{d}s_j}{\mathrm{d}t} = D\left(s_j^{\mathrm{f}} - s_j\right) - c_1 r_{1j} - c_2 r_{2j}, \quad j = 1,2, \tag{11}$$

$$\frac{\mathrm{d}e_{ij}}{\mathrm{d}t} = r_{ij}^{\mathrm{e}}(s_j, e_{ij}) - e_{ij} r_i^{\mathrm{g}}(s_1, s_2, e_{i1}, e_{i2}) = R_{ij}(s_1, s_2, e_{i1}, e_{i2}), \quad i,j = 1,2, \tag{12}$$

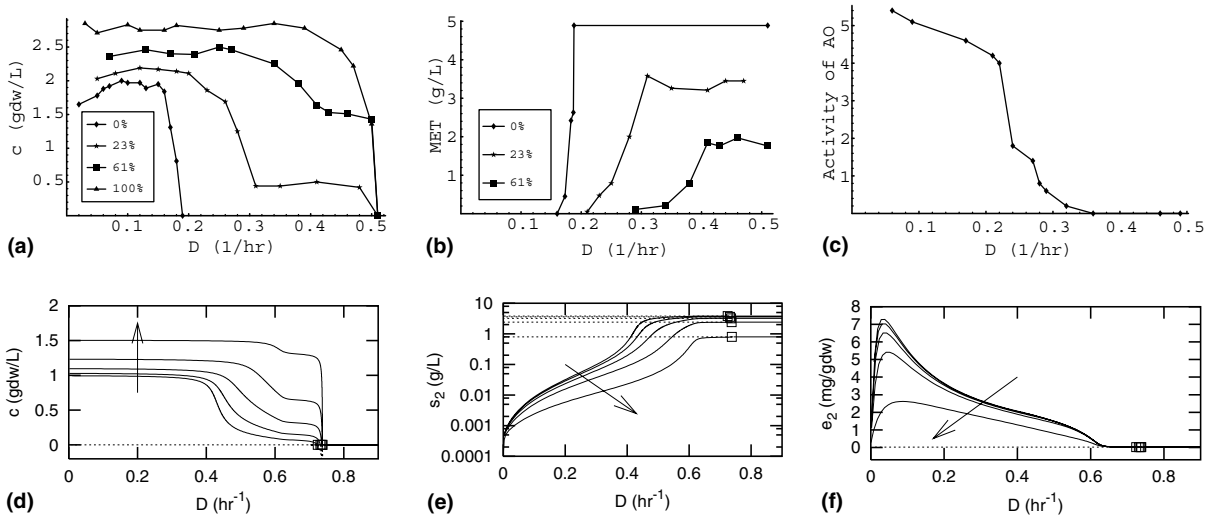

Fig. 3. Variation of the steady state concentrations when the dilution rate is varied at fixed feed concentrations. *Upper panel*: Experimental data for growth of *H. polymorpha* on various mixtures of glucose and methanol. The feed composition varies from 0% glucose (pure methanol) to 100% glucose by weight (from [15,16]). (a) Cell density, (b) methanol concentration, (c) peripheral enzyme (alcohol oxidase) level for 61% glucose in the feed. *Lower panel*: Model simulations from [11] showing the (d) cell density, (e) concentration of the 'less preferred' substrate (S_2), (f) level of the peripheral enzyme for the 'less preferred' substrate. The arrow points in the direction of increasing proportion of S_1 in the feed. The open square shows the bifurcation point corresponding to washout of the culture.

$$\frac{\mathrm{d}c_i}{\mathrm{d}t} = c_i(r_i^{\mathrm{g}}(s_1,s_2,e_{i1},e_{i2}) - D), \quad i = 1,2, \tag{13}$$

where we assume that

(H1) The functions $r_{ij}^{\mathrm{e}}(s_j,e_{ij})$ are such that

$$\frac{\partial r_{ij}^{\mathrm{e}}(s_j,e_{ij})}{\partial s_j} > 0, \quad \frac{\partial}{\partial e_{ij}}\left(\frac{r_{ij}^{\mathrm{e}}(s_j,e_{ij})}{e_{ij}}\right) < 0.$$

(H2) For any $s_j > 0$,

$$\lim_{e_{ij}\to 0} r_{ij}^{\mathrm{e}}(s_j,e_{ij}) = k_{ij}^* > 0,$$

where k_{ij}^* represents the rate of constitutive enzyme synthesis. In addition, there exists a unique value $e_{ij}^0(s_j) > 0$ such that $r_{ij}^{\mathrm{e}}(s_j,e_{ij}^0(s_j)) = 0$. We also assume that at high enzyme levels, the enzymes are degraded faster than their production rate, e.g. the net production rate is negative ($r_{ij}^{\mathrm{e}}(s_j,e_{ij}) < 0$) when $e_{ij} > e_{ij}^0(s_j)$.

(H3) The functions r_{ij} are given by $e_{ij}\sigma_{ij}(s_j)$ where $\sigma_{ij}(s)$ are such that

$$\sigma_{ij}(0) = 0, \quad \sigma_{ij}'(s) > 0, \quad \lim_{s\to\infty} \sigma_{ij}(s) < +\infty.$$

For mathematical convenience, we will treat the coefficient V_{ij}^{s} in (8) as a part of σ_{ij}, so that

$$r_i^g = Y_{i1}e_{i1}\sigma_{i1} + Y_{i2}e_{i2}\sigma_{i2}, \quad i = 1,2. \tag{14}$$

One can easily see that Eqs. (5)–(8) are a special case of (11)–(14).

The assumption (H1) states that an increase in the specific substrate S_j enhances the production of the corresponding enzyme E_{ij}. Furthermore, the rate of production of E_{ij} is a sublinear function of the enzyme level e_{ij}. In addition, the assumption (H2) states that at low levels, the enzyme E_{ij} is still produced via the constitutive enzyme synthesis.

In the next two Lemmas, we show that under steady state conditions, the enzyme levels and the specific growth rates are uniquely determined by the substrate concentrations. In addition, we show that the specific uptake rates exhibit mutual inhibition, but the specific growth rates are increasing functions of each substrate.

Lemma 1. *At steady state, the values of e_{ij} and r_i^g are uniquely determined by the values s_1 and s_2.*

Proof. First, consider Eq. (12) at steady state, that is,

$$r_{ij}^e(s_j,e_{ij}) - e_{ij}r_i^g = 0 \sim r_i^g = \frac{r_{ij}^e(s_j,e_{ij})}{e_{ij}},$$

where $s_j > 0$ is fixed. By assumptions (H1) and (H2), the function $\psi_{ij}(s_j,e_{ij}) = \frac{r_{ij}^e(s_j,e_{ij})}{e_{ij}}$ is a monotonically decreasing function of e_{ij} such that

$$\lim_{e_{ij}\to 0}\psi_{ij}(s_j,e_{ij}) = +\infty, \quad \psi_{ij}\left(s_j,e_{ij}^0(s_j)\right) = 0.$$

Therefore, $\psi_{ij}(s_j,e_{ij})$ is an invertible function from the interval $(0,e_{ij}^0(s_j))$ to the interval $(0,+\infty)$. We denote the inverse of $\psi_{ij}(s_j,e_{ij})$ by $\tilde{e}_{ij}(r_i^g,s_j)$. Assumption (H1) implies that

$$\frac{\partial\tilde{e}_{ij}\left(r_i^g,s_j\right)}{\partial r_i^g} < 0, \quad \frac{\partial\tilde{e}_{ij}\left(r_i^g,s_j\right)}{\partial s_j} > 0.$$

Furthermore, assumption (H2) implies that $\tilde{e}_{ij}(r_i^g,s_j)$ is defined for all $r_i^g \in (0,+\infty)$ and

$$\lim_{r_i^g\to+\infty}\tilde{e}_{ij}\left(r_i^g,s_j\right) = 0, \quad \lim_{r_i^g\to 0}\tilde{e}_{ij}\left(r_i^g,s_j\right) = e_{ij}^0(s_j).$$

Second, consider Eq. (14) at steady state, that is,

$$r_i^g = Y_{i1}\tilde{e}_{i1}(r_i^g,s_1)\sigma_{i1}(s_1) + Y_{i2}\tilde{e}_{i2}(r_i^g,s_2)\sigma_{i2}(s_2), \tag{15}$$

and treat r_i^g as the unknown. As r_i^g increases from 0 to $+\infty$ on the left hand side, the right hand side of (15) decreases from the positive value

$$Y_{i1}e_{i1}^0(s_1)\sigma_{i1}(s_1) + Y_{i2}e_{i2}^0(s_2)\sigma_{i2}(s_2)$$

to 0. Thus, for any pair $s_1 > 0$, $s_2 > 0$ Eq. (15) admits a unique positive solution r_i^g which we denote by $\tilde{r}_i^g(s_1,s_2)$. Finally, it follows that the steady state values of e_{ij} are given by $\tilde{e}_{ij}(\tilde{r}_i^g(s_1,s_2),s_j)$ and thus are uniquely determined by s_1 and s_2. □

Definition. The functions $\tilde{r}_i^{\mathrm{g}}(s_1,s_2)$ are called the *steady state specific growth rates*. The functions $\tilde{e}_{ij}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_j)$ are called the *steady state enzyme levels*. The functions

$$\tilde{r}_{ij}(s_1,s_2) = \tilde{e}_{ij}\big(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_j\big)\sigma_{ij}(s_j)$$

are called the *steady state specific uptake rates*.

Lemma 2. (a) *The steady state specific growth rates are increasing functions of each substrate, that is,*

$$\frac{\partial \tilde{r}_i^{\mathrm{g}}(s_1,s_2)}{\partial s_1} > 0, \quad \frac{\partial \tilde{r}_i^{\mathrm{g}}(s_1,s_2)}{\partial s_2} > 0, \qquad i = 1,2.$$

(b) *The steady state specific uptake rates are such that*

$$\frac{\partial \tilde{r}_{i1}^{\mathrm{s}}(s_1,s_2)}{\partial s_1} > 0, \quad \frac{\partial \tilde{r}_{i1}^{\mathrm{s}}(s_1,s_2)}{\partial s_2} < 0, \qquad i = 1,2,$$

and

$$\frac{\partial \tilde{r}_{i2}^{\mathrm{s}}(s_1,s_2)}{\partial s_1} < 0, \quad \frac{\partial \tilde{r}_{i2}^{\mathrm{s}}(s_1,s_2)}{\partial s_2} > 0, \qquad i = 1,2.$$

Proof. (a) Replacing r_i^{g} by $\tilde{r}_i^{\mathrm{g}}(s_1,s_2)$ in Eq. (15), we obtain

$$\tilde{r}_i^{\mathrm{g}}(s_1,s_2) = Y_{i1}\tilde{e}_{i1}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_1)\sigma_{i1}(s_1) + Y_{i2}\tilde{e}_{i2}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_2)\sigma_{i2}(s_2).$$

Differentiating both sides with respect to s_1, we obtain

$$\begin{aligned}\frac{\partial \tilde{r}_i^{\mathrm{g}}}{\partial s_1}(s_1,s_2) = {} & Y_{i1}\left(\frac{\partial \tilde{e}_{i1}}{\partial r_i^{\mathrm{g}}}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_1)\frac{\partial \tilde{r}_i^{\mathrm{g}}}{\partial s_1}(s_1,s_2) + \frac{\partial \tilde{e}_{i1}}{\partial s_1}(r_i^{\mathrm{g}}(s_1,s_2),s_1)\right)\sigma_{i1}(s_1) \\ & + Y_{i1}\tilde{e}_{i1}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_1)\sigma'_{i1}(s_1) + Y_{i2}\frac{\partial \tilde{e}_{i2}}{\partial r_i^{\mathrm{g}}}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_2)\frac{\partial \tilde{r}_i^{\mathrm{g}}}{\partial s_1}(s_1,s_2)\sigma_{i2}(s_2).\end{aligned}$$

Solving for $\frac{\partial \tilde{r}_i^{\mathrm{g}}}{\partial s_1}(s_1,s_2)$, we obtain

$$\frac{\partial \tilde{r}_i^{\mathrm{g}}}{\partial s_1}(s_1,s_2) = \frac{Y_{i1}\left(\frac{\partial \tilde{e}_{i1}}{\partial s_1}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_1)\sigma_{i1}(s_1) + \tilde{e}_{i1}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_1)\sigma'_{i1}(s_1)\right)}{1 - Y_{i1}\frac{\partial \tilde{e}_{i1}}{\partial r_i^{\mathrm{g}}}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_1)\sigma_{i1}(s_1) - Y_{i2}\frac{\partial \tilde{e}_{i2}}{\partial r_i^{\mathrm{g}}}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_2)\sigma_{i2}(s_2)}.$$

By Lemma 1,

$$\frac{\partial \tilde{e}_{ij}}{\partial r_i^{\mathrm{g}}}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_j) < 0, \quad \frac{\partial \tilde{e}_{i1}}{\partial s_1}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_1) > 0,$$

thus $\frac{\partial \tilde{r}_i^{\mathrm{g}}}{\partial s_1}(s_1,s_2) > 0$. A similar argument shows that $\frac{\partial \tilde{r}_i^{\mathrm{g}}}{\partial s_2}(s_1,s_2) > 0$.

(b) Using the definition of the specific uptake rate, we find that

$$\frac{\partial \tilde{r}_{i1}}{\partial s_2}(s_1,s_2) = \frac{\partial \tilde{e}_{i1}}{\partial r_i^{\mathrm{g}}}(\tilde{r}_i^{\mathrm{g}}(s_1,s_2),s_1)\frac{\partial \tilde{r}_i^{\mathrm{g}}}{\partial s_2}(s_1,s_2)\sigma_{i1}(s_1).$$

By part (a) of this Lemma, the factor $\frac{\partial \tilde{r}_i^g}{\partial s_2}(s_1,s_2)$ is positive. By Lemma 1, the factor $\frac{\partial \tilde{e}_{i1}}{\partial \tilde{r}_i^g}(\tilde{r}_i^g(s_1,s_2),s_1)$ is negative. Therefore, $\frac{\partial \tilde{r}_{i1}}{\partial s_2}(s_1,s_2) < 0$. A similar argument shows that $\frac{\partial \tilde{r}_{i2}}{\partial s_1}(s_1,s_2) < 0$.

Since $\tilde{r}_i^g(s_1,s_2) = Y_{i1}\tilde{r}_{i1}(s_1,s_2) + Y_{i2}\tilde{r}_{i2}(s_1,s_2)$, we have that

$$\frac{\partial \tilde{r}_i^g}{\partial s_1}(s_1,s_2) = Y_{i1}\frac{\partial \tilde{r}_{i1}}{\partial s_1}(s_1,s_2) + Y_{i2}\frac{\partial \tilde{r}_{i2}}{\partial s_1}(s_1,s_2).$$

By part (a) of this Lemma, $\frac{\partial \tilde{r}_i^g}{\partial s_1}(s_1,s_2) > 0$. The argument in the previous paragraph implies that $\frac{\partial \tilde{r}_{i2}}{\partial s_1}(s_1,s_2) < 0$. Therefore, we conclude that

$$\frac{\partial \tilde{r}_{i1}}{\partial s_1}(s_1,s_2) = \frac{1}{Y_{i1}}\left(\frac{\partial \tilde{r}_i^g}{\partial s_1}(s_1,s_2) - Y_{i2}\frac{\partial \tilde{r}_{i2}}{\partial s_1}(s_1,s_2)\right) > 0.$$

A similar argument shows that $\frac{\partial \tilde{r}_{i2}}{\partial s_2}(s_1,s_2) > 0$. □

3. Dynamics of enzymes

We begin the analysis of the model (11)–(14) by studying several important properties of the enzyme dynamics. The next Lemma shows that the enzyme system for the ith species is globally stable and it exhibits competitive dynamics.

Lemma 3. *Suppose that the substrate concentrations $s_j > 0$ are fixed. Let $i \in \{1,2\}$ and consider the dynamics of the system*

$$\frac{de_{i1}}{dt} = r_{i1}^e(s_1,e_{i1}) - (Y_{i1}e_{i1}\sigma_{i1} + Y_{i2}e_{i2}\sigma_{i2})e_{i1}, \tag{16}$$

$$\frac{de_{i2}}{dt} = r_{i2}^e(s_2,e_{i2}) - (Y_{i1}e_{i1}\sigma_{i1} + Y_{i2}e_{i2}\sigma_{i2})e_{i2}, \tag{17}$$

where $\sigma_{ij} = \sigma_{ij}(s_j)$ and s_j, $j = 1, 2$. The system (16) and (17) is strictly competitive and it admits a unique globally asymptotically stable equilibrium in the positive quadrant.

Proof. In the positive quadrant,

$$\frac{\partial}{\partial e_{i2}}\frac{de_{i1}}{dt} = -Y_{i2}\sigma_{i2}e_{i1} < 0,$$

$$\frac{\partial}{\partial e_{i1}}\frac{de_{i2}}{dt} = -Y_{i1}\sigma_{i1}e_{i2} < 0,$$

therefore (16) and (17) is strictly competitive. Solving for the nullclines in (16) and (17), we obtain two functions

$$e_{i2} = W_1(e_{i1}) \equiv \frac{1}{Y_{i2}\sigma_{i2}}\left(\frac{r_{i1}^e(s_1,e_{i1})}{e_{i1}} - Y_{i1}e_{i1}\sigma_{i1}\right),$$

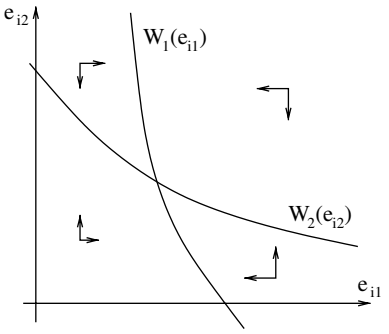

Fig. 4. Phase portrait of (16) and (17). The curves labeled $W_1(e_{i1})$ and $W_2(e_{i2})$ represent the nullclines for e_{i1} and e_{i2}, respectively. The nullclines partition the $e_{i1}e_{i2}$-quadrant into four regions; the arrows show the orientation of the vector field of (16) and (17) in these regions.

$$e_{i1} = W_2(e_{i2}) \equiv \frac{1}{Y_{i1}\sigma_{i1}}\left(\frac{r^{e}_{i2}(s_2,e_{i2})}{e_{i2}} - Y_{i2}e_{i2}\sigma_{i2}\right).$$

The functions $W_1(e_{i1})$ and $W_2(e_{i2})$ describe the e_{i1}- and e_{i2}-nullclines respectively. We observe that

- Both W_1 and W_2 are decreasing functions by assumption (H1);
- $W_1 \to +\infty$ as $e_{i1} \to 0$ and $W_1 \to -\infty$ as $e_{i1} \to \infty$; similarly $W_2 \to +\infty$ as $e_{i2} \to 0$ and $W_2 \to -\infty$ as $e_{i2} \to \infty$ by assumption (H2);
- Since s_1 and s_2 are fixed, Eqs. (16) and (17) admit a unique positive equilibrium as guaranteed by Lemma 1, that is, the graphs of W_1 and W_2 intersect exactly once in the positive quadrant;
- $de_{i1}/dt < 0$ for $e_{i2} > W_1(e_{i1})$ and $de_{i1}/dt > 0$ for $e_{i2} < W_1(e_{i1})$; similarly $de_{i2}/dt < 0$ for $e_{i1} > W_2(e_{i2})$ and $de_{i2}dt > 0$ for $e_{i1} < W_1(e_{i2})$.

These observations imply that the only possible phase diagram of (16) and (17) is as shown in Fig. 4.

An important conclusion of the phase plane analysis is that the positive equilibrium of (16) and (17) is globally asymptotically stable and the variational matrix

$$E_i = \frac{\partial(R_{i1},R_{i2})}{\partial(e_{i1},e_{i2})} \tag{18}$$

of (16) and (17) at this equilibrium has two strictly negative real eigenvalues. [3] □

Remark. The pair of steady state enzyme values $(\tilde{e}_{i1},\tilde{e}_{i2})$ is the positive solution of the system

$$R_{i1}(s_1,s_2,\tilde{e}_{i1},\tilde{e}_{i2}) = 0, \quad R_{i2}(s_1,s_2,\tilde{e}_{i1},\tilde{e}_{i2}) = 0. \tag{19}$$

The partial derivatives of $\tilde{e}_{ij}$ with respect to s_j can be obtained by implicit differentiation in (19), that is,

[3] The eigenvalues are real because the system (16) and (17) is competitive.

$$0 = R_i + E_i \cdot \frac{\partial(\tilde{e}_{i1},\tilde{e}_{i2})}{\partial(s_1,s_2)}, \quad R_i = \frac{\partial(R_{i1},R_{i2})}{\partial(s_1,s_2)}, \quad E_i = \frac{\partial(R_{i1},R_{i2})}{\partial(e_{i1},e_{i2})}.$$

Therefore,

$$\frac{\partial(\tilde{e}_{i1},\tilde{e}_{i2})}{\partial(s_1,s_2)} = -E_i^{-1}R_i. \tag{20}$$

Recall that the steady state value of the ith specific growth rate is given by

$$\tilde{r}_i^{\mathrm{g}}(s_1,s_2) = Y_{i1}\tilde{e}_{i1}(s_1,s_2)\sigma_{i1}(s_1) + Y_{i2}\tilde{e}_{i2}(s_1,s_2)\sigma_{i2}(s_2).$$

Using (20), we find that

$$\frac{\partial(\tilde{r}_1^{\mathrm{g}},\tilde{r}_2^{\mathrm{g}})}{\partial(s_1,s_2)} = \begin{pmatrix} Y_{11}\tilde{e}_{11}\sigma'_{11} & Y_{12}\tilde{e}_{12}\sigma'_{12} \\ Y_{21}\tilde{e}_{21}\sigma'_{21} & Y_{22}\tilde{e}_{22}\sigma'_{22} \end{pmatrix} - \begin{pmatrix} Y_{11}\sigma_{11} & Y_{12}\sigma_{12} \\ 0 & 0 \end{pmatrix} E_1^{-1}R_1 - \begin{pmatrix} 0 & 0 \\ Y_{21}\sigma_{21} & Y_{22}\sigma_{22} \end{pmatrix} E_2^{-1}R_2. \tag{21}$$

Using the results of Lemma 3, we can prove that single species cultures (e.g., monocultures) can only exhibit equilibrium behavior in the long term. The dynamics of monocultures is governed by the following system:

$$\dot{s}_1 = D\left(s_1^{\mathrm{f}} - s_1\right) - ce_1\sigma_1(s_1), \tag{22}$$

$$\dot{s}_2 = D\left(s_2^{\mathrm{f}} - s_2\right) - ce_2\sigma_2(s_2), \tag{23}$$

$$\dot{c} = c(r^{\mathrm{g}} - D), \tag{24}$$

$$\dot{e}_1 = r_1^{\mathrm{e}}(s_1,e_1) - r^{\mathrm{g}}e_1, \tag{25}$$

$$\dot{e}_2 = r_2^{\mathrm{e}}(s_2,e_2) - r^{\mathrm{g}}e_2, \tag{26}$$

where we dropped the subscript i in all appropriate terms and used the dot to denote the time derivative. The specific growth rate in (24) is given by $r^g = Y_1e_1\sigma_1(s_1) + Y_2e_2\sigma_2(s_2)$.

Theorem 4. *If the dynamics of the enzymes is either fast or slow on the time scale of the chemostat, then all solutions of (22)–(26) converge to an equilibrium.*

Proof. Suppose that the enzyme dynamics is fast. Then the enzyme levels rapidly settle at their quasi steady state levels $(\tilde{e}_{i1}(s_1,s_2), \tilde{e}_{i2}(s_1,s_2))$. We use this QSSA and reduce Eqs. (22)–(26) to obtain

$$\dot{s}_1 = D\left(s_1^{\mathrm{f}} - s_1\right) - c\tilde{r}_1(s_1,s_2), \tag{27}$$

$$\dot{s}_2 = D\left(s_2^{\mathrm{f}} - s_2\right) - c\tilde{r}_2(s_1,s_2), \tag{28}$$

$$\dot{c} = c(\tilde{r}^{\mathrm{g}} - D), \tag{29}$$

where $\tilde{r}_j(s_1,s_2) = \tilde{e}_j(s_1,s_2)\sigma_j(s_j)$, and $\tilde{r}^g = Y_1\tilde{r}_1 + Y_2\tilde{r}_2$. Lemma 2 implies that the reduced system (27)–(29) belongs to the class of phenomenological models studied in the first part of this paper [6]. Therefore, every solution of (27)–(29) converges to an equilibrium.

Now suppose that the enzyme dynamics is slow. First, we consider the dynamics of the subsystem (22)–(24) assuming that the enzymes are constant. This limiting case also belongs to the class of phenomenological models studied in [6]. Using the global convergence of such systems, we introduce the following quasi steady state assumption:

$$F = \left(s_1^f - s_1\right)e_2\sigma_2 - \left(s_2^f - s_2\right)e_1\sigma_1 = 0, \quad Y_1e_1\sigma_1 + Y_2e_2\sigma_2 = D, \tag{30}$$

which states that (22)–(24) is at the positive equilibrium for a given combination (e_1, e_2) of the enzyme levels. Using (30), we treat $s_1 = \hat{s}_1(e_1,e_2)$ and $s_2 = \hat{s}_2(e_1,e_2)$ as implicit functions of e_1 and e_2. The quasi steady state assumption (30) reduces the system (22)–(26) to two equations

$$\dot{e}_1 = r_1^e(\hat{s}_1(e_1,e_2),e_1) - De_1, \tag{31}$$

$$\dot{e}_2 = r_2^e(\hat{s}_2(e_1,e_2),e_2) - De_2. \tag{32}$$

Implicitly differentiating (30) with respect to e_2, we find that

$$Y_1e_1\sigma_1'\frac{\partial\hat{s}_1}{\partial e_2} + Y_2e_2\sigma_2'\frac{\partial\hat{s}_2}{\partial e_2} = -Y_2\sigma_2,$$

$$\frac{\partial F}{\partial s_1}\frac{\partial\hat{s}_1}{\partial e_2} + \frac{\partial F}{\partial s_2}\frac{\partial\hat{s}_2}{\partial e_2} = -\frac{\partial F}{\partial e_2}.$$

Since $\frac{\partial F}{\partial s_1} = -e_2\sigma_2 - (s_2^f - s_2)e_1\sigma_1' < 0$ and $\frac{\partial F}{\partial s_2} = (s_1^f - s_1)e_2\sigma_2' + e_1\sigma_1 > 0$, we have that

$$\mathscr{D} = \det\begin{pmatrix} Y_1e_1\sigma_1' & Y_2e_2\sigma_2' \\ \frac{\partial F}{\partial s_1} & \frac{\partial F}{\partial s_2} \end{pmatrix} > 0,$$

and therefore

$$\frac{\partial\hat{s}_1}{\partial e_2} = \frac{-Y_2\sigma_2\frac{\partial F}{\partial s_2} + Y_2e_2\sigma_2'\frac{\partial F}{\partial e_2}}{\mathscr{D}} = -\frac{Y_2\sigma_2e_1\sigma_1}{\mathscr{D}} < 0.$$

A symmetrical argument shows that $\frac{\partial\hat{s}_2}{\partial e_1} < 0$. Since each function r_j^e in (31) and (32) is strictly increasing in $\hat{s}_j$ due to assumption (H2), we conclude that the reduced system (31) and (32) is strictly competitive in the positive quadrant. Therefore, all solutions of (31) and (32) converge to an equilibrium. □

Remark. When the enzyme dynamics is fast on the time scale of the chemostat, the physiological model is well approximated by the phenomenological model. Therefore, all analytical results regarding existence, uniqueness, and stability of equilibria and global convergence of solutions that we presented in [6] can be directly extended to the case of the physiological model.

4. Existence and uniqueness of equilibria

In the physiological model (11)–(13), we distinguish three types of equilibria,

- the trivial equilibrium, denoted ϕ_{00}, where $c_1 = c_2 = 0$;
- the semitrivial (single species) equilibria, denoted ϕ_{10} if $c_1 > 0$, $c_2 = 0$ and ϕ_{01} if $c_1 = 0$, $c_2 > 0$;
- the non-trivial (coexistence) equilibrium, denoted ϕ_{11}, where $c_1 > 0$, $c_2 > 0$.

Definition. In the context of the physiological model (11)–(13), we define the *growth isocline* G_i of the ith species as the locus of all points (s_1,s_2) such that $\tilde{r}_i^{\mathrm{g}}(s_1,s_2) = D$ with $s_j \geqslant 0$, and the *consumption curve* Φ_i of the ith species as the locus of all points (s_1,s_2) such that

$$\frac{s_1^{\mathrm{f}} - s_1}{\tilde{r}_{i1}(s_1,s_2)} = \frac{s_2^{\mathrm{f}} - s_2}{\tilde{r}_{i2}(s_1,s_2)}, \tag{33}$$

with $0 \leqslant s_j \leqslant s_j^{\mathrm{f}}$. The *envelope of coexistence* is the set of all points in the (s_1,s_2) plane that lie between the curves Φ_1 and Φ_2.

Lemma 5. (a) *The growth isocline G_i is a graph of a smooth function $s_2 = q_i(D,s_1)$ which is monotonically increasing in D and monotonically decreasing in s_1. For any positive D there exist values $0 \leqslant s_{ji}^-(D) < s_{ji}^+(D)$, $j = 1,2$ such that $q_i(D,s_1)$ is defined for all $s_{1i}^-(D) < s_1 < s_{1i}^+(D)$ and*

$$\lim_{s_1 \to s_{1i}^-(D)} q_i(D,s_1) = s_{2i}^+(D), \qquad \lim_{s_1 \to s_{1i}^+(D)} q_i(D,s_1) = s_{2i}^-(D).$$

Furthermore, $s_{1i}^-(D) = 0$ if and only if $s_{2i}^+(D) < +\infty$, and $s_{2i}^-(D) = 0$ if and only if $s_{1i}^+(D) < +\infty$.

(b) *The consumption curve Φ_i is a graph of a smooth function $s_2 = \eta_i(s_1^{\mathrm{f}},s_2^{\mathrm{f}},s_1)$ which is monotonically increasing in s_1 and s_2^{f} and monotonically decreasing in s_1^{f}. In addition, $s_2 = \eta_i(s_1^{\mathrm{f}},s_2^{\mathrm{f}},s_1)$ is defined for all $0 \leqslant s_1 \leqslant s_1^{\mathrm{f}}$ and*

$$\lim_{s_1 \to 0} \eta_i\left(s_1^{\mathrm{f}},s_2^{\mathrm{f}},s_1\right) = 0, \qquad \lim_{s_1 \to s_1^{\mathrm{f}}} \eta_i\left(s_1^{\mathrm{f}},s_2^{\mathrm{f}},s_1\right) = s_2^{\mathrm{f}}.$$

Proof. (a) The equation defining G_i is obtained by substituting $\tilde{r}_i^{\mathrm{g}}$ into Eq. (15)

$$D = Y_{i1}\tilde{e}_{i1}(D,s_1)\sigma_{i1}(s_1) + Y_{i2}\tilde{e}_{i2}(D,s_2)\sigma_{i2}(s_2).$$

Equivalently,

$$1 = h_{i1}(D,s_1) + h_{i2}(D,s_2), \quad h_{ij}(D,s_j) = \frac{Y_{ij}}{D}\tilde{e}_{ij}(D,s_j)\sigma_{ij}(s_j). \tag{34}$$

By Lemma 1, $h_{ij}(D,0) = 0$ and $h_{ij}(D,s_j)$ is monotonically increasing in s_j and monotonically decreasing in D for all $D > 0$ and $s_j > 0$. For any $D > 0$, Eq. (34) implicitly defines a smooth function $s_2 = q_i(D,s_1)$ which is monotonically decreasing in s_1. Substituting $s_2 = q_i(D,s_1)$ into (34) and differentiating with respect to D, we find that

$$\frac{\partial h_{i1}}{\partial D}(D,s_1) + \frac{\partial h_{i2}}{\partial D}(D,q_i(D,s_1)) + \frac{\partial h_{i2}}{\partial s_2}(D,q_i(D,s_1))\frac{\partial q_i}{\partial D}(D,s_1) = 0.$$

Since $\frac{\partial h_{ij}}{\partial D} < 0$ and $\frac{\partial h_{i2}}{\partial s_2} > 0$, we conclude that $\frac{\partial q_i}{\partial D}(D,s_1) > 0$.

Consider the equation $h_{ij}(D,x) = 1$. If this equation admits a positive solution x, then we define $s_{ji}^{+}(D) = x$. If $h_{ij}(D,x)<1$ for all $x>0$, we define $s_{ji}^{+}(D) = +\infty$. Now consider the equation $h_{i1}(D,y) + h_{i2}(D,s_{2i}^{+}(D)) = 1$. If there exists a non-negative solution y, we define $s_{1i}^{-}(D) = y$, otherwise we define $s_{1i}^{-}(D) = 0$. Similarly, we define $s_{2i}^{-}(D)$. A straightforward argument shows that the quantities $s_{ji}^{\pm}(D)$ are as claimed. This concludes the proof of (a).

The proof of part (b) is identical to the proof of Lemma 1 in [6]. □

Remarks

1. As s_1 is decreased along the growth isocline G_i, then depending on the value of D, the growth isocline G_i either intersects the s_2-axis at $(0,s_{2i}^{+}(D))$ (if $s_{2i}^{+}(D)$ is finite) or it has a vertical asymptote at $s_1 = s_{1i}^{-}(D) \geqslant 0$ (if $s_{2i}^{+}(D)$ is infinite). As s_1 is increased along the growth isocline G_i, then depending on the value of D, the growth isocline G_i either intersects the s_1-axis at $(s_{1i}^{+}(D),0)$ (if $s_{1i}^{+}(D)$ is finite) or it has a horizontal asymptote at $s_2 = s_{2i}^{-}(D) \geqslant 0$ (if $s_{1i}^{+}(D)$ is infinite). Biologically, the fact that $s_{1i}^{+}(D)$ is finite means that the first substrate s_1 can alone sustain the steady state specific growth rate $\tilde{r}_i^{g}$ of the ith species at the level D, and thus c_i can persist in the chemostat even if $s_2 = 0$. Similarly, c_i can persist on s_2 alone if and only if $s_{2i}^{+}(D)$ is finite.
2. The inequalities $\frac{\partial \tilde{r}_i^{g}}{\partial s_j} \geqslant 0$ for $i,j = 1,2$ obtained in Lemma 2 imply that $\nabla \tilde{r}_i^{g} \in R_{+}^{2}$. Consequently, the substrates s_1 and s_2 are always locally synergistic in the model (11)–(13). For the exact definition of synergistic vs. antagonistic interaction between the substrates, we refer the reader to the first part of this paper [6].

Theorem 6

(a) *The trivial equilibrium is unique and it exists for all combinations of the dilution rate D and the feed concentrations* $s_j^{f}, j = 1,2$.
(b) *The semitrivial equilibrium* ϕ_{10} *exists if and only if* $\tilde{r}_1^{g}(s_1^{f},s_2^{f}) > D$ *and it is unique whenever it exists. Similarly, the semitrivial equilibrium* ϕ_{01} *exists if and only if* $\tilde{r}_2^{g}(s_1^{f},s_2^{f}) > D$ *and it is unique whenever it exists.*
(c) *The non-trivial equilibrium* ϕ_{11} *exists whenever the growth isoclines* G_1 *and* G_2 *intersect within the envelope of coexistence. Existence of both* ϕ_{10} *and* ϕ_{01} *is necessary for existence of* ϕ_{11}.

Proof

(a) Since $c_1 = c_2 = 0$ at ϕ_{00}, Eq. (11) implies that $s_j = s_j^{f}, j = 1,2$. The enzyme levels e_{ij} are uniquely defined by Lemma 1.
(b) Eqs. (11)–(13) imply that the projection of ϕ_{10} onto the (s_1,s_2) plane must be an intersection of G_1 and Φ_1. By Lemma 5, G_1 is a graph of a decreasing function, and Φ_1 is a graph of an increasing function, therefore G_1 and Φ_1 can intersect at most once. The coordinates (s_1,s_2) of such intersection uniquely determine e_{ij} by Lemma 1. Thus, ϕ_{10} is unique whenever it exists. By Lemma 2, $\tilde{r}_1^{g}(s_1,s_2)$ is an increasing function of both s_1 and s_2. As the point (s_1,s_2) travels

along Φ_1 from $(0,0)$ to (s_1^f,s_2^f), the value of $\tilde{r}_1^g(s_1,s_2)$ continuously increases from $\tilde{r}_1^g = 0$ to $\tilde{r}_1^g = \tilde{r}_1^g(s_1^f,s_2^f)$. Consequently, G_1 intersects Φ_1 if and only if $\tilde{r}_1^g(s_1^f,s_2^f) > D$. A similar argument applies to the existence and uniqueness of ϕ_{01}.

(c) The proof of the first part of this assertion is identical to the proof of Theorem 5 in [6]. Now suppose that ϕ_{11} exists, that is, G_1 and G_2 intersect within the envelope of coexistence. The results of Lemma 5 (a) imply that each growth isocline G_i must then intersect the corresponding consumption curve Φ_i and thus both ϕ_{10} and ϕ_{01} must exist. □

Remarks

1. It is possible that multiple coexistence equilibria ϕ_{11} exist because the growth isoclines G_1 and G_2 can intersect more than once. We have constructed examples of both phenomenological and physiological models with two distinct coexistence equilibria.
2. The relative geometry of growth isoclines and the consumption curves established in Lemma 5 also provides sufficient conditions for existence of ϕ_{11}. For instance, it follows that ϕ_{11} exists whenever
   - both ϕ_{10} and ϕ_{01} exist with $\tilde{r}_2^g > D$ at ϕ_{10} and $\tilde{r}_1^g > D$ at ϕ_{01} in which case *each* competitor can invade the reactor in presence of the other competitor;
   - both ϕ_{10} and ϕ_{01} exist with $\tilde{r}_2^g < D$ at ϕ_{10} and $\tilde{r}_1^g < D$ at ϕ_{01} in which case *neither* competitor can invade the reactor in presence of the other competitor.

5. Stability of equilibria

Lemma 7. *The trivial equilibrium ϕ_{00} is stable if and only if $\tilde{r}_i^g(s_1^f,s_2^f) < D$ for $i = 1,2$.*

Proof. The variational matrix of (11)–(13) at ϕ_{00} is given by

$$J(\phi_{00}) = \begin{pmatrix} -D & 0 & 0 & 0 & 0 & 0 & -\tilde{r}_{11} & -\tilde{r}_{21} \\ 0 & -D & 0 & 0 & 0 & 0 & -\tilde{r}_{12} & -\tilde{r}_{22} \\ \frac{\partial(R_{11},R_{12})}{\partial(s_1,s_2)} & & \frac{\partial(R_{11},R_{12})}{\partial(e_{11},e_{12})} & & 0 & 0 & 0 & 0 \\ & & & & 0 & 0 & 0 & 0 \\ \frac{\partial(R_{21},R_{22})}{\partial(s_1,s_2)} & & 0 & 0 & \frac{\partial(R_{21},R_{22})}{\partial(e_{21},e_{22})} & & 0 & 0 \\ & & 0 & 0 & & & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \tilde{r}_1^g - D & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \tilde{r}_2^g - D \end{pmatrix}.$$

The first two eigenvalues of $J(\phi_{00})$ are $\lambda_1 = \lambda_2 = -D$. Each of the blocks

$$E_1 = \frac{\partial(R_{11},R_{12})}{\partial(e_{11},e_{12})} \quad \text{and} \quad E_2 = \frac{\partial(R_{21},R_{22})}{\partial(e_{21},e_{22})}$$

contributes two negative eigenvalues so that λ_3, λ_4, λ_5, $\lambda_6 < 0$. The last two eigenvalues are given by $\lambda_7 = \tilde{r}_1^{\mathrm{g}}(s_1^{\mathrm{f}}, s_2^{\mathrm{f}}) - D$ and $\lambda_8 = \tilde{r}_2^{\mathrm{g}}(s_1^{\mathrm{f}}, s_2^{\mathrm{f}}) - D$. We conclude that ϕ_{00} is stable if and only if $\tilde{r}_1^{\mathrm{g}}(s_1^{\mathrm{f}}, s_2^{\mathrm{f}}) < D$ and $\tilde{r}_2^{\mathrm{g}}(s_1^{\mathrm{f}}, s_2^{\mathrm{f}}) < D$. □

Corollary. *The trivial equilibrium is stable if and only none of the semitrivial equilibria exist.*

In the next Lemma, we analyze the stability of the semitrivial equilibrium ϕ_{10}. [4]

Lemma 8. *Suppose that ϕ_{10} exists. The necessary condition for stability of ϕ_{10} is that $\tilde{r}_2^{\mathrm{g}}$ evaluated at ϕ_{10} is strictly less than D.*

Proof. The variational matrix of (11)–(13) at ϕ_{10} is given by

$$J(\phi_{10}) = \begin{pmatrix} -A & -S_1 & 0 & -B \\ R_1 & E_1 & 0 & 0 \\ R_2 & 0 & E_2 & 0 \\ C & T & 0 & G \end{pmatrix}, \tag{35}$$

where

$$A = \begin{pmatrix} D + c_1 e_{11} \sigma'_{11} & 0 \\ 0 & D + c_1 e_{12} \sigma'_{12} \end{pmatrix}, \quad G = \begin{pmatrix} 0 & 0 \\ 0 & \tilde{r}_2^{\mathrm{g}} - D \end{pmatrix}, \quad B = \begin{pmatrix} \tilde{r}_{11} & \tilde{r}_{21} \\ \tilde{r}_{12} & \tilde{r}_{22} \end{pmatrix},$$

$$C = \begin{pmatrix} c_1 Y_{11} e_{11} \sigma'_{11} & c_1 Y_{12} e_{12} \sigma'_{12} \\ 0 & 0 \end{pmatrix}, \quad S_1 = \begin{pmatrix} c_1 \sigma_{11} & 0 \\ 0 & c_1 \sigma_{12} \end{pmatrix}, \quad T = \begin{pmatrix} c_1 Y_{11} \sigma_{11} & c_1 Y_{12} \sigma_{12} \\ 0 & 0 \end{pmatrix},$$

$$R_i = \frac{\partial(R_{i1}, R_{i2})}{\partial(s_1, s_2)}, \quad E_i = \frac{\partial(R_{i1}, R_{i2})}{\partial(e_{i1}, e_{i2})}.$$

A direct examination of (35) shows that one eigenvalue of $J(\phi_{10})$ is given by $\lambda_8 = \tilde{r}_2^{\mathrm{g}} - D$ and two more eigenvalues λ_6 and λ_7 are eigenvalues of E_2 thus λ_6, $\lambda_7 < 0$ as we established in section 3. The remaining five eigenvalues of $J(\phi_{10})$ are the eigenvalues of the submatrix

$$Q = \begin{pmatrix} -D - c_1 e_{11} \sigma'_{11} & 0 & -c_1 \sigma_{11} & 0 & -\tilde{r}_{11} \\ 0 & -D - c_1 e_{12} \sigma'_{12} & 0 & -c_1 \sigma_{12} & -\tilde{r}_{12} \\ & R_1 & & E_1 & \begin{matrix} 0 \\ 0 \end{matrix} \\ c_1 Y_{11} e_{11} \sigma'_{11} & c_1 Y_{12} e_{12} \sigma'_{12} & c_1 Y_{11} \sigma_{11} & c_1 Y_{12} \sigma_{12} & 0 \end{pmatrix}.$$

Let

$$N = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ Y_{11} & Y_{12} & 0 & 0 & 1 \end{pmatrix}.$$

[4] The stability analysis of ϕ_{01} is completely analogous and is not presented here.

Then

$$NQN^{-1} = \begin{pmatrix} -W & -\Sigma & 0 \\ R_1 & E_1 & 0 \\ 0 & 0 & -D \end{pmatrix},$$

where

$$W = \begin{pmatrix} Y_{12}\tilde{r}_{12} + c_1 e_{11}\sigma'_{11} & -Y_{12}\tilde{r}_{11} \\ -Y_{11}\tilde{r}_{12} & Y_{11}\tilde{r}_{11} + c_1 e_{12}\sigma'_{12} \end{pmatrix}, \quad \Sigma = \begin{pmatrix} c_1\sigma_{11} & 0 \\ 0 & c_1\sigma_{12} \end{pmatrix}.$$

Clearly, one eigenvalue of Q is given by $\lambda_5 = -D$. The remaining four eigenvalues are the eigenvalues of the submatrix

$$Q' = \begin{pmatrix} -W & -\Sigma \\ R_1 & E_1 \end{pmatrix}.$$

In the remainder of the proof, we compute the sign of $\det Q'$. Since E_1 is non-singular, $\det Q' = \det(E_1)\det(W - \Sigma E_1^{-1} R_1)$. Furthermore,

$$-E_1^{-1}R_1 = \frac{\partial(\tilde{e}_{11},\tilde{e}_{12})}{\partial(s_1,s_2)}$$

implies that

$$-\Sigma E_1^{-1}R_1 = \begin{pmatrix} c_1\sigma_{11}\frac{\partial\tilde{e}_{11}}{\partial s_1} & c_1\sigma_{11}\frac{\partial\tilde{e}_{11}}{\partial s_2} \\ c_1\sigma_{12}\frac{\partial\tilde{e}_{12}}{\partial s_1} & c_1\sigma_{12}\frac{\partial\tilde{e}_{12}}{\partial s_2} \end{pmatrix},$$

and thus

$$W - \Sigma E_1^{-1}R_1 = \begin{pmatrix} Y_{12}\tilde{r}_{12} + c_1\frac{\partial\tilde{r}_{11}}{\partial s_1} & -Y_{12}\tilde{r}_{11} + c_1\frac{\partial\tilde{r}_{11}}{\partial s_2} \\ -Y_{11}\tilde{r}_{12} + c_1\frac{\partial\tilde{r}_{12}}{\partial s_1} & Y_{11}\tilde{r}_{11} + c_1\frac{\partial\tilde{r}_{12}}{\partial s_2} \end{pmatrix}.$$

Using elementary row operations, we can show that

$$\det\left(W - \Sigma E_1^{-1}R_1\right) = \det\begin{pmatrix} c_1\frac{\partial\tilde{r}_1^{\mathrm{g}}}{\partial s_1} & c_1\frac{\partial\tilde{r}_1^{\mathrm{g}}}{\partial s_2} \\ -\tilde{r}_{12} + \frac{c_1}{Y_{11}}\frac{\partial\tilde{r}_{12}}{\partial s_1} & \tilde{r}_{11} + \frac{c_1}{Y_{11}}\frac{\partial\tilde{r}_{12}}{\partial s_2} \end{pmatrix},$$

so that

$$\det(W - \Sigma E_1^{-1}R_1) = c_1\frac{\partial\tilde{r}_1^{\mathrm{g}}}{\partial s_1}\left(\tilde{r}_{11} + \frac{c_1}{Y_{11}}\frac{\partial\tilde{r}_{12}}{\partial s_2}\right) + c_1\frac{\partial\tilde{r}_1^{\mathrm{g}}}{\partial s_2}\left(\tilde{r}_{12} - \frac{c_1}{Y_{11}}\frac{\partial\tilde{r}_{12}}{\partial s_1}\right).$$

Since $c_1 = Y_{11}(s_1^{\mathrm{f}} - s_1) + Y_{12}(s_2^{\mathrm{f}} - s_2)$ at ϕ_{10}, the quantity $\beta = \frac{s_2^{\mathrm{f}} - s_2}{Y_{11}}$ satisfies the relations

$$\left(\frac{c_1}{Y_{11}} - \beta Y_{12}\right) = s_1^{\mathrm{f}} - s_1, \quad \beta Y_{11} = s_2^{\mathrm{f}} - s_2. \tag{36}$$

Now we rewrite $\det(W - \Sigma E_1^{-1} R_1)$ as

$$\det(W - \Sigma E_1^{-1} R_1) = c_1 \frac{\partial \tilde{r}_1^g}{\partial s_1}\left(\tilde{r}_{11} + \frac{c_1}{Y_{11}}\frac{\partial \tilde{r}_{12}}{\partial s_2} - \beta \frac{\partial \tilde{r}_1^g}{\partial s_2}\right) + c_1 \frac{\partial \tilde{r}_1^g}{\partial s_2}\left(\tilde{r}_{12} - \frac{c_1}{Y_{11}}\frac{\partial \tilde{r}_{12}}{\partial s_1} + \beta \frac{\partial \tilde{r}_1^g}{\partial s_1}\right).$$

Equivalently,

$$\begin{aligned}\det(W - \Sigma E_1^{-1} R_1) = {} & c_1 \frac{\partial \tilde{r}_1^g}{\partial s_1}\left(\tilde{r}_{11} + \left(\frac{c_1}{Y_{11}} - \beta Y_{12}\right)\frac{\partial \tilde{r}_{12}}{\partial s_2} - \beta Y_{11}\frac{\partial \tilde{r}_{11}}{\partial s_2}\right) \\ & + c_1 \frac{\partial \tilde{r}_1^g}{\partial s_2}\left(\tilde{r}_{12} - \left(\frac{c_1}{Y_{11}} - \beta Y_{12}\right)\frac{\partial \tilde{r}_{12}}{\partial s_1} + \beta Y_{11}\frac{\partial \tilde{r}_{11}}{\partial s_1}\right).\end{aligned}$$

Using (36), the above expression can be written as

$$\begin{aligned}\det(W - \Sigma E_1^{-1} R_1) = {} & c_1 \frac{\partial \tilde{r}_1^g}{\partial s_1}\left(\tilde{r}_{11} + \left(s_1^f - s_1\right)\frac{\partial \tilde{r}_{12}}{\partial s_2} - \left(s_2^f - s_2\right)\frac{\partial \tilde{r}_{11}}{\partial s_2}\right) \\ & + c_1 \frac{\partial \tilde{r}_1^g}{\partial s_2}\left(\tilde{r}_{12} - \left(s_1^f - s_1\right)\frac{\partial \tilde{r}_{12}}{\partial s_1} + \left(s_2^f - s_2\right)\frac{\partial \tilde{r}_{11}}{\partial s_1}\right).\end{aligned}$$

In the proof of Theorem 3 in [6], we obtained the same expression and demonstrated that it actually equals

$$\det\left(W - \Sigma E_1^{-1} R_1\right) = c_1 \nabla \tilde{r}_1^g \cdot \widetilde{F}^1,$$

where $\widetilde{F}^1 \in R_+^2$ is the tangent vector to the consumption curve Φ_1. Since $\nabla \tilde{r}_1^g \in R_+^2$, $\det(W - \Sigma E_1^{-1} R_1) > 0$ and the substrates act synergistically. Using the fact that $\det(E_1) > 0$, we find that $\det Q' > 0$. The trace of Q' is given by

$$\operatorname{tr} Q' = -D - c_1 e_{11}\sigma'_{11} - c_1 e_{12}\sigma'_{12} + \operatorname{tr} E_1 < 0.$$

Therefore, the eigenvalues $\lambda_1, \ldots, \lambda_4$ of Q' must be such that

$$\lambda_1 \cdots \lambda_4 = \det Q' > 0, \quad \lambda_1 + \cdots + \lambda_4 = \operatorname{tr} Q + D < 0.$$

Only two combinations are possible: either $\Re\lambda_i < 0$ for $i = 1, \ldots, 4$ or $\Re\lambda_i < 0$ for $i = 1, 2$ and $\Re\lambda_i > 0$ for $i = 3, 4$. □

Conjecture. $\Re\lambda_i < 0$ for $i = 1, \ldots, 4$. If this conjecture holds, then ϕ_{10} has seven eigenvalues $\lambda_1, \ldots, \lambda_7$ with negative real parts, and the last eigenvalue is given by $\lambda_8 = \tilde{r}_2^g - D$. Then ϕ_{10} is stable if and only if $\tilde{r}_2^g - D < 0$.

In the next Lemma, we analyze the stability of ϕ_{11} assuming that its existence has been established.

Lemma 9. *Suppose that ϕ_{11} exists. The necessary condition for stability of ϕ_{11} is that one of the following hold*:

1. *The (s_1, s_2) projection of ϕ_{11} lies below Φ_1 and above Φ_2* and *the vector pair $(\nabla \tilde{r}_1^g, \nabla \tilde{r}_2^g)$ has positive orientation*;

2. *The* (s_1, s_2) *projection of* ϕ_{11} *lies above* Φ_1 *and below* Φ_2 and *the vector pair* $(\nabla\tilde{r}_1^{\mathrm{g}}, \nabla\tilde{r}_2^{\mathrm{g}})$*has negative orientation.*

Proof. The variational matrix of (11)–(13) at ϕ_{11} is given by

$$J(\phi_{11}) = \begin{pmatrix} -A & -S_1 & -S_2 & -B \\ R_1 & E_1 & 0 & 0 \\ R_2 & 0 & E_2 & 0 \\ C & T_1 & T_2 & 0 \end{pmatrix}, \tag{37}$$

where

$$A = \begin{pmatrix} D + c_1 e_{11}\sigma'_{11} + c_2 e_{21}\sigma'_{21} & 0 \\ 0 & D + c_1 e_{12}\sigma'_{12} + c_2 e_{22}\sigma'_{22} \end{pmatrix}, \quad B = \begin{pmatrix} \tilde{r}_{11} & \tilde{r}_{21} \\ \tilde{r}_{12} & \tilde{r}_{22} \end{pmatrix},$$
$$C = \begin{pmatrix} c_1 Y_{11} e_{11}\sigma'_{11} & c_1 Y_{12} e_{12}\sigma'_{12} \\ c_2 Y_{21} e_{21}\sigma'_{21} & c_2 Y_{22} e_{22}\sigma'_{22} \end{pmatrix}, \quad S_1 = \begin{pmatrix} c_1\sigma_{11} & 0 \\ 0 & c_1\sigma_{12} \end{pmatrix}, \quad S_2 = \begin{pmatrix} c_2\sigma_{21} & 0 \\ 0 & c_2\sigma_{22} \end{pmatrix},$$
$$T_1 = \begin{pmatrix} c_1 Y_{11}\sigma_{11} & c_1 Y_{12}\sigma_{12} \\ 0 & 0 \end{pmatrix}, \quad T_2 = \begin{pmatrix} 0 & 0 \\ c_2 Y_{21}\sigma_{21} & c_2 Y_{22}\sigma_{22} \end{pmatrix},$$
$$R_i = \frac{\partial(R_{i1}, R_{i2})}{\partial(s_1, s_2)}, \quad E_i = \frac{\partial(R_{i1}, R_{i2})}{\partial(e_{i1}, e_{i2})}.$$

The characteristic polynomial $p(\lambda)$ of (37) can be written as

$$p(\lambda) = \det \begin{pmatrix} -A - \lambda I & -S_1 & -S_2 & -B \\ R_1 & E_1 - \lambda I & 0 & 0 \\ R_2 & 0 & E_2 - \lambda I & 0 \\ C & T_1 & T_2 & -\lambda I \end{pmatrix}. \tag{38}$$

After a series of elementary row operations, we obtain

$$p(\lambda) = \det\left\{\lambda(A + \lambda I) + BC - (\lambda S_1 + BT_1)(E_1 - \lambda I)^{-1}R_1 - (\lambda S_2 + BT_2)(E_2 - \lambda I)^{-1}R_2\right\} \cdot \det(E_1 - \lambda I) \cdot \det(E_2 - \lambda I). \tag{39}$$

It is clear that $p(\lambda) = \lambda^8 + \mathrm{O}(\lambda^7)$ for $\lambda \gg 1$. Therefore, a necessary condition for stability is that $p(0) > 0$. Conversely, a sufficient condition for instability is that $p(0) < 0$.

Now we study the sign of $p(0)$. According to (39),

$$p(0) = \det(BC - BT_1E_1^{-1}R_1 - BT_2E_2^{-1}R_2)\det(E_1)\det(E_2). \tag{40}$$

In Section 3, we have demonstrated that $\det(E_i) > 0$. According to (21),

$$\det(BC - BT_1E_1^{-1}R_1 - BT_2E_2^{-1}R_2) = c_1c_2\det(B)\det\frac{\partial(\tilde{r}_1^{\mathrm{g}}, \tilde{r}_2^{\mathrm{g}})}{\partial(s_1, s_2)}.$$

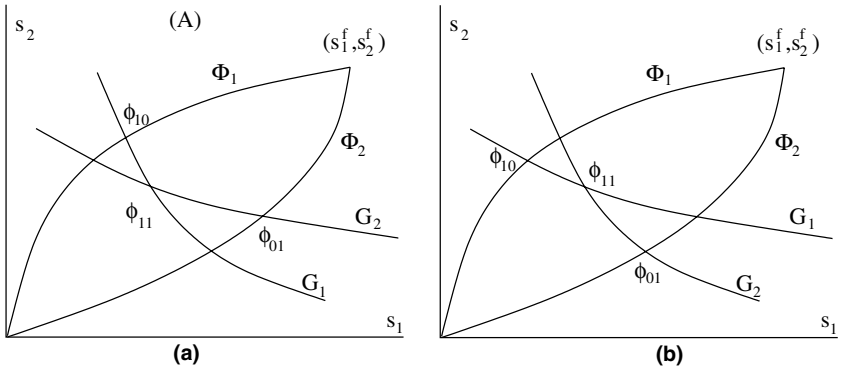

Fig. 5. (a) Both ϕ_{10} and ϕ_{01} exist, and $\tilde{r}_2^g > D$ at ϕ_{10} and $\tilde{r}_1^g > D$ at ϕ_{01} so that both ϕ_{10} and ϕ_{01} are unstable. The growth isoclines G_1 and G_2 must intersect within the envelope of coexistence. Furthermore, the intersection ϕ_{11} satisfies the necessary condition for stability (41). (b) Both ϕ_{10} and ϕ_{01} exist, and $\tilde{r}_2^g < D$ at ϕ_{10} and $\tilde{r}_1^g < D$ at ϕ_{01} so that both ϕ_{10} and ϕ_{01} are stable. The growth isoclines G_1 and G_2 must intersect within the envelope of coexistence. Furthermore, the intersection ϕ_{11} violates the condition (41) and thus ϕ_{11} is unstable.

Since c_1, $c_2 > 0$, the necessary condition $p(0) > 0$ for stability of ϕ_{11} can be expressed as

$$\det(B) \det \frac{\partial(\tilde{r}_1^g, \tilde{r}_2^g)}{\partial(s_1, s_2)} > 0. \tag{41}$$

As we demonstrated in [6], the sign of $\det(B)$ is determined by the position of ϕ_{11} relative to Φ_1 and Φ_2. Specifically, $\det(B) > 0$ if the projection of ϕ_{11} onto the (s_1, s_2) plane lies below Φ_1 and above Φ_2, and $\det(B) < 0$ if such projection lies below Φ_2 and above Φ_1. The sign of the second determinant in (41) corresponds to the orientation of the vector pair $(\nabla\tilde{r}_1^g, \nabla\tilde{r}_2^g)$. □

Remark. Now we can strengthen the existence criteria formulated at the end of Section 4. If both ϕ_{10} and ϕ_{01} exist with $\tilde{r}_2^g > D$ at ϕ_{10} and $\tilde{r}_1^g > D$ at ϕ_{01} (refer to Fig. 5(a)) then there exists a non-trivial equilibrium ϕ_{11} which satisfies the necessary condition for stability (41). If both ϕ_{10} and ϕ_{01} exist with $\tilde{r}_2^g < D$ at ϕ_{10} and $\tilde{r}_1^g < D$ at ϕ_{01} (refer to Fig. 5(b)) then there exists an unstable non-trivial equilibrium ϕ_{11}.

6. Discussion

In this paper, we have analyzed the existence, uniqueness, and stability of equilibria of the physiological model of mixed microbial growth on a mixture of two substitutable substrates. This physiological model explicitly includes the dynamics of the peripheral enzymes that are involved in the uptake and initial metabolism of the substrates. We argued that the use of such physiological models is necessary in trying to understand the dynamic responses of continuous cultures to various environmental perturbations.

The biological significance of the physiological model is that it provides the explicit mechanism for competition between enzymes specific to distinct substrates. In our model, each enzyme level is determined by the balance of the rate at which this enzyme is synthesized and

the rate at which the cell biomass increases (e.g., the specific growth rate). An upregulation of one enzyme level results in the overall increase of the specific growth rate and thus other enzymes become more dilute. The same mechanism is responsible for the mutual downregulation of the substrate uptake rates which we studied in our previous work in the context of a phenomenological model [6].

In the analysis of the model, we made three rather general assumptions (H1)–(H3) regarding the kinetics of substrate uptake, enzyme synthesis, and cell growth. The most important assumption was (H1) postulating that the enzyme synthesis rate is a sublinear function of the enzyme level which increases with the concentration of the specific substrate. Based on this assumption, we have demonstrated that

1. For any fixed combination of substrate concentrations, the enzyme levels converge to a unique globally stable equilibrium. This result depends strongly on the assumption (H1). If one needs to formulate a model where the enzyme equations can have more than one attractor (multistability), then this assumption would have to be modified.
2. At steady state, the enzyme levels are enhanced by their specific substrate and inhibited by the non-specific substrate(s). The inhibition is weak so that increasing each substrate results in a higher specific growth rate. Using this property, we showed that each growth isocline G_i is a graph of a decreasing function in the (s_1, s_2) plane.
3. We showed that the consumption curve Φ_i that describes the balanced steady state growth of the ith species is a graph of an increasing function in the (s_1, s_2) plane.
4. Each semitrivial equilibrium is represented by the intersection of the corresponding growth isocline and the consumption curve. It is unique whenever exists. The stability of the semitrivial equilibria is determined by the ability of one species to invade the reactor where the other species is resident.
5. The non-trivial (coexistence) equilibrium ϕ_{11} is represented by an intersection of two growth isoclines if and only if it occurs within the envelope of coexistence, that is, in the region between the two consumption curves. A necessary condition for coexistence is that both semitrivial equilibria exist. Multiple non-trivial equilibria may exist.
6. The necessary condition for ϕ_{11} to be stable is given by (41). This condition is a generalization of the Gilpin–Justice criterion [1]. If both semitrivial equilibria exist and are unstable, then there exists at least one coexistence equilibrium that satisfies the necessary condition for stability.

All of these criteria are related to the geometric properties of the growth isoclines and the consumption curves that can be obtained experimentally. For a given dilution rate D, the growth isocline can be obtained by measuring both residual substrate concentrations s_1 and s_2 while varying the feed concentrations s_1^f and s_2^f. For a given combination of the feed concentrations s_1^f and s_2^f, the consumption curve can be obtained by measuring both residual substrate concentrations s_1 and s_2 while varying the dilution rate D in the reactor inhabited with a single microbial species. These experiments can also be used to validate the model.

In the study of local stability of semi- and non-trivial equilibria of the physiological model, we did not provide any sufficient conditions for stability. These mathematical questions currently remain open.

We have also studied the global dynamics of single species cultures. We analyzed the limiting case when the enzyme dynamics is fast or slow on the time scale of the chemostat. If the enzymes are fast, then the physiological model can be reduced to the phenomenological model by a quasi steady state assumption that replaces the actual enzyme levels by their steady state analogues. If the enzymes are slow, we used global convergence of the phenomenological model to prove that the enzyme dynamics is competitive, and therefore, globally convergent.

Finally, we found that the physiological model under assumption (H1) exhibits the dynamics that is similar to that of the phenomenological model with weak mutual inhibition of substrate uptake rates. We have found no cases when the dynamics of enzymes would affect the stability of the phenomenological model. In fact, assumption (H1) must be relaxed to allow for multistability of the enzyme equations, in order for the physiological model to have more complex dynamics.

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