Dynamic analysis of the cybernetic model for diauxic growth

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Abstract—The cybernetic framework is a mathematical formalism developed by Ramkrishna and coworkers for modelling microbial systems. In this work, we analyze the dynamics of the cybernetic model for diauxic growth (Kompala et al., 1986, Biotechnol. Bioengng. 28 1044–1055). Recent data shows, however, that microbial growth on binary mixtures is frequently nondiauxic. The goal of this work is to:

(1) Understand why the model captures the diauxie.
(2) Determine if the model can embrace the nondiauxic growth patterns.

The analysis shows that the global dynamics of the cybernetic model agree with diauxic behavior, and the autocatalytic nature of enzyme synthesis plays a key role in ensuring this agreement. But there exists no set of parameter values for which the model embraces the nondiauxic growth patterns; this observation is specific to the model of Kompala et al. (1986) and does not apply to the more recent cybernetic model of Ramakrishna et al. (1997, Biotechnol. Bioengng 52, 141–151). © 1997 Elsevier Science Ltd

Keywords: Microbial growth; cybernetic model; diauxie; autocatalysis; nondiauxic growth patterns.

1. INTRODUCTION

The cybernetic framework is a mathematical formalism developed by Ramkrishna and coworkers for modelling microbial systems. The distinguishing feature of the cybernetic approach is the manner in which it accounts for metabolic regulation. It replaces the description of the diverse regulatory mechanisms with a control-theoretic formulation of their ultimate goal: maximization of the growth rate (Ramkrishna et al., 1987).

In this work, we revisit the cybernetic model for diauxic growth (Kompala et al., 1986). The diauxic refers to the phenomenon in which a batch culture of bacteria growing on a mixture of two substrates preferentially utilizes only one of the substrates. Such a substrate utilization pattern results in the appearance of two successive exponential growth phases, with each growth phase corresponding to the consumption of only one of the two substrates. It is characteristic of the diauxic that the very same substrate is consumed preferentially, no matter how the inoculum is precultured. That is to say, the identity of the 'preferred' substrate is independent of the initial physiological state of the cells. In general, this is the substrate that, by itself, supports a higher growth rate. Kompala et al. showed numerically that the cybernetic model mirrored these characteristic properties of diauxic growth (Kompala et al., 1986). The first goal of this work is to show analytically that the global dynamics of the model are consistent with the diauxie. This global analysis helps in abstracting those essential properties that enable the model to capture the diauxie.

The cybernetic model was based on the assumption that the growth pattern in a batch culture is always diauxic, and the preferred substrate is the one that supports a higher single-substrate growth rate (Ramkrishna et al., 1987). Experimental data in the literature indicates that the substrates are often utilized simultaneously, and, in some cases, the substrate utilization pattern depends on the preculturing conditions. The data on simultaneous utilization has been summarized in a recent review article (Egli, 1995). The summary shows that the phenomenon of simultaneous utilization is quite common, and has been reported for various bacteria and yeast growing under aerobic, anaerobic, and thermophilic conditions. The substrates yielding simultaneous substrate utilization...
often support relatively low maximum specific growth rates during single-substrate growth. Instances of the preculture-dependent growth pattern are less common, and have not been reviewed. A short discussion can be found in Narang et al. (1997a). The second goal of this work is to determine whether the cybernetic model admits of these nondiauxic growth patterns. More precisely, we wish to determine whether there exists a set of parameter values for which the cybernetic model exhibits the nondiauxic growth patterns.

2. THE CYBERNETIC MODEL

We begin by recalling the main features of the cybernetic model. Note that the nomenclature used here differs from that used by Kompala et al. (1986). Reaction rates are denoted here by \( r \), with appropriate subscripts appended to identify the reaction. If the reaction follows saturation kinetics, its maximum velocity and saturation constant are denoted by appropriately subscripted \( V \) and \( K \), respectively. If the reaction follows zeroth- or first-order kinetics, its rate constant is denoted by an appropriately subscripted \( k \).

The kinetic structure of the model is shown in Fig. 1. Here \( S_i \) denotes the \( i \)th substrate, \( E_i \) denotes the ‘lumped’ system of inducible enzymes associated with growth on \( S_i \), and \( B \) denotes the biomass. Thus, the model views growth on a binary mixture of substrates as two parallel enzyme-catalyzed growth ‘reactions’.

It was assumed, furthermore, that

(1) The specific growth rate on the \( i \)th substrate, \( r_{g,i} \), obeys modified Monod kinetics:

\[
r_{g,i} = \frac{V_{g,i} e_i}{K_{s,i} + s_i} \tag{1}
\]

The modified kinetics include a proportional dependence on the enzyme level, \( e_i \), in addition to the usual saturation factor \( s_i/(K_{s,i} + s_i) \).

(2) The synthesis of \( E_i \) is induced directly by the substrate \( S_i \) (Fig. 1), and satisfies the kinetic law

\[
r_{e,i} = \frac{V_{e,i} s_i}{K_{e,i} + s_i} \tag{2}
\]

(3) Enzyme degradation is a first-order process:

\[
r_{d,i} \equiv k_d e_i \tag{3}
\]

(4) The fraction of substrate uptake, \( r_{s,i} \), channelled into biomass, denoted \( Y_i \), is a fixed ‘stoichiometric’ constant. That is

\[
r_{s,i} = \frac{1}{Y_i} r_{g,i} \tag{4}
\]

The value of \( Y_i \) was assumed to be the same as the yield of biomass during single-substrate growth.

(5) The effects of regulation can be captured by the cybernetic variables:

\[
u_i = \frac{r_{g,i}}{\sum_{j=1}^{2} r_{g,j}} \tag{5}
\]

\[
e_i = \frac{r_{e,i}}{\max_{1 < j < 2} V_{g,j} e_j} \tag{6}
\]

where \( u_i \) regulates the rate of enzyme synthesis, and \( e_i \) regulates the growth rate. These two laws of regulation were referred to as the matching and proportional laws, respectively. They were derived from microeconomic principles that microbes are purported to follow.

Upon taking mass balances, we obtain the following initial-value problem:

\[
\frac{dS_i}{dt} = - \frac{1}{Y_i} \left( \frac{V_{g,i} e_i}{K_{s,i} + s_i} \right) c \tag{7}
\]

\[
\frac{de_i}{dt} = V_{e,i} \frac{s_i}{K_{e,i} + s_i} - \left( \sum_{j=1}^{2} \frac{V_{g,j} e_j}{K_{s,j} + s_j} e_j - k_d e_i \right) \tag{8}
\]

\[
\frac{dc}{dt} = \left( \sum_{j=1}^{2} \frac{V_{g,j} e_j}{K_{s,j} + s_j} e_j \right) c - \left( \sum_{j=1}^{2} \frac{V_{g,j} e_j}{K_{s,j} + s_j} e_j \right) c \tag{9}
\]

where

\[
u_i = \frac{V_{g,i} e_i}{K_{s,i} + s_i} \tag{10}
\]

\[
\sum_{j=1}^{2} \frac{V_{g,j} e_j}{K_{s,j} + s_j}
\]

\[
e_i = \frac{r_{e,i} s_i}{K_{e,i} + s_i} \tag{11}
\]

max_{1 < j < 2} \frac{2 V_{g,j} e_j}{K_{s,j} + s_j}

3. REDUCTION

Throughout this work, we consider the example of growth of *Klebsiella oxytoca* on a mixture of glucose and xylose. Kompala et al. (1986) observed that the growth pattern for this system was diauxic with preferential utilization of glucose, no matter which substrate, glucose or xylose, was used to grow the inoculum. They also showed that the cybernetic model mimics this behavior. Figure 2 shows simulations of the model done with the parameter values listed in Table 1, and two sets of initial conditions.
Table 1. Parameter values used by Kompala et al. (1986) for simulating the growth of Klebsiella oxytoca on a mixture of glucose ($S_1$) and xylose ($S_2$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter value</th>
<th>Parameter</th>
<th>Parameter value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{e,1}$</td>
<td>1220.4 g/gdw h</td>
<td>$V_{e,2}$</td>
<td>713.4 g/gdw h</td>
</tr>
<tr>
<td>$Y_1$</td>
<td>0.52 gdw/g</td>
<td>$Y_2$</td>
<td>0.50 gdw/g</td>
</tr>
<tr>
<td>$V_{c,1}$</td>
<td>0.001 g/gdw h</td>
<td>$V_{c,2}$</td>
<td>0.001 g/gdw h</td>
</tr>
<tr>
<td>$K_{s,1}$</td>
<td>0.01 g/l</td>
<td>$K_{s,2}$</td>
<td>0.20 g/l</td>
</tr>
<tr>
<td>$K_{e,1}$</td>
<td>0.01 g/l</td>
<td>$K_{e,2}$</td>
<td>0.20 g/l</td>
</tr>
<tr>
<td>$k_{d,1}$</td>
<td>0.05 l/h</td>
<td>$k_{d,2}$</td>
<td>0.05 l/h</td>
</tr>
<tr>
<td>$k_{e,1}$</td>
<td>0.00001 g/gdw h</td>
<td>$k_{e,2}$</td>
<td>0.00001 g/gdw h</td>
</tr>
</tbody>
</table>

Fig. 2. The dynamics of the cybernetic model. The full lines show the evolution of the variables when the inoculum has been grown on $S_1$, the dashed lines when the inoculum has been grown on $S_2$.

The goal of the analysis is to determine whether the motion of the enzyme levels during the first growth phase shows the trend described above for all possible initial enzyme levels. That is, we seek the global dynamics of the enzyme levels during the first growth phase. It is essential to show this if we wish to prove that there is preferential utilization of glucose no matter how the inoculum is precultured.

The evolution of the enzyme levels during the first growth phase can be approximated by a reduced system of only two differential equations. This reduction rests upon the fact that the enzyme levels evolve during the first growth phase without 'seeing' any changes in the environmental state variables, $s_i$ and $c$. Indeed, eq. (8) shows that the only environmental variables influencing the evolution of the enzyme levels are the substrate concentrations $s_i$. They exert their influence on the enzyme levels through the ratios, $s_i/(K_{e,i} + s_i)$ and $s_i/(K_{s,i} + s_i)$. Since $K_{e,i}, K_{s,i}$ are on the order of 0.1 g/l, and $s_{i,0}$ is on the order of 1 g/l, these ratios are approximately unity until one of the substrate concentrations approaches 0.1 g/l. Thus, the cell 'see' a constant environment throughout almost the entire first growth phase. In the face of this quasiconstant environment, the physiological state variables, $e_i$, approach a quasistationary state. Microbiologists refer to this quasistationary state as the state of balanced growth (Ingraham et al., 1983). The evolution of the enzyme levels toward the state of balanced growth can be approximated by eq. (8) with $s_i/(K_{e,i} + s_i)$ and $s_i/(K_{s,i} + s_i)$ replaced by unity.

This intuitive argument is made rigorous below by appealing to the theorem of continuous dependence on initial conditions. We note first of all that the cell density is linearly dependent on the substrate concentrations. For it follows from eqs (7) and (9) that

$$ \frac{d}{dt} (Y_1 s_1 + Y_2 s_2 + c) = 0. \tag{12} $$

Hence

$$ c = c_0 + Y_1 (s_{1,0} - s_1) + Y_2 (s_{2,0} - s_2). \tag{13} $$

That is, the instantaneous cell density is the sum of the initial cell density plus the density of the cells created by consumption of the two substrates. This conservation law is a consequence of the 'stoichiometric' relationship expressed by eq. (4).

Upon substituting eq. (13) into eq. (7), we obtain the simpler initial-value problem:

$$ \frac{ds_i}{dt} = - \frac{1}{Y_i} \left( V_{c,i} e^r e^i \frac{s_i}{K_{s,i} + s_i} \right) \left[ c_0 + \sum_{j=1}^{2} Y_j (s_{j,0} - s_j) \right] \tag{14} $$
\[
\frac{\text{d}v_i}{\text{d}t} = \frac{V_i}{K_i + s_i} - \left( \frac{1}{2} \sum_{j=1}^{2} V_{ij}^2 \frac{s_i}{K_{ij} + s_j} \right) e^{\text{d}_i} - k_{d_i} e_i \tag{15}
\]

Further simplification obtains from the fact that we are interested in the evolution of the enzyme levels during the finite time interval consisting of the approach to the first exponential growth phase. It is easier to explain this reduction for the special case of single-substrate growth, since it can be shown graphically.

### 3.1. Single-substrate dynamics

In the special case of single-substrate growth, \( u_i = v_i = 1 \), and we obtain the two-dimensional initial-value problem:

\[
\frac{\text{d}s_i}{\text{d}t} = - \frac{1}{Y_i} \left( V_i e_i \frac{s_i}{K_i + s_i} \right) \left[ c_0 + Y_i (s_{i,0} - s_i) \right] \tag{17}
\]

\[
\frac{\text{d}e_i}{\text{d}t} = \frac{V_i}{K_i + s_i} - \left( V_i e_i \frac{s_i}{K_i + s_i} \right) e^{\text{d}_i} - k_{d_i} e_i \tag{18}
\]

\[ t = 0: \quad s_i = s_{i,0}, \quad e_i = e_{i,0} \tag{19} \]

We wish to show that the evolution of the enzyme level over a certain finite time interval is well approximated by a one-dimensional initial-value problem. Let us begin by specifying the finite time interval of interest. Figure 3 shows the dynamics of single-substrate growth on glucose \((s_i)\). The evolution of the enzyme level is classified into three phases:

- **A-phase** during which the enzyme accumulates.
- **Q-phase** during which the enzyme level is quasistationary. This coincides with the state of balanced growth.
- **D-phase** during which the enzyme is depleted by degradation.

The finite time interval of interest is the time interval consisting of the A-phase during which the enzyme approaches its quasistationary level.

We show next that over this finite time interval of interest, the evolution of the enzyme level predicted by eqs (17)–(19) is well approximated by a one-dimensional initial-value problem. To do this, it is useful to plot the solution in state space (Fig. 4). The solution has the shape of an inverted U, and the three phases of enzyme evolution correspond to the three 'edges' of this inverted U. The reduction we have in mind obtains by a slight variation of the initial conditions. Thus, instead of solving eqs (17)–(19), we solve the 'neighboring' initial-value problem:

\[
\frac{\text{d}s_i}{\text{d}t} = - \frac{1}{Y_i} \left( V_i e_i \frac{s_i}{K_i + s_i} \right) \left[ c_0 + Y_i (s_{i,0} - s_i) \right] \tag{20}
\]

\[
\frac{\text{d}e_i}{\text{d}t} = \frac{V_i}{K_i + s_i} - \left( V_i e_i \frac{s_i}{K_i + s_i} \right) e^{\text{d}_i} - k_{d_i} e_i \tag{21}
\]

\[ t = 0: \quad s_i = s_{i,0}^*, \quad e_i = e_{i,0} \tag{22} \]

which differs from eqs (17)–(19) only with respect to the initial conditions. The new initial conditions (22) are obtained by orthogonal projection of the actual initial condition (19) onto the line (Fig. 4, Inset):

\[
c_0 + Y_i (s_{i,0} - s_i) = 0. \tag{23}
\]

The solution of this new initial-value problem is shown as a dashed line in Fig. 4.

The initial value problem (20)–(22) is simpler than the original initial-value problem, since it is
The exact and the approximate solutions of the initial-value problem (17)-(18). Figure 4 shows that the dashed line is also a good approximation to the solution of the original initial-value problem over the finite time interval of interest. This is because the initial conditions of the two initial-value problems are close: the distance between the two initial conditions is \( c_0/Y_e \), which is significantly smaller than \( \sqrt{s_{i,0}^2 + e_{i,0}^2} \sim s_{i,0} \), the distance of the original initial condition from the origin. The theorem of continuous dependence on initial conditions assures us that the solutions of the two initial-value problems will remain close for a length of time which depends on the magnitude of \( c_0/(Y_e s_{i,0}) \): the smaller the value of this ratio, the longer the time interval over which the approximation is valid.

The finite-time global behavior of the enzyme level during its approach to balanced growth can be depicted by constructing the phase portrait of eq. (24). Figure 5 shows that no matter what the initial enzyme level, it tends to a certain nonzero value as the cells approach the exponential growth phase.

The final approximation further simplifies the structure of eq. (24). During the A-phase, the influence of degradation on the enzymatic dynamics is negligible compared to the effects of induction and dilution. Thus, we arrive at the reduced initial-value problem:

\[
\frac{de_i}{dt} = V_{e,i} - V_{g,i} e_i^2
\]

\[ t = 0: \quad e_i = e_{i,0} \]

where

\[
V_{e,i} = V_{e,i} \frac{s_{i,0}^*}{K_{e,i} + s_{i,0}^*} \approx V_{e,b}
\]

\[
V_{g,i} = V_{g,i} \frac{s_{i,0}^*}{K_{g,i} + s_{i,0}^*} \approx V_{g,f}
\]

The reduced initial-value problem approximates the motion of the enzyme level toward its quasistationary value from any given initial condition \( e_{i,0} \). Figure 6 shows that the phase portrait of eq. (26) is qualitatively the same as the phase portrait of eq. (24). There is a unique stable steady state at:

\[
e_i = \frac{V_{e,i}^*}{V_{g,i}^*} \approx \frac{V_{e,i}}{V_{g,i}}
\]
Fig. 5. The phase portrait of (24).

Fig. 6. The phase portrait of the reduced equation for single-substrate growth.
Hence, no matter what the initial enzyme level, it tends to a nonzero value as the cells approach the exponential growth phase.

The parameters \( V_{g,i} \) were chosen to ensure agreement between the predicted and experimental exponential growth rates during single-substrate growth. Since the enzyme level during the exponential growth phase is given by (28), this implies

\[
V_{g,i} = \frac{\mu_i^{\max}}{V_{g,i}}
\]

where \( \mu_i^{\max} \) denotes the experimentally determined exponential growth rate on \( S_i \). Hence

\[
V_{g,i} = (\mu_i^{\max})^2 / V_{g,i}
\]  

3.2. Mixed-substrate dynamics

The same sequence of approximations will now be applied to the mixed-substrate model. The finite time interval of interest here is the time interval during which the enzyme levels approach their quasisteady-state during the first growth phase. Once again, instead of solving the initial problem (14)-(16), we solve the 'neighboring' initial-value problem:

\[
\frac{ds_i}{dt} = - \frac{1}{Y_i} \left( V_{g,i} e_i s_i \right) \frac{1}{K_{s,i} + s_i} - \frac{2}{j=1} Y_j (s_j,0 - s_j)
\]

The initial conditions (32) are obtained by orthogonal projection of the original initial condition (16) onto the affine hyperplane:

\[
c_0 + \sum_{j=1}^{2} Y_j (s_j,0 - s_j) = 0.
\]

The solution of eqs (30)-(32) is a good approximation to the solution of eqs (14)-(16) over the finite time interval of interest. This is because the new initial condition (32) is very close to the original initial condition (16): the distance between the two initial conditions is only \( c_0 / \sqrt{Y_i^2 + Y_j^2} \ll s_{j,0}^* + s_{j,0} \). The new initial condition lies on the affine hyperplane, which is invariant under the flow of eqs (30) and (31). Hence, the initial-value problem (30)-(32), is equivalent to the two-dimensional initial-value problem

\[
\frac{d e_i}{dt} = V_{g,i} e_i \left( \frac{s_i}{K_{s,i} + s_i} \right) - \frac{2}{j=1} Y_j (s_j,0 - s_j)
\]

Fig. 7. The phase portrait of the reduced equations of the cybernetic model. All initial conditions lead to preferential utilization of \( S_1 \).
where

\[ t = 0: \quad e_i = e_{i,0} \]

Substituting eq. (36) in eq. (34), and neglecting enzyme degradation, we obtain

\[
\frac{d e_i}{dt} = V_{g,i}^{*,*} e_i \frac{s_i^{*,0}}{K_{s,i} + s_i^{*,0}} - \left( \sum_{j=1}^{2} V_{g,j}^{*,*} e_j \frac{s_j^{*,0}}{K_{s,j} + s_j^{*,0}} \right) e_i
\]

\[ t = 0: \quad e_i = e_{i,0} \] (38)

Equations (37) will be referred to as the reduced equations of the model. They approximate the motion of the enzyme levels from any given initial levels \((e_{1,0}, e_{2,0})\) to their quasisteady-state values during the first growth phase.

Figure 7 shows the phase portrait of the reduced equations when the parameter values are the same as those used in Fig. 2. The phase portrait contains two axial steady states (i.e. occurring on the axes), only one of which is stable – the nonzero steady state on the \(e_1\)-axis. All orbits, except the stable manifolds of the saddle point, approach the stable steady state. This corresponds to preferential utilization of glucose (\(S_1\)), since \(e_2 = 0\) on this steady state.

Thus, we have shown that the evolution of the enzyme levels during the first growth phase is independent of their initial values. There is preferential utilization of glucose no matter how the inoculum is precultured.

4. ANALYSIS

The questions to be addressed here are:

(1) Why does the model capture the diauxic growth pattern?
(2) Can the model capture the simultaneous growth pattern?
(3) Which parameters determine the identity of the preferred substrate in the diauxic growth pattern?
(4) How are the dynamics affected by inclusion of constitutive enzyme synthesis?
Fig. 9. The null-clines for the reduced equations of the cybernetic model. The full lines represent the null cline for $e_1$, and the dashed lines represent the null cline for $e_2$.

trivial and nontrivial branches, respectively. It follows from these definitions that (Fig. 9):

1. The steady states are the points of intersection of the null-clines for distinct $e_i$.
2. Axial steady states are obtained only if this intersection involves at least one trivial branch. (The apparent intersection of the trivial branches at the origin is not a steady state. This is because $f_i(e_1, e_2)$ is not defined at the origin.)
3. Nonaxial steady states are obtained if two nontrivial branches intersect.

4.1. Diauxic growth pattern

To capture the diauxie, it is necessary that axial steady states exist. It follows from the characterizations above that the existence of the axial steady states hinges upon the existence of the trivial branches of the null-clines. We show below that the trivial branches exist only because enzyme synthesis is autocatalytic. It will then follow that the autocatalytic nature of enzyme synthesis plays a key role in capturing the dynamics of the diauxie.

Let us therefore consider how the trivial and nontrivial branches arise. The null-clines for $e_i$ are defined by the equation $f_i(e_1, e_2) = 0$, which implies that either:

$$e_i = 0$$

or

$$V_{e,i} = \frac{V_{e,i} \sum_{j=1}^{2} V_{e,j}^{*} e_j}{\sum_{j=1}^{2} V_{e,j}^{*} e_j} \frac{V_{e,j}^{*} e_j}{\max_{1 \leq k \leq 2} V_{e,k}^{*} e_k}$$

Equations (41) and (42) define the trivial and nontrivial branches, respectively. This derivation shows that the trivial branches exist only because the regulated rate of enzyme synthesis:

$$V_{e,i} = \frac{V_{e,i} \sum_{j=1}^{2} V_{e,j}^{*} e_j}{\sum_{j=1}^{2} V_{e,j}^{*} e_j}$$

is proportional to $e_i$. These kinetics are characteristic of autocatalytic processes: The product $(e_i)$ of the process (enzyme synthesis) promotes its own synthesis, and in the absence of this product, its rate of synthesis decline to zero.

In the cybernetic model, the autocatalytic nature of enzyme synthesis rests upon the introduction of regulation via the cybernetic variable $u_i$: $e_i$ appears in the numerator of eq. (43) through the cybernetic variable $u_i$. But inducible enzyme synthesis is inherently autocatalytic: Autocatalysis is already embedded in the kinetics of inducible enzyme synthesis. The inducible enzyme catalyzes the synthesis of the inducer associated with it, which in turn is required for synthesis of the enzyme. This mutually dependent cycle of reactions, by itself, implies that the enzyme catalyzes its own synthesis. In the kinetic model formulated in
Narang et al. (1997b), we include the inducers as state variables, and postulate a kinetic structure which reproduces the mutually dependent relationship between an inducible enzyme and its associated inducer. This kinetic model captures both the diauxic and the simultaneous growth patterns.

4.2. Simultaneous growth pattern

The model will embrace the simultaneous growth pattern only if it admits of a nonaxial steady state. Since nonaxial steady states are obtained by the intersection of the nontrivial branches, we are led to consider to question of whether there exist parameter values for which the nontrivial branches intersect.

By making the following coordinate change in eq. (42)

\[
e_1 = \frac{r}{V_{e1}} \cos t, \quad e_2 = \frac{r}{V_{e2}} \sin t
\]

it can be shown that the nontrivial branch for \( e_1 \) has the parametric representation:

\[
\left(\frac{V_{e1}^*}{V_{e1}^* \sqrt{1 + \tan t}} \cos t, \frac{V_{e2}^*}{V_{e2}^* \sqrt{1 + \tan t}} \sin t\right)
\]

if \( 0 \leq t \leq \pi/4 \) \hspace{1cm} (44)

\[
\left(\frac{V_{e1}^*}{V_{e1}^* \sqrt{1 + \cot t}} \cos t, \frac{V_{e2}^*}{V_{e2}^* \sqrt{1 + \cot t}} \sin t\right)
\]

if \( \pi/4 \leq t \leq \pi/2 \) \hspace{1cm} (45)

while that for \( e_2 \) has the representation:

\[
\left(\frac{V_{e2}^*}{V_{e2}^* \sqrt{1 + \tan t}} \cos t, \frac{V_{e2}^*}{V_{e2}^* \sqrt{1 + \tan t}} \sin t\right)
\]

if \( 0 \leq t \leq \pi/4 \) \hspace{1cm} (46)

\[
\left(\frac{V_{e2}^*}{V_{e2}^* \sqrt{1 + \cot t}} \cos t, \frac{V_{e2}^*}{V_{e2}^* \sqrt{1 + \cot t}} \sin t\right)
\]

if \( \pi/4 \leq t \leq \pi/2 \) \hspace{1cm} (47)

It follows that the ratio of the distances from the origin of the nontrivial null-clines for \( e_1 \) and \( e_2 \) is given by

\[
\sqrt{V_{e1}^* V_{e2}^*} \sqrt{V_{e1}^* V_{e2}^*}
\]

Hence, the nontrivial branches do not intersect unless \( \sqrt{V_{e1}^* V_{e2}^*} = \sqrt{V_{e1}^* V_{e2}^*} \). In this case, however, the two nontrivial branches coincide, resulting in infinitely many steady states, which contradicts the experimental data. Thus, the model cannot capture the simultaneous growth pattern. We note, however, that this does not preclude the slow utilization of \( S_2 \) that occurs either in the presence of nonzero constitutive enzyme synthesis (Section 4.4) or during the approach to balanced growth of cells precultured on \( S_2 \) (dashed line in Fig. 2).

4.3. Identity of the preferred substrate

In general, \( \sqrt{V_{e1}^* V_{e1}^*} \neq \sqrt{V_{e2}^* V_{e2}^*} \), and the nontrivial branches do not intersect. Two axial steady states are obtained at the points where the nontrivial branches intersect the trivial branches. It can be shown that only one of these steady states is stable, this being the one that is further from the origin. It follows from eqs (29) and (48) that the ratio of the distances from the origin of the steady states on the \( e_1 \)- and \( e_2 \)-axes is

\[
\frac{\sqrt{V_{e1}^* V_{e2}^*}}{\sqrt{V_{e2}^* V_{e2}^*}} \approx \frac{\sqrt{V_{e1}^* V_{e1}^*}}{\sqrt{V_{e2}^* V_{e2}^*}} = \frac{\mu_{\text{max}}}{\mu_{\text{max}}^*}
\]

Hence, the preferred substrate is the one that, by itself, supports a higher maximum specific growth rate.

4.4. Constitutive enzyme synthesis

In general, most of the enzyme is synthesized by induction only in the presence of the substrate. However, a relatively small fraction of the enzyme is synthesized even in the absence of the substrate. This is referred to as constitutive enzyme synthesis. Turner and Ramkrishna (1988) modified the model by including a term accounting for constitutive enzyme synthesis. The reduced equations then become

\[
\frac{d e_i}{d t} = V_{e_i}^* e_i - \sum_{j=1}^{2} \max_{1 < k < 2} \frac{(V_{e_j}^* e_j)^2}{V_{e_k}^* e_k} - k_i e_i + k_i^* e_i
\]

\[
t = 0: \quad e_i = e_{i0}
\]

where \( k_i^* \) denotes the zero-order rate of constitutive enzyme synthesis.

Figure 10 shows that, despite this modification, the global dynamics are essentially the same as the global dynamics without constitutive enzyme synthesis (Fig. 7). Inclusion of constitutive enzyme synthesis causes both steady states to shift slightly. The saddle-point on the \( e_2 \)-axis is shifted to the left, whereas the stable node on the \( e_1 \)-axis is shifted up. Consequently, there is a unique steady state in the first quadrant to which all orbits converge. This is revealed more clearly by the null-clines shown in Fig. 11. Perturbing the model with constitutive enzyme synthesis separates the intersecting branches of the null-clines for \( e_i \) into two non-intersecting branches. The trivial branches (not shown in the figure) are shifted outside the first quadrant. There is a unique steady state in the first quadrant to which all orbits converge. This is revealed more clearly by the null-clines shown in Fig. 11. Perturbing the model with constitutive enzyme synthesis separates the intersecting branches of the null-clines for \( e_i \) into two non-intersecting branches. The trivial branches (not shown in the figure) are shifted outside the first quadrant. The nontrivial branches for \( e_i \) approach the \( e_j \)-axis asymptotically instead of intersecting it. Thus, the saddle point on the \( e_2 \)-axis in Fig. 9 is shifted outside the first quadrant, and the stable node on the \( e_1 \)-axis in Fig. 9 becomes an interior point of the first quadrant.

Strictly speaking, the unique steady state obtained in the presence of constitutive enzyme synthesis corresponds to simultaneous utilization, since both enzyme levels are nonzero. However, for reasonable values of the constitutive enzyme synthesis rate \( k_i^* \ll V_{e_i}^* \), the substrate utilization pattern is ‘almost sequential’.

\[
\sqrt{V_{e1}^* V_{e2}^*} \approx \sqrt{V_{e2}^* V_{e2}^*}
\]
Fig. 10. The phase portrait of the reduced equations of the cybernetic model in the presence of enzyme degradation and constitutive enzyme synthesis. All initial conditions lead to preferential utilization of $S_1$.

Fig. 11. The null-clines for eq. (50). The full line represents the null cline for $e_1$, and the dashed line represents the null cline for $e_2$. 
5. CONCLUSIONS

Based on the analysis above, we arrive at the following conclusions:

(1) The global dynamics of the cybernetic model are consistent with the experimental data on diauxic growth. The model predicts preferential utilization of the preferred substrate for all initial conditions. This global behavior endures even if constitutive enzyme synthesis is included in the model.

(2) The model cannot accommodate the simultaneous substrate utilization pattern, except for very special sets of parameter values that result in infinitely many physiological steady states.

NOTATION

- $c$: cell density
- $c_0$: initial cell density
- $e_i$: concentration of $i$th enzyme
- $e_{i,0}$: initial concentration of $i$th enzyme
- $K_{e,i}$: saturation constant for inducible synthesis of $i$th enzyme
- $K_{x,i}$: saturation constant for growth on $i$th substrate
- $k_{d,i}$: rate constant for degradation on $i$th enzyme
- $k_{r,c,i}$: rate constant for constitutive synthesis of $i$th enzyme
- $r_{d,i}$: degradation rate of $i$th enzyme
- $r_{e,i}$: inducible synthesis rate of $i$th enzyme
- $r_{r,c,i}$: constitutive synthesis rate of $i$th enzyme
- $r_{x,i}$: specific growth rate on $i$th substrate
- $s_i$: concentration of $i$th substrate
- $s_{i,0}$: initial concentration of $i$th substrate
- $t$: time
- $u_i$: cybernetic variable for controlling synthesis of $i$th enzyme
- $V_{e,i}$: maximum rate of inducible synthesis of $i$th enzyme
- $V_{r,c,i}$: maximum growth rate on $i$th substrate

Greek letters

- $\mu_{i,\text{max}}$: experimentally observed maximum specific growth rate on $i$th substrate

REFERENCES


