The Dynamics of Microbial Growth on Mixtures of Substrates in Batch Reactors

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A quantitative understanding of microbial growth on mixtures of substrates is of considerable biological and practical interest. Microbes growing in mixed-substrate environments display a rich spectrum of dynamics. The goal of this work is to show that it is possible to capture these dynamics by a judicious choice of only a few physiological variables. The central idea of the mathematical model is that the autocatalytic nature of enzyme induction is the basis of the dynamical behavior associated with mixed-substrate systems. We formulate a model taking due account of the cyclic structure of enzyme synthesis. By applying the quasisteady-state approximation to the model, we show how this cyclic structure makes enzyme synthesis autocatalytic. Simulations of the model show that it successfully captures the experimental data for both the diauxic and the simultaneous substrate utilization patterns. Further insight into these simulations is obtained by performing a complete analysis of the dynamics of the model. The model is first reduced by appealing to the theory of regular and singular perturbations. The reduced equations thus obtained are then analysed by the method of null clines.

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1. Introduction

Microbial growth on mixtures of substrates has been one of the most fertile areas of experimental biology. Indeed, many of the key developments of molecular biology, such as allosteric and transcriptional control, were inspired by macroscopic phenomena, like the diauxie, observed during mixed-substrate growth. Mixed-substrate phenomena are also of great ecological and engineering interest (Egli, 1995). In nature, and in several man-made environments such as wastewater purification systems and commercial fermenters, microorganisms grow on a diverse mixture of substrates (Harder & Dijkhuizen, 1982). Thus, there are compelling reasons for developing a mathematical theory of mixed-substrate growth. Such a theory could play a useful role in determining the relative importance of the various regulatory mechanisms involved in mixed-substrate growth. It could also enhance our understanding of microbial ecology, and enable the rational design of bioreactors in biochemical and environmental engineering.

Mixed-substrate phenomena in nature and man-made systems occur in media containing extremely complex mixtures of substrates. The formulation of a mathematical model of growth on such media is a daunting task. The model system we consider here consists of a batch culture growing on a synthetic medium containing a mixture of only two carbon and energy sources. Although this system is simple, it exhibits a rich spectrum of dynamics. Let us therefore circumscribe the class of mixed-substrate phenomena we wish to capture through our model. During batch growth on a binary mixture, one of the substrates is generally exhausted before the other, leading to the appearance of two exponential growth phases. Based on the growth pattern observed during the first growth phase, the experimental data on binary mixtures can be classified into three categories (Narang et al., 1996b):

(i) Sequential growth pattern independent of preculturing (diauxie). Only one of the two substrates...
is consumed during the first growth phase. Moreover, the order in which the substrates are consumed does not depend on the preculturing conditions.

(ii) **Simultaneous growth pattern independent of preculturing.** Both the substrates are consumed during the first growth phase. Furthermore, the very same specific growth rate and specific uptake rates are obtained, no matter how the inoculum is grown.

(iii) **Growth pattern dependent on preculturing.** The growth rate and substrate utilization pattern during the first growth phase depend on the manner in which the inoculum has been precultured.

The mathematical model formulated here attempts to capture the first two growth patterns; it does not embrace the preculture-dependent growth pattern.

Mathematical models of microbial growth have been classified into **unstructured** and **structured** models (Tsuchiya *et al*., 1966). Unstructured models assume that either the physiological state of the microbial population is not changing in time, or else that the specific growth rate is unaffected by changes in the population’s physiological state. Structured models take into account the changes in the population’s physiological state, and its effects on the specific growth rate.

There are several unstructured models of mixed-substrate growth in the literature (Lee *et al*., 1974; Gondo *et al*., 1978; Hegwald & Knorre, 1978; Toda & Yabe, 1979). These models assume that the growth on each substrate follows Monod kinetics (Monod, 1942), and the interaction between the growth rates is mutually inhibitory. However, they provide no physiological basis for this mutually inhibitory interaction.

Following the discovery of the induction and repression mechanisms controlling mRNA synthesis, steady state models were developed to explain the variation of the enzyme level, $E$, with respect to the dilution rate, $D$, in single-substrate continuous cultures (Toda, 1976; Imanaka & Aiba, 1976; Gondo *et al*., 1978). The logical structure of these models can be schematized as follows:

$$D \Rightarrow S \Rightarrow S_r \Rightarrow Q \Rightarrow E$$

where $S$, $S_r$, and $S_I$ denote the concentrations of the substrate, inducer, and corepressor, respectively, and $Q$ denotes the fraction of free or unbound operator genes. It was assumed that $S$ is related to $D$ via Monod’s model, $S_r$ and $S_I$ are proportional to $S$, and $E$ is proportional to $Q$. The missing link, contained in the function $Q(S, S_r)$, was derived by postulating various models for induction and repression of mRNA synthesis (Koch, 1967; Yagil & Yagil, 1971). These steady-state single-substrate models cannot capture the dynamical phenomena observed during batch growth on mixtures of substrates.

Van Dedem & Moo-Young proposed the first structured dynamic model of the diauxie (Van Dedem & Moo-Young, 1975). The model assumes a priori that the enzyme for the preferred substrate is constitutive, while that for the less preferred substrate is inducible. The diauxie is captured by postulating catabolite repression via ATP. In the presence of the preferred substrate, the level of ATP is high, and represses the synthesis of the inducible enzyme. Bajpai & Ghose formulated a similar model, the only difference being that the repression was exerted by the preferred substrate itself rather than by one of its products (Bajpai & Ghose, 1978). Instances of the diauxie wherein the enzymes for both the substrates are inducible (Monod, 1947) are beyond the scope of these models. An entirely different approach was taken by Kompala *et al*., who developed the cybernetic model for diauxic growth (Kompala *et al*., 1986). A dynamic analysis of this model shows that it does not capture the simultaneous growth pattern (Narang *et al*., 1996a).

An important goal of the mathematical model presented here is to shed light on the relative importance of the various mechanisms underlying the dynamics of the environmental observables, cell density and exogenous substrate concentrations. Experimental studies, particularly those concerning the diauxie, have shown that these dynamics are dictated by the dynamics of the inducible enzymes catalyzing the transport and peripheral catabolism of the substrates. The enzymatic dynamics are in turn determined by the rate of synthesis of the enzymes by induction, and the rate of dilution of the enzymes by growth. Superimposed on the processes of enzyme induction and dilution are regulatory mechanisms, such as catabolite inhibition (Magasanik, 1961) and inducer exclusion (Postma, 1987). In the literature, the dynamical behavior of the environmental variables, such as complete cessation of substrate uptake during the diauxie, is invariably attributed to regulatory mechanisms. Here, we wish to highlight the role of a key nonlinearity associated with enzyme induction. The seat of this nonlinearity is the autocatalytic nature of inducible enzyme synthesis; that is, the inducible enzyme, in effect, catalyzes its own synthesis.

The autocatalytic nature of inducible enzyme synthesis is rooted in the kinetic structure associated...
with it. In Section 2, we formulate a model that properly accounts for this kinetic structure. In Section 3, we show that for a suitable choice of parameter values, the model captures the preculture-independent growth patterns. In Section 4, we show that the essential dynamics of the model can be approximated by a reduced system of only two differential equations. This formulates mathematically the following empirical observation: The dynamics of mixed-substrate growth is determined essentially by the dynamics of the inducible enzymes. The reduction to a two-dimensional system enables us to perform a detailed global analysis of the model. The phase portraits obtained from this global analysis are discussed in Section 5.

2. Formulation

The kinetic scheme is shown in Fig. 1. Here, $S_i$ denotes the $i$th substrate, $E_i$ denotes the inducible enzyme or "lumped" system of enzymes uniquely associated with the uptake and peripheral catabolism of $S_i$, $X_i$ denotes the inducer for $E_i$, $P$ denotes the "lumped" pool of biosynthetic precursors, and $B$ denotes the biomass. The concentrations of these entities are denoted by the lower-case letters $s_i$, $e_i$, $x_i$, $p$, and $c$, respectively. The yield of biomass on $S_i$, denoted $Y_i$, is the fraction of the stream from $X_i$ that is channeled into biomass, the remainder being oxidized to produce the energy required for growth.

The kinetic scheme assumes that the inducer, $X_i$, is a product of the inducible enzyme(s) $E_i$. The autocatalytic nature of enzyme synthesis is buried in this structure, and is forcefully brought out by the following intuitive argument:

1. The inducible enzyme catalyzes the synthesis of the inducer associated with it.
2. The inducer in turn stimulates the synthesis of the inducible enzyme.

This mutually dependent cycle of reactions, by itself, suggests that the enzyme effectively catalyzes its own synthesis. In Section 4, we show that the kinetic structure assumed does result in autocatalytic enzyme synthesis. Here, we pause to consider the empirical basis for the assumption. The inducible enzymes associated with lactose and glycerol are well-known examples that support this assumption: Allolactose is a product of $\beta$-galactosidase, and glycerol-3-phosphate is a product of glycerol-3-kinase (Lin, 1987). It is important to note, however, that this assumption is trivially true if the enzyme catalyzing the transport of a substrate is inducible, a condition that is satisfied by the transport enzymes of most substrates (Lin, 1987).

The following assumptions are made about the kinetics of the various processes:

1. The specific rate of uptake of the $i$th substrate, denoted $r_{ui}$, satisfies the kinetic law:

$$r_{ui} \equiv V_{ui} e_i \frac{s_i}{K_{ui} + s_i}. \quad (1)$$

2. The specific rate of breakdown of $X_i$ into energy and the precursor pool $P$, denoted $r_{xi}$, is given by:

$$r_{xi} \equiv V_{xi} \frac{x_i}{K_{xi} + x_i}. \quad (2)$$

3. The yield, $Y_i$, is a fixed "stoichiometric" coefficient. This coefficient is the same as the yield observed during single-substrate growth.

4. The specific growth rate, denoted $r_g$, is given by:

$$r_g \equiv V_g \frac{p}{K_g + p}. \quad (3)$$

5. The specific rate of induced enzyme synthesis, denoted $r_{ei}$, is hyperbolic with respect to the inducer $X_i$:

$$r_{ei} \equiv V_{ei} \frac{x_i}{K_{ei}(p) + x_i}. \quad (4)$$

where $K_{ei}(p)$ is given by:

$$K_{ei} = K_{ei}^0 \left[ 1 + \left( \frac{p}{K_{ei}} \right) \right]. \quad (5)$$

This dependence of $K_{ei}$ on the precursor level $p$ is intended to reflect the effect of catabolite repression. As precursor levels increase, the
The order of magnitude of \( V_s \) and \( V_x \) is on the order of 0.001 g/(g-hr), respectively. Since the enzyme level is on the order of 0.001 g/gdw (Inghram et al., 1983), \( V_e \) is on the order of 1000 g/(g-hr). \( K_s \) is on the order of 0.01 g/gdw. Assumed \( K_x \) is 1/100 of \( 10^{-4} \) g/dw/hr, the inducer concentration under substrate-sufficient conditions. ** By definition, \( K_i^0 \) is the saturation constant for enzyme synthesis under substrate-deficient conditions (p < 0). Assumed \( K_i^0 \) is 1/100 of \( 10^{-4} \) g/dw/hr, the inducer concentration under substrate-sufficient conditions.

†† Assumed to be the value of \( p \) at which \( V_e(p)(K_s + p) \) equals the experimentally observed maximum specific growth rate on \( S_i \), respectively (Narang et al., 1996b).

§§ The maximum specific growth rate of \( E. coli \) in a synthetic medium is on the order of 1 hr⁻¹. * Measured experimentally.

** Precursor concentrations are on the order of 0.01 g/gdw (Inghram et al., 1983).

The parameter values used in the simulations are parameter values are assigned the subscript 1 if they listed in Table 1. Note that the substrate-specific parameter values are assigned the subscript 1 if they

Thus, we arrive at the following initial-value problem:

\[
\frac{dS_j}{dt} = -\left( V_e \frac{x_i}{K_s + x_j} \right) c
\]

\[
\frac{dx_i}{dt} = V_e \frac{x_j}{K_s + x_i} - V_k \frac{x_i}{K_k + x_i} - \left( V_e \frac{p}{K_p + p} \right) x_i
\]

\[
\frac{dp}{dt} = \sum_{j=1}^{m} Y_j V_{aj} \frac{x_j}{K_{aj} + x_j} - V_e \frac{p}{K_p + p} - \left( V_e \frac{p}{K_p + p} \right) p
\]

\[
\frac{dc_i}{dt} = V_e \frac{x_i}{K_i(p) + x_i} + k^{\star} - k_{di} c_i
\]

\[
\frac{dc}{dt} = \left( V_e \frac{p}{K_p + p} \right) c
\]

\[ t = 0: S_i = S_{i,0}, x_i = X_{i,0}, p = P_0, c_i = c_{i,0}, c = c_0 \]

where the last terms in eqns (9–11), are the dilution rates of \( X_i \), \( P \), and \( E_i \), respectively, and account for the rates at which these intracellular entities are channelled into biomass.

### 3. Simulation

It was shown in Narang et al. (1996b), that a mixture of glucose and fumarate led to diauxic growth of \( E. coli \) K12; glucose was consumed preferentially no matter which substrate, glucose or fumarate, was used to cultivate the inoculum. On the other hand, the mixture of fumarate and pyruvate resulted in simultaneous utilization of the substrates, regardless of the preculturing conditions. We show below that for a suitable choice of parameter values and initial conditions, the model successfully mimics this behavior.

The parameter values used in the simulations are listed in Table 1. Note that the substrate-specific parameter values are assigned the subscript 1 if they

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Glucose (S₁)</th>
<th>Fumarate (S₂)</th>
<th>Pyruvate (S₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{i,*} )</td>
<td>g/(g-hr)</td>
<td>1650</td>
<td>1450</td>
<td>660</td>
</tr>
<tr>
<td>( K_{s,*} )</td>
<td>g/L</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>( V_{i,†} )</td>
<td>g/(g-dw-hr)</td>
<td>16</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>( V_{i,‡} )</td>
<td>g/dw/g</td>
<td>0.41</td>
<td>0.24</td>
<td>0.15</td>
</tr>
<tr>
<td>( K_{i} )</td>
<td>g/(g-dw-hr)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>( K_{i,‡‡} )</td>
<td>g/gdw</td>
<td>10⁻⁶</td>
<td>5 × 10⁻⁶</td>
<td>10⁻⁶</td>
</tr>
<tr>
<td>( K_{i,††} )</td>
<td>g/dw</td>
<td>0.027</td>
<td>0.007</td>
<td>0.004</td>
</tr>
<tr>
<td>( k_{di,‡‡} )</td>
<td>g/(g-dw-hr)</td>
<td>10⁻⁵</td>
<td>10⁻⁵</td>
<td>10⁻⁵</td>
</tr>
</tbody>
</table>
| \( k_{di,‡†} \) | /
| \( k_{di,§§} \) | g/gdw      | 0.05        | 0.05          | 0.05          |

\( * \) Adjusted to fit the experimental data (Narang et al., 1996b).

\( † \) On the order of 10 mg/L (Button, 1985).

\( ‡ \) Assumed \( V_{i,*} = 0.01 V_{i,†} \).

\( § \) Assumed \( K_{s,*} \) is on the order of the inducer concentration under substrate-excess conditions (10⁻⁴ g/dw) (Yagil & Yagil, 1971).

\( ¶ \) Measured experimentally.

\( ‡‡ \) g/(g-hr) 10⁻⁵ 10⁻⁵ 10⁻⁵

\( † † \) g/(g-hr) 16 15 7

\( § § \) g/(g-hr) 0.41 0.24 0.15

\( † ‡ \) g/(g-hr) 0.001 0.001 0.001

\( ‡ † \) g/(g-hr) 16 15 7

\( §§ \) g/(g-hr) 10⁻⁵ 10⁻⁵ 10⁻⁵

\( † † † \) g/(g-hr) 0.41 0.24 0.15

\( ∗ ∗ \) g/(g-hr) 10⁻⁵ 10⁻⁵ 10⁻⁵

\( † † † † \) g/(g-hr) 0.001 0.001 0.001

\( §§ §§ \) g/(g-hr) 10⁻⁵ 10⁻⁵ 10⁻⁵

\( † † † † † \) g/(g-hr) 0.001 0.001 0.001

\( ¶ ¶ \) g/(g-hr) 0.027 0.007 0.004

\( §§ §§ §§ \) g/(g-hr) 10⁻⁵ 10⁻⁵ 10⁻⁵

\( † † † † † † \) g/(g-hr) 0.001 0.001 0.001

\( ¶ ¶ ¶ ¶ \) g/(g-hr) 0.05 0.05 0.05
belong to glucose, 2 if they belong to fumarate, and 3 if they belong to pyruvate. The parameter values for these substrates are not available in the literature. Thus, the orders of magnitude of the parameters were estimated as shown in Table 1. All parameters, except $V_i$ and $K_{0,i}$, were then fixed. These two parameters were adjusted to fit the experimental data (Narang et al., 1996b).

Figure 2 shows two simulations of the model for the set of parameter values corresponding to the glucose-fumarate system. We wish to show here that no matter which substrate, glucose ($S_1$) or fumarate ($S_2$), is used to grow the inoculum, the model predicts preferential utilization of glucose. Thus, the initial values for the substrate concentrations and the cell density are the same for both simulations. The only difference between these two simulations is in the choice of the initial values of $x_i$, $p$, and $e_i$. This is intended to reflect the fact that the initial physiological state depends on the substrate used to grow the inoculum. The full lines in Fig. 2 show the evolution of the model when the inoculum has been precultured on $S_1$, so that $e_{1,0}$ is high and $e_{2,0}$ is low. The dashed lines show the evolution of the model when the initial physiological state represents an inoculum grown on $S_2$ (low $e_{1,0}$, high $e_{2,0}$). In both cases, $e_1$ and $e_2$ tend toward the very same levels during the first growth phase: $e_1$ approaches a certain fixed, but non-zero level, while $e_2$ tends to zero, so that $S_1$ is preferentially utilized during the first growth phase.

---

**Fig. 2.** Dynamics of the model: sequential utilization. The full lines show the evolution of the variables when the inoculum has been precultured on $S_1$: $x_{1,0} = 10^{-4}$ g/gdw, $x_{2,0} = 10^{-6}$ g/gdw, $p_0 = 0.03$ g/gdw, $e_{1,0} = 10^{-3}$ g/gdw, $e_{2,0} = 0.05 \times 10^{-3}$ g/gdw. The dashed lines show the evolution when the inoculum has been grown on $S_2$: $x_{1,0} = 10^{-4}$ g/gdw, $x_{2,0} = 10^{-4}$ g/gdw, $p_0 = 0.007$ g/gdw, $e_{1,0} = 0.05 \times 10^{-3}$ g/gdw, $e_{2,0} = 1.12 \times 10^{-3}$ g/gdw. The initial values of the substrate concentrations and cell density are the same in both simulations: $s_{1,0} = s_{2,0} = 2$ g/L, $e_0 = 0.001$ gdw/L.
Figure 3 shows the agreement between the model simulations and the experimentally observed dynamics for the mixture of fumarate ($S_2$) and pyruvate ($S_3$). Once again, we have chosen two distinct sets of initial conditions to reflect the preculturing of the inoculum on $S_2$ and $S_3$, respectively. In both cases, the enzyme levels $e_2$ and $e_3$ approach the same non-zero values during the first growth phase. Thus, the same non-zero specific uptake rates of the two substrates are obtained regardless of the substrate on which the inoculum was precultured.

Figures 2 and 3 suggest that the evolution of the enzyme levels during the first growth phase is independent of the physiological state of the inoculum. We emphasize the word “suggest” since the dynamics of the model were simulated for only two distinct sets of initial values for $x_i$, $p$, and $e$. We now ask: Is the evolution of the enzyme levels during the first growth phase the same for any choice of $x_i$, $p$, and $e$? It is necessary to show this if we wish to establish that the evolution of the enzyme levels during the first growth phase is indeed independent of the initial physiological state.

4. Reduction

The global behavior of the enzyme levels during the first growth phase can be approximated by a reduced system of only two differential equations. The benefit of this reduction is that we can construct a phase
Hence, we obtain the equations:

\[
\frac{ds_i}{dt} = - V_{ij} e_i \frac{s_i}{K_{ij} + s_i} c
\]  

(14)

The evolution of the physiological (biotic) state variables \( x_i \), \( p \), and \( e_i \), is influenced by the environmental (abiotic) state variables, \( s \), and \( c \). According to the model, this influence is exerted only by the substrate concentrations through the ratios, \( s_i/(K_{ij} + s_i) \), appearing in the evolution equations (9) for the physiological state variables \( x \). Since \( s_{0i} \) is on the order of 1 g/L, and \( K_{ij} \) is on the order of 0.01 g/L, these ratios are initially almost equal to one. Moreover, they do not deviate significantly from this value until \( s \) becomes as small as \( K_{ij} \). Hence, the cells “see” a constant environment throughout almost the entire first growth phase. In the face of this quasiconstant environment, the physiological state variables tend toward a quasisteady-state. Their approach toward this quasisteady state occurs in stages. The fast variables, \( x_i \) and \( p \), achieve quasisteady-state almost instantaneously. This is followed by the slow evolution of the enzyme levels, \( e_i \), toward their quasisteady-state values. In typical batch cultures, the first growth phase lasts long enough for the enzyme levels to also reach their quasisteady-state values. Indeed, since the time constants for the cell density and the enzyme levels are the same \((1/V_g)\)*, the enzyme levels approach quasisteady-state in 2–3 cell doublings. In typical batch cultures, \( c_{0i}, s_{0i}, \) and \( Y_i \) are on the order of 0.01–0.001 g dry weight (gdw) per liter, 1.0 g/L, and 0.5 g/gdw, respectively. Hence, \( c_{0i}/(Y_i s_{0i}) \) is on the order of 0.01–0.001, and the cells undergo at least 7–10 doublings before the end of the first growth phase. This represents sufficient time for the enzyme levels to reach quasisteady-state. We are interested in the motion of the enzyme levels toward the quasisteady-state values reached during the first growth phase. This can be approximated by studying the dynamics of the reduced system obtained by letting \( \tilde{x}_i = \tilde{p} = 0 \) and \( s_i = s_{0i} \).

We begin the process of simplifying these equations by expressing mathematically the fact that the fast variables \( x_i \) and \( p \) rapidly achieve quasisteady-state. Hence, we obtain the equations:

\[
\frac{de_i}{dt} = V_{ei} \frac{\tilde{x}_i}{K_{ei}(\tilde{p}) + \tilde{x}_i} - \left( \sum_{j=1}^{2} Y_j V_{ej} e_j \frac{s_j}{K_{ej} + s_j} \right) e_i - k_{ej} e_i + k_{eij}^s
\]  

(15)

\[
\frac{dc}{dt} = \sum_{j=1}^{2} Y_j V_{ej} e_j \frac{s_j}{K_{ej} + s_j} c
\]  

(16)

\[
s_i(0) = s_{0i}, \quad e_i(0) = e_{0i}, \quad c(0) = c_0
\]  

(17)

where:

\[
\tilde{x}_i = K_{ei} (V_{ei}/V_{ej}) c_{0i} \tilde{s}_i/(K_{ei} + \tilde{s}_i)
\]

(18)

\[
\tilde{p} = K_{p} \left[ 1 - \sum_{j=1}^{2} (Y_j V_{ej}/V_{p}) \tilde{s}_j/(K_{p} + \tilde{s}_j) \right]
\]  

(19)

are the quasisteady-state concentrations of the inducers and precursors, respectively. Equation (18), which shows that \( \tilde{x}_i \) is a monotonically increasing function of \( e_i \), is the key to the autocatalytic nature of enzyme synthesis. Indeed, if we substitute \( \tilde{x}_i \) in the kinetic expression for enzyme synthesis, we see that the quasisteady-state rate of synthesis of \( e_i \):

\[
\tilde{r}_{ei} \equiv \frac{V_{ei}}{K_{ei}(\tilde{p}) + \tilde{x}_i} = \frac{e_i}{K_{ei}(\tilde{p}) + \tilde{x}_i} \]

(20)

is zero whenever \( e_i \) is zero. Such kinetics are characteristic of autocatalytic processes. The product \( (e_i) \) of the process (enzyme synthesis) is essential for its own synthesis; in the absence of the product, the rate of the process is zero.*

* The term “autocatalytic” is generally used to describe the kinetics of single reactions that catalyze their own synthesis. The fact that we are using this term to describe a process (i.e. a set of reactions) calls for some clarification. In a process consisting of a closed network of reactions, “substances which are by no means self-replicating in their own right may nevertheless increase according to the law of autocatalysis which makes them appear autoasynthetic” (Dean & Hinselwood, 1966). Neither the rate of enzyme induction, nor the rate of inducer formation are, by themselves, autocatalytic. However, due to cyclic structure of enzyme synthesis, the process of enzyme synthesis exhibits properties characteristic of autocatalytic reactions.
The next simplification results from the existence of a linear dependence between the cell density and the substrate concentrations. It follows from eqns (14) and (16) that:

\[
\frac{d}{dt}(Y_1s_1 + Y_2s_2 + c) = 0
\]

whence:

\[
c = c_0 + Y_1(s_{1,0} - s_1) + Y_2(s_{2,0} - s_2)
\]

which says that the instantaneous cell density is the initial cell density plus the density of the cells created by consumption of the two substrates.

Substituting (22) in (14), we obtain:

\[
\frac{ds_i}{dt} = -V_{s,i}e_i \frac{s_i}{K_{s,i} + s_i} \\
(c_0 + Y_1s_{1,0} + Y_2s_{2,0} - Y_1s_1 - Y_2s_2)
\]

\[
\frac{de_i}{dt} = V_{e,i} \frac{\bar{x}_i}{K_{e,i}(\bar{\rho}) + \bar{x}_i} - \left( \sum_{j=1}^{2} Y_jV_{e,j} \frac{s_j}{K_{s,j} + s_j} \right) e_i - k_{d,i}e_i + k_{e,i}^\ast
\]

\[
s_i(0) = s_{i,0}, \quad e_i(0) = e_{i,0}
\]

Further reduction of these equations obtains from the fact that for a certain finite interval of time, the enzyme levels evolve without “seeing” the changes in the substrate concentrations. It is easier to explain this for the special case of single substrate growth, since the reduction can be shown graphically.

4.1. SINGLE-SUBSTRATE GROWTH

In the special case of single-substrate growth, the equations become:

\[
\frac{ds_1}{dt} = -V_{s,1}e_1 \frac{s_1}{K_{s,1} + s_1} (c_0 + Y_1s_{1,0} - Y_1s_1)
\]

\[
\frac{de_1}{dt} = V_{e,1} \frac{\bar{x}_1}{K_{e,1}(\bar{\rho}) + \bar{x}_1} - \left( Y_1V_{e,1} \frac{s_1}{K_{s,1} + s_1} \right) e_1 - k_{d,1}e_1 + k_{e,1}^\ast
\]

where we use the subscript 1 since the substrate-specific parameter values used in the following simulations belong to glucose.

We stated above that we are interested in the evolution of the enzyme level for only a finite interval of time. Let us begin by specifying precisely just how long is this finite interval of time. Figure 4 shows the results of a numerical simulation of the initial-value problem (26–28). The evolution of the enzyme level can be classified into three phases:

Fig. 4. The dynamics of single substrate growth.
(i) **A-phase** during which the enzyme level accumulates

(ii) **Q-phase** during which the enzyme level (and the inducer and precursor concentrations) are quasistationary. Deviation from the quasistationary levels occurs only when $s_i$ approaches $K_{s,i}$. Thus, the **Q-phase** represents the state of balanced growth during which all physiological variables are constant (Ingraham et al., 1983).

(iii) **D-phase** during which the enzyme level is slowly depleted.

The finite time interval of interest is the **A-phase** during which the enzyme level evolves from its initial level to its quasisteady-state value in the **Q-phase**. The obvious way to determine the enzymatic dynamics during this time interval is to integrate the initial-value problem (26–28). What we wish to show here is that these dynamics are well approximated by a one-dimensional differential equation.

To show this, it is instructive to plot the solution of the initial-value problem (26–28) in the $(s_1, e_1)$ state space. The full line in Fig. 5 shows that when the solution is thus plotted, it 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 the initial-value problem (26–28), we solve the “neighboring” initial-value problem:

\[
\frac{ds_i}{dt} = -V_{s,i}e_1 \frac{s_i}{K_{s,i} + s_i} (c_0 + Y_{s,0} - Y_{s,i})
\]

\[
\frac{de_1}{dt} = V_{e,i} \frac{e_1}{K_{e,i}(\hat{p}) + \hat{x}_i} - \left( Y_{s,i}e_1 \frac{s_i}{K_{s,i} + s_i} \right) e_1 - k_d e_1 + k^* e_1
\]
\[ s_1(0) = s_{1,0} + \frac{c_0}{Y_1}, \quad e_1(0) = e_{1,0}. \]  

(31)

The initial conditions (31) are obtained by orthogonal projection of the actual initial condition \((s_{1,0}, e_{1,0})\) onto the line:

\[ c_0 + Y_1s_{1,0} - Y_1s_1 = 0. \]

The solution of this new initial-value problem is shown as a dashed line in Fig. 5.

The new initial-value problem is simpler than the original initial-value problem, yet its solution is a good approximation over the finite time interval of interest. Let us substantiate this claim. It is simpler than the original initial-value problem since it is equivalent to the one-dimensional initial-value problem:

\[ s_1(t) = s_{1,0} + \frac{c_0}{Y_1}. \]  

(32)

This is because the new initial conditions (31) lie on the line, \(c_0 + Y_1s_{1,0} - Y_1s_1 = 0\), which is invariant under the flow of (29–30), i.e., a solution starting at any point on this line will remain on it subsequently. The solution of the new initial value problem is also a good approximation over the finite time interval of interest, since the new initial-value problem is obtained from the original initial-value problem by a very slight modification of the initial conditions. The distance between the two sets of initial conditions is only \(c_0/Y_1\), which is substantially less than \((s_{2,0} + e_{2,0}Y_1)\), the distance of the original initial conditions from the origin. Hence, the theorem of continuous dependence on initial conditions assures us that the solutions will remain close for a finite period of time whose length depends on the magnitude of the dimensionless parameter \(c_0/(Y_1s_{1,0})\). The smaller the magnitude of \(c_0/(Y_1s_{1,0})\), the larger the time interval over which the approximation is valid. In typical batch experiments, \(c_0/(Y_1s_{1,0})\) is on the order of 0.01–0.001.

The theorem guarantees the proximity of the exact and approximate solutions only over a finite time interval; it does not preclude the possibility that these solutions ultimately diverge. Figure 5 shows that their asymptotic behavior is indeed quite different. The approximate solution ultimately approaches the unstable steady state. The exact solution passes through a neighborhood of this unstable steady state, but ultimately approaches the stable steady state near the origin \((s_1 = 0, e_1 = 0.0002)\). However, throughout the finite time interval of interest, the approximate solution is very close to the exact solution.

It is now easy to deduce the global behavior of the enzyme level during its approach to balanced growth.

---

**Fig. 6.** Phase portrait for single-substrate growth: with enzyme degradation and constitutive enzyme synthesis [eqn (33)].

**Fig. 7.** Phase portrait for single-substrate growth: without enzyme degradation and constitutive enzyme synthesis [eqn (36)].
from any initial condition. The phase portrait for the reduced eqn (33) shows that no matter what the initial enzyme level, it tends to a certain non-zero level (Fig. 6).

The last approximation simplifies the structure of the initial-value problem (32–34), thus revealing the autocatalytic nature of enzyme synthesis. Indeed, over sufficiently small finite time intervals, (32–34) is close to the problem:

\[
\frac{ds_i}{dt} = -Y_iV_i e_i \frac{s_i}{K_{i,i} + s_i},
\]

\[
\frac{de_i}{dt} = V_e e_i \frac{\bar{x}_i}{K_e(\bar{p}) + \bar{s}_i} - \left( \sum_{j=1}^{2} Y_j V_j e_j \frac{s_j}{K_{j,i} + s_j} \right) e_i,
\]

\[
s_i(0) = s_{i,0} + \frac{c_0}{Y_i}. \tag{35}
\]

\[
e_i(0) = e_{i,0} \tag{37}
\]

obtained from (32–34) by letting \(k_{d1} = k_{d1} = 0\). The intuitive justification for this approximation is that for the first few hours, the high rates of inducible enzyme synthesis and dilution overwhelm the relatively small rates of enzyme degradation and constitutive enzyme synthesis. Figure 7 shows that the phase portraits for eqns (33) and (36) are almost the same, the only difference being that the latter has a steady state at \(e_i = 0\); this is a manifestation of the autocatalytic nature of inducible enzyme synthesis. Figure 6 lacks this steady state because constitutive enzyme synthesis leads to small but non-zero enzyme synthesis rates even in the absence of the enzyme. We shall refer to eqn (36) as the reduced equation of the model for single-substrate growth. It approximates the finite-time global dynamics of the enzyme for all non-zero initial conditions.

4.2. MIXED-SUBSTRATE GROWTH

The real benefit of the reduction process described above will now be realized in the mixed-substrate case, where it leads to a two-dimensional system of equations. The finite time interval of interest here is the time interval during which the enzyme levels approach their quasisteadystate during the first growth phase. Once again, instead of solving initial-value problem (23–25), we solve the “neighboring” initial-value problem:

\[
\frac{ds_i}{dt} = -Y_iV_i e_i \frac{s_i}{K_{i,i} + s_i}, \quad (c_0 + Y_1 s_{1,0} + Y_2 s_{2,0} - Y_1 s_1 - Y_2 s_2) \tag{38}
\]

\[
\frac{de_i}{dt} = V_e e_i \frac{\bar{x}_i}{K_e(\bar{p}) + \bar{s}_i} - \left( \sum_{j=1}^{2} Y_j V_j e_j \frac{s_j}{K_{j,i} + s_j} \right) e_i - k_{d1} e_i + k_{d1}^* \tag{39}
\]

\[
s_i(0) = s_{i,0} + \frac{Y_i}{Y_1^2 + Y_2^2} e_{i,0}, \quad e_i(0) = e_{i,0}. \tag{40}
\]

The initial conditions (40) are obtained by orthogonal projection of the point \((s_{1,0}, s_{2,0}, e_{1,0}, e_{2,0})\) onto the affine hyperplane:

\[
Y_1 s_1 + Y_2 s_2 - (c_0 + Y_1 s_{1,0} + Y_2 s_{2,0}) = 0. \tag{41}
\]

The solution of this new initial-value problem is a good approximation to the solution of the original initial-value problem because the distance between the new and original initial conditions is only \(c_0/\sqrt{Y_1^2 + Y_2^2}\), which is substantially less than the distance of the actual initial condition from the origin, \((s_{1,0}^2 + s_{2,0}^2 + e_{1,0}^2 + e_{2,0}^2)^{1/2} \approx (s_{1,0}^2 + s_{2,0}^2)^{1/2}\). It is also equivalent to the simpler two-dimensional problem:

\[
s_i(t) = s_{i,0} + \left( \frac{Y_i}{Y_1^2 + Y_2^2} \right) c_0 \text{ for all } t \tag{42}
\]
\[
\frac{de}{dt} = V_{ei} \frac{\bar{x}_i}{K_{s_i}(\bar{p}) + \bar{x}_i} - \left( \sum_{j=1}^{2} Y_j V_s e_j \frac{s_j}{K_{s_j} + s_j} \right) e_i - k_{dj} e_i + k_{ej} e_j^{*} \tag{43}
\]
\[
e_i(0) = e_{i,0} \tag{44}
\]

since the initial conditions (40) lie on the affine hyperplane (41), which is invariant under the flow of eqns (38–39). The solution of (42–44) is well-approximated over the finite time interval of interest by the solution of:

\[
x_i(t) = s_{i,0} + \left( \frac{Y_i}{Y_1 + Y_2} \right) c_0 \text{ for all } t \tag{45}
\]

\[
\frac{de}{dt} = V_{ei} \frac{\bar{x}_i}{K_{s_i}(\bar{p}) + \bar{x}_i} - \left( \sum_{j=1}^{2} Y_j V_s e_j \frac{s_j}{K_{s_j} + s_j} \right) e_i \tag{46}
\]
\[
e_i(0) = e_{i,0}. \tag{47}
\]

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

We are now ready to show that there is nothing special about the initial physiological state, \((x_{i,0}, p_0, e_{i,0})\), chosen for the simulations depicted in Figs 2 and 3. The enzyme levels approach the same values during the first growth phase, no matter what the choice of \((x_{i,0}, p_0, e_{i,0})\).

Figure 8 shows the phase portrait for the reduced equations obtained when the parameter values are the same as those used to simulate sequential utilization in Fig 2. The phase portrait contains three axial steady states, only one of which is stable, namely, the non-zero steady state on the \(e_1\)-axis. All orbits in Fig. 8 approach this stable steady state. Since \(e_2 = 0\) on this steady state, this corresponds to preferential utilization of \(S_1\) during the first growth phase.*

The phase portrait for the case of simultaneous utilization is shown in Fig. 9. Here, there is a globally stable non-axial steady state. Hence, no matter how the inoculum is precultured, both enzymes approach non-zero levels, resulting in simultaneous utilization of the substrates during the first growth phase.

We conclude that the evolution of the enzyme levels during the first growth phase is indeed independent of the initial physiological state.

5. Analysis

In this section, we wish to:

(1) Show the crucial role of the autocatalytic nature of enzyme synthesis in determining the dynamics of the diauxie. To this end, we shall analyse the reduced eqns (46) by the method of null-clines (Murray, 1989).

(2) Elucidate the roles of enzyme dilution and catabolite repression during mixed-substrate growth.

* The initial conditions in Fig. 8 have been chosen inside the triangle enclosed by the two axes and the dashed curve running across the phase portrait because the enzyme levels must satisfy the conditions:

\[
\sum_{j=1}^{2} Y_j V_s e_j \frac{s_j}{K_{s_j} + s_j} < V_e \tag{48}
\]
\[
e_1, e_2 > 0. \tag{49}
\]

That is, the enzyme levels must be such that the quasisteady-state growth rate, i.e., the growth rate reached after \(x_i\) and \(p\) have reached quasisteady-state, is less than the maximum attainable growth rate \(V_e\).
5.1. THE ROLE OF AUTOCATALYSIS IN THE DIAUXIE

The null-cline for \( e_i \) refers to the curves defined by the zero set of the function:

\[
f_i(e_1, e_2) \equiv \frac{V_{e_i}}{K_{e_i}(\bar{p}) + \bar{x}_i} - \left( \sum_{j=1}^{2} Y_j V_{e_j} e_j \frac{s_j}{K_{e_j} + s_j} \right) e_i
\]  

(50)

where:

\[
\bar{x}_i = \frac{(V_{e_i}/V_x) e_i s_i (K_{e_i} + s_i)}{1 - (V_{e_i}/V_x) e_i s_i (K_{e_i} + s_i)}
\]

(51)

\[
\bar{p} = K_x \left( 1 - \sum_{j=1}^{2} (Y_j V_{e_j}/V_x) \bar{x}_j / (K_{e_j} + \bar{x}_j) \right)
\]

(52)

\[
s_i = s_{i0} + \left( \frac{Y_i}{Y_1 + Y_2} \right) c_0.
\]

(53)

These \( f_i \)'s are the functions appearing on the r.h.s. of (46); that is, \( \frac{de_i}{dt} = f_i(e_1, e_2) \).

Figures 10 and 11 show the null-clines for sequential and simultaneous utilization, respectively. The null-cline for each \( e_i \) consists of two branches, one of which coincides with an axis (\( e_i = 0 \)), and the other cuts across the \( e_1 e_2 \)-plane. It is useful to make a distinction between these two branches of a null-cline. We refer to a branch as the trivial branch of the null-cline for \( e_i \) if it coincides with an axis; if the branch cuts across the \( e_1 e_2 \)-plane, we call it the non-trivial branch.

The autocatalytic nature of enzyme synthesis plays a key role in the existence of the trivial branches. Since enzyme synthesis is autocatalytic, the quasisteady-state enzyme synthesis rate satisfies:

\[
1e_i = 0 \Rightarrow V_{e_i} \frac{\bar{x}_i}{K_{e_i}(\bar{p}) + \bar{x}_i} = 0.
\]

(54)

Hence, we can factor out \( e_i \) from (50), and rewrite \( f_i(e_1, e_2) \) in the form:

\[
f_i(e_1, e_2) = e_i g_i(e_1, e_2)
\]

(55)

where \( g_i(e_1, e_2) \) is some function of \( e_1 \) and \( e_2 \). The null-cline for \( e_i \) is therefore given by the conditions:

\[
either e_i = 0 \ or \ g_i(e_1, e_2) = 0
\]

(56)

The first condition, \( e_i = 0 \), yields the trivial branch, and the second condition, \( g_i(e_1, e_2) = 0 \), yields the non-trivial branch.

Having decomposed a null-cline into its trivial and non-trivial branches, we can now give a simple...
characterization of the axial and non-axial steady states appearing in the phase portraits. The steady states of the reduced equations are the points of intersection of the null-clines for distinct enzymes. Axial steady states occur only if the intersection of the null-clines involves at least one trivial branch (Figs 10 and 11). Non-axial steady states occur only if the intersection of the null-clines involves no trivial branches, i.e., by the intersection of the two non-trivial null-clines (Fig. 11).

For the diauxie to occur, there must exist an axial steady state. Such axial steady states exist only because enzyme synthesis is autocatalytic. Indeed, suppose this was not true. Then the null clines for $e_i$ would contain no trivial branches. But axial steady states are obtained only if the intersection of the null-clines involves at least one trivial null-cline. It follows that if there are no trivial null-clines, there are no axial steady states, and hence, no diauxie. Therefore, insofar as our model is concerned, the autocatalytic nature of inducible enzyme synthesis plays a critical role in capturing the diauxie: It guarantees the existence of axial steady states.

5.2. THE ROLE OF CATABOLITE REPRESSION

The role of catabolite repression is revealed by considering the reduced equations obtained when the effect of catabolite repression is nullified ($K_{inh,i} \to \infty$):
quasisteadystate level is zero, leading to the diauxic growth pattern.

In the presence of catabolite repression, the addition of $S_i$ not only increases the dilution rate of $E_2$, but also suppresses the rate of synthesis of $E_2$. Thus, insofar as the net rate of enzyme synthesis is concerned, enzyme dilution and catabolite repression act in concert: Both inhibit the net rate of enzyme synthesis. If this dual inhibitory effect is not too strong, $E_2$ “survives”, leading to the simultaneous growth pattern. If the dual effect is sufficiently strong, $E_2$ is rendered “extinct”, resulting in the diauxic growth pattern.

Thus, according to the model, the substrate utilization patterns are the outcome of the mutually inhibitory interaction between the two inducible enzymes: Each enzyme inhibits the net rate of synthesis of the other enzyme. Such an interaction results partly because each enzyme promotes the dilution rate of the other enzyme, and is accentuated by catabolite repression which causes each enzyme to also repress the synthesis rate of the other enzyme.

6. Discussion

We have constructed a simple model that abstracts from known biochemical details, but captures most of the experimental behavior described in the literature (Harder & Dijkhuizen, 1982; Narang et al., 1996b). We have not accounted for all the metabolic pathways and regulatory mechanisms. Any attempt to do so soon leads to a model that makes analysis difficult. The simplicity of our model permitted us to perform a detailed dynamic analysis that elucidated the role of what seem to be the most important mechanisms, namely, induction of enzyme synthesis and catabolite repression. Having shown how the model works in a simple setting, we wish to discuss modifications required to bring it into closer correspondence with reality.

6.1. ENERGETICS

The most serious deficiency of the model is the absence of energetics. It was assumed in the model that the fraction of $r_{ij}$ converted to energy is a fixed “stoichiometric” coefficient, and that this coefficient is given by $(1 - Y_i)$, where $Y_i$ is the single substrate yield of biomass on $S_i$. Continuous culture experiments with single substrates have shown that the yield is not constant. As the dilution rate is decreased, the fraction of the input stream channeled into energy increases, resulting in progressively lower yields (Herbert, 1982; Schultz & Lipe, 1964). Further evidence of a variable yield follows from certain mixed-substrate experiments. Linton and coworkers have shown that when formate, an almost pure energy source, is added to a culture of Beneckea natriegens growing on glucose, the energy derived from formate supplements the energy derived from glucose, so that the mixed-substrate yield on glucose is significantly higher than the single-substrate yield on it (Linton et al., 1984). If a pure energy source, such as formate, can alter the partitioning of a substrate into energy and biosynthetic precursors, then so can any carbon and energy source. The assumption of a constant yield must therefore be relaxed. The yields are functions of the prevailing physiological state, not parameters that can be fixed a priori.

6.2. MACROMOLECULAR MACHINERY

The second shortcoming of the model is the simplistic representation of the growth process. We have ignored the elaborate macromolecular machinery that catalyzes the conversion of biosynthetic precursors to biomass. One consequence of this omission is that the growth rate changes as fast as the precursor levels. Experiments show, however, that the time-scale for changes in precursor levels are on the order of seconds, whereas perceptible changes in the growth rate occur on the time-scale of minutes (Maaløe & Kjeldgaard, 1966). This contradiction is resolved once it is admitted that it is not merely the supply of the biosynthetic precursors that determines the growth rate. Before the increased supply of precursors can be exploited to support an increased growth rate, the cell must increase its capacity for converting these precursors to biomass. Since the synthesis of this ribosomal machinery has a time-scale of minutes, the growth rate will change on this slower time-scale. In the model, this might be accounted for by incorporating the ribosomal machinery as a new state variable, and making suitable changes in the growth kinetics to account for the role of the ribosomes in determining the capacity for growth.

6.3. REGULATION OF ENZYME ACTIVITY

The only regulatory mechanism accounted for in the model is catabolite inhibition, which modulates enzyme synthesis. However, there exist two regulatory mechanisms that influence enzyme activity, namely, feedback inhibition by energy or/and precursors (Sanwal, 1970) and inducer exclusion (Postma, 1987). To account for these mechanisms, it suffices to modify the kinetics of uptake as follows:

$$r_{ij} = V_{rj}e_i \frac{s_i}{K_{rj} + s_i} \phi(e_i) \psi(p, [ATP]), \ j \neq i$$
where $\phi(e)$ and $\psi(p,[\text{ATP}])$ are suitable functions defined to capture the effects of inducer exclusion and feedback inhibition.

These extensions will enhance the scope of the model. We note, however, that even though the model has been formulated for two carbon and energy sources, it applies to any pair of substrates satisfying identical nutritional requirements. The problem of growth on, say, two nitrogen sources, such as nitrate and ammonia (Dean & Hinshelwood, 1966, p. 233), is even simpler. Unlike carbon and energy sources, these substrates fulfill exactly one nutritional requirement. Hence, we do not have to contend with the distribution of the substrate toward fulfillment of different nutritional requirements.

7. Conclusions

The key conclusions resulting from this work are:

1. A simple model accounting only for enzyme induction and catabolite repression is capable of capturing the preculturing-independent growth patterns.

2. In the process of reducing this model, a dynamical interpretation was given to the state of balanced growth in batch cultures. It corresponds to almost the entire $Q$-phase shown in Fig. 5.

3. The autocatalytic nature of inducible enzyme synthesis plays a crucial role in mixed-substrate dynamics. In particular, it offers a natural explanation for the all-or-none type of behavior observed during diauxic growth.

REFERENCES


