Complex Growth Dynamics in Batch Cultures: Experiments and Cybernetic Models

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The cybernetic framework developed by Ramkrishna and co-workers is shown to encompass the regulation of nutrient transport processes as well as the effect of nutrient transport on the biotic phase. A structured model which accounts for both an abiotic or environmental phase and a biotic or cellular phase is proposed to describe bacterial growth on lactose as the limiting carbon and energy source. In the presence of lactose, competing uptake mechanisms are proposed. At low lactose concentrations, an energy-requiring transport process is the preferred uptake mechanism. The coupling between cellular energetics and nutrient uptake results in an interesting intermittent growth phenomenon. As the concentration of lactose increases, a nonenergetic transport process is preferred and cellular growth ceases to be intermittent. Model simulations are compared with previously reported experimental results and exhibit good agreement over the entire range of initial lactose concentrations.

Key words: cybernetic model • regulation • transport • lactose inhibition

INTRODUCTION

During transient periods in environmental conditions a microorganism must manipulate its internal composition in order to adapt to the prevailing environment. Such adaptation requires the implementation of appropriate metabolic regulatory processes by the cell. Rather than incorporating detailed information on cellular regulatory processes while maintaining a high degree of predictability the cybernetic perspective, as developed by Ramkrishna and co-workers, identifies control or cybernetic variables which describe the outcome of these regulatory processes as being consistent with a desired goal. Regulatory processes are not neglected; however, the implementational details of these control mechanisms, which are associated with molecular processes, are important only for the values of the cybernetic variables. Using process rates and concentration variables the cybernetic variables may be computed and derived from simple microeconomic principles, e.g., resource investment and product maximization. The application of this cybernetic or goal-seeking perspective to describe metabolic regulation during diauxic growth and maintenance metabolism has demonstrated a high level of success.

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The experimental results obtained by Kompala et al. during batch growth involving lactose have indicated a need to include the transport of nutrients across the cell membrane and a need for multiple key enzymes to metabolize a single limiting substrate. When a microorganism adapts to the existing environment, it relies upon nutrient transport between the abiotic and the biotic phases as perhaps the primary means of adjusting nutrient levels within the biotic phase. Once this transport process has occurred, the microorganism must be prepared to commit some or all of its resources to the metabolism of the nutrient; therefore, to maximize the utilization of limited resources it is to the advantage of the organism to regulate the admissance of abiotic nutrients. Such metabolic regulation may become apparent in the choice of nutrient that is transported such as in the presence of substitutable substrates where the regulation of nutrient transport processes has been found to play an important if not primary role during growth or in the mechanism of transport, e.g., high-affinity versus low-affinity uptake mechanisms.

We may take advantage of several aspects of lactose metabolism. One, lactose transport has been observed to limit bacterial growth on lactose when present as the carbon and energy source. Two, the lac operon is perhaps the most well known and studied multiple enzyme system in the literature. Finally, as a disaccharide the hydrolysis of lactose within the cellular phase creates a substitutable substrate environment (i.e., glucose and galactose). This allows the utilization of the original cybernetic framework as developed by Kompala et al. when describing the diauxic growth phenomenon in the presence of substitutable substrates. Due to the energetic nature of active lactose transport, bacterial growth on lactose offers an additional area of expansion to the cybernetic framework besides the incorporation of nutrient transport. The use of key cellular resources can now be extended to include energy resources. The effect of the coupling between the proton-motive force (a key cellular energy resource) and the lac permease has been observed to produce growth inhibition and stoppage. It is proposed that growth and transport processes must compete for a limited supply of energy resources. In the absence of a sufficient energy
resource level, maintenance processes are proposed to increase the production of the limiting resource in order to meet the current demand for resource.

The objective of this article is to demonstrate that experimental results obtained in batch culture during bacterial growth on lactose are consistent with the concepts of the cybernetic framework when metabolic regulation associated with the interaction between transport, growth, and maintenance processes is accounted for. Within the proposed model, metabolic regulation of enzyme synthesis and enzyme activity is described from a cybernetic perspective. The experimental data described by the model has been reported previously and produced by *Klebsiella oxytoca* growing aerobically in batch culture on lactose as the limiting carbon and energy source. Growth inhibition in the form of intermittent growth was evident in the cell density profiles when the initial lactose concentration was 1 g/L or less. At higher initial lactose concentrations, growth was not intermittent but it proceeded at a significantly reduced rate.

**MODEL DEVELOPMENT**

The incorporation of transport processes necessitates the description of two distinct phases: an environmental or abiotic phase and a cellular or biotic phase. Since a distinction between abiotic and biotic phases must be made, the subsequent development will denote all biotic species with a carat ('). The concentrations of all biotic or abiotic components are expressed on a biotic or abiotic volume basis, respectively. In addition all key enzymes and kinetic parameters will be denoted with an "i, j" subscript to identify the i<sup>th</sup> process and the j<sup>th</sup> substrate associated with the particular enzyme or parameter. Before we discuss the details of the model, it is helpful to present an overview of the processes occurring within the abiotic and the biotic phases. Figure 1 describes the pertinent enzyme mediated processes which determine the primary carbon flow. Complete details are described within the text. The following development is divided into three sections. First, we present the pertinent concentration variables, process equations, and kinetics. Second, the incorporation of metabolic regulation is discussed. Lastly, the complete mass balances are presented with some discussion on parameter estimation.

**Process Equations and Kinetics**

With the incorporation of nutrient transport processes, the accumulation of substrate within the biotic phase and its assimilation into biomass must be explicitly accounted for. With regards to abiotic lactose, $S_L$, three uptake mechanisms are identified. The first two processes, given as

$$ S_L + Y_{pl} \dot{S}_p + B \xrightarrow{\dot{e}_{pl}} \dot{S}_L + B $$

(1)

$$ 2S_L + B \xrightarrow{\dot{e}_{pl}} 2\dot{S}_L + B $$

(2)

![Figure 1](image-url)  
**Figure 1.** Identification of the enzyme mediated processes which determine the primary carbon flow within the proposed model. In addition to the appropriate key enzymes described within the text, the following components are incorporated within the model: abiotic lactose, $S_L$; biotic lactose, $\dot{S}_L$; biotic glucose, $\dot{S}_G$; biotic galactose, $\dot{S}_G$; a biotic key energy resource, $\dot{S}_p$; a by-product, $\dot{S}_x$; and biomass, $B$.  

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are the primary transport mechanisms when a key internal energy resource \( \hat{S}_P \) is at or near an optimal level as denoted by \( \hat{S}_P^* \). Equation (1) describes a high-affinity process which consumes the key resource \( \hat{S}_P \) during transport while eq. (2) describes a low-affinity process which does not consume \( \hat{S}_P \) but requires a higher level of \( S_L \) to be fully active. Each transport process is assumed to be catalyzed by the same key enzyme, \( \hat{E}_{T,L} \). The high-affinity process may be identified as an active transport process which requires an energy input represented here by the key energy resource \( \hat{S}_P \), and the low-affinity process represents what is commonly referred to as a "slip" transport process since the uncoupling between the transport enzyme and the energy resource involves the same enzyme. Both transport processes have been suggested from experimental results within the literature. The rate equations for these two processes can be written as

\[
\begin{align*}

r_{TA,L} &= \frac{\mu_{TA,L} \hat{E}_{T,L} \text{CVS}_P \hat{S}_P}{(K_{TA,L} + s_i)(K_{TA,P} + \hat{S}_P)} \\

r_{TS,L} &= \frac{\mu_{TS,L} \hat{E}_{T,L} \text{CVS}_P \hat{S}_P}{s_i^2 + K_{TS,L} \hat{S}_P + K_{TS,P} \hat{S}_P}
\end{align*}
\]

for active transport and slip transport, respectively. The biomass concentration is denoted by \( c \); \( s_i \) is the abiotic lactose concentration; \( \hat{S}_P \) is the biotic resource concentration; the concentration of \( \hat{E}_{T,L} \) is denoted by \( \hat{E}_{T,L} \); \( v \) is the specific cell volume; \( \mu_{TA,L} \) and \( \mu_{TS,L} \) are the rate constants for active and slip transport, respectively; \( K_{TA,L} \) and \( K_{TA,P} \) are saturation constants for \( \hat{S}_P \) and \( \hat{S}_P \) during active transport, respectively, while \( K_{TS,L} \) and \( K_{TS,P} \) are saturation constants for \( \hat{S}_P \) during slip transport.

The kinetics in eq. (4) can be derived from a mechanism, proposed by Jost, \(^1\) which assumes that the key enzyme \( \hat{E}_{T,L} \) has multiple active sites for lactose. We adopt this kinetic form for the slip transport process in order to be consistent with the view of multiple active sites already proposed by eq. (1). When \( \hat{E}_{T,L} \) is in the "energized" state, two active sites are assumed: one for lactose and one for the energy resource. Therefore, a dual Monod kinetic form, as given by eq. (3), can be derived. As the concentration of lactose increases we assume a second active site can be occupied by lactose which permits the enzyme to exist in the "nonenergized" or slip state. The resulting kinetic form, eq. (4), is equivalent to the most general case derived by Jost. \(^1\) The control of the distribution between the active and slip states is discussed later in the section on metabolic regulation.

As \( \hat{S}_P \) is reduced from its optimal value due to consumption by active transport, the cell responds by minimizing the loss of this key resource by activating a third mechanism of lactose transport, eq. (5), which is catalyzed by \( \hat{E}_{T,L} \) and does not consume \( \hat{S}_P \):

\[
S_L + B \xrightarrow{\hat{E}_{T,L}} \hat{S}_P + B
\]

Equation (5) is viewed as a "leak" transport \(^3\) of lactose since the uncoupling between the transport enzyme and energy resource involves a separate enzyme. The conversion of \( \hat{E}_{T,L} \) to \( \hat{E}_{T,L} \) is symbolically described as

\[
\hat{E}_{T,L} \xrightarrow{\hat{S}_P} \hat{E}_{T,L}
\]

where \( \hat{S}_P \) represents the absence of \( \hat{S}_P \). Such a conversion of the lac permease, possibly from a dimeric form to a monomeric form, in the absence of a sufficient energy level, as described here by \( \hat{S}_P \), has been suggested by Villarejo. \(^25\) The rate of the leak transport process is given by

\[
r_{TL,L} = \frac{\mu_{TL,L} \hat{E}_{T,L} \text{CVS}_1}{K_{TL,L} + s_i}
\]

where \( \mu_{TL,L} \) is the rate constant and \( K_{TL,L} \) is the saturation constant for the leak lactose transport; the concentration of \( \hat{E}_{T,L} \) is denoted by \( \hat{E}_{T,L} \). The rate of \( \hat{E}_{T,L} \) to \( \hat{E}_{T,L} \) transformation is given by

\[
r_{TR} = \frac{\mu_{TR} \hat{E}_{T,L} \hat{S}_P}{\hat{S}_P + k_{TR} \hat{S}_P}
\]

where \( \mu_{TR} \) and \( k_{TR} \) are the associated kinetic constants. From a mechanistic perspective, this process has not been well described within the literature; therefore, a kinetic form which reflects the experimental observations has been taken. To prevent excessive accumulation of glucose and galactose within the biotic phase, feedback inhibition of lactose transport by glucose and galactose is incorporated. The transport rates are therefore modified as

\[
r_{TL,i} = r_{TL} \left( \frac{K_{LI}}{K_{LI} + \sum \hat{S}_j} \right) ; \quad i = A, S, L, \quad \text{and} \quad j = gl, ga
\]

where \( K_{LI} \) is the inhibition constant and \( \hat{S}_j \) is the biotic phase concentration of glucose or galactose. The inhibition of nutrient uptake by the biotic phase nutrients has been previously referred to as transinhbition. \(^9\)

From the parameter values listed in Table I, it is evident that the slip transport process has the highest maximum rate and a low affinity for lactose while the leak transport process has the lowest maximum rate as well as the lowest affinity for lactose. These parameters identify the leak process as an emergency measure capable of maintaining a minimum rate of lactose transport at all times. Finally, since the active transport process is an energized process it has the highest affinity for lactose with a maximum rate intermediate between the slip and the leak processes. Therefore, the microorganism can not maintain a high affinity for lactose without the presence of the key resource \( \hat{S}_P \).

Once within the biotic phase lactose is hydrolyzed by \( \hat{E}_{H,L} \) to glucose, \( \hat{S}_G \), and galactose, \( \hat{S}_G \):

\[
\hat{S}_L + B \xrightarrow{\hat{E}_{H,L}} Y_G \hat{S}_G + Y_Ga \hat{S}_Ga + B
\]

The rate of lactose hydrolysis is given by

\[
r_{H,L} = \frac{\mu_{H,L} \hat{E}_{H,L} \hat{S}_L}{K_{H,L} + \hat{S}_L}
\]
where $\mu_{H,j}$ is the rate constant and $K_{H,j}$ is the saturation constant for lactose hydrolysis; the concentration of $\hat{E}_{H,L}$ is denoted by $\hat{E}_{H,L};$ $\hat{s}_i$ is the biotic phase lactose concentration. The key enzymes $\hat{E}_{T,L}$ and $\hat{E}_{H,L}$ which catalyze transport and hydrolysis processes, respectively, are induced by the presence of lactose in the biotic phase. The induction process is described simply as

$$B \xrightarrow{\hat{s}_i} B^- + \hat{E}_{H,L} + \hat{E}_{T,L} \quad (12)$$

where $B^-$ is the biomass, excluding the key enzymes. Since enzyme catalysis generally predominates in biological systems, we assume that saturation kinetics, i.e., typical enzyme kinetics, are also applicable to the induction of key enzymes for the utilization of lactose, glucose, and galactose where the inducing substrates are $\hat{s}_L, \hat{s}_G,$ and $\hat{s}_G,$ respectively. The rate expression for the synthesis of $\hat{E}_{H,L}$ and $\hat{E}_{T,L}$ is therefore given by

$$r_{E,J} = \frac{\mu_{E,J} \hat{s}_E \hat{s}_i}{K_{E,J} + \hat{s}_i} \quad (13)$$

where $\mu_{E,J}$ is the rate constant and $K_{E,J}$ is the saturation constant for enzyme synthesis. Equation (13) also assumes that the energy resource $\hat{S}_P$ is required for enzyme synthesis. In order to simplify the development first order kinetics with respect to $\hat{S}_P$ are assumed for the induction of key enzymes for lactose, glucose, and galactose utilization as well as for processes discussed later in the development. Once present within the biotic phase, glucose and galactose are utilized for growth, maintenance, and resource synthesis.

In accordance with Kompala et al.,

$$\hat{s}_J + B \xrightarrow{\hat{E}_{G,J}} (1 + Y_B)B; \quad J = Gl, Ga \quad (14)$$

while the growth rate on $\hat{s}_G$ or $\hat{s}_G$ is given by

$$r_{G,J} = \frac{\mu_{G,J} \hat{E}_{G,J} \hat{s}_G}{K_{G,J} + \hat{s}_G}; \quad J = gl, ga \quad (15)$$

where $\mu_{G,J}$ is the rate constant and $K_{G,J}$ is the saturation constant for growth on glucose or galactose; the concentration of $\hat{E}_{G,J}$ is denoted by $\hat{E}_{G,J};$ $\hat{s}_j$ is the biotic concentration of glucose or galactose. The synthesis of $\hat{E}_{G,J}$ is completely analogous to eq. (12); therefore, the enzyme synthesis rate, $r_{E,J}$ is equivalent to $r_{E,J}$ by replacing the subscript "L" with "J" in eq. (13) where $J = gl, ga.$

As is apparent from the previous discussion, we have accounted for cellular energetics in a number of different process types by identifying a key energy resource, $\hat{S}_P.$ In our first attempt at considering the importance of cellular energetics, we have chosen a simple approach by considering $\hat{S}_P$ as a lumped species. Since this energy resource is assumed to be required for both active transport and many biotic phase processes, which have been established as requiring some form of energy such as ATP or the same ion gradients required for active transport, $\hat{S}_P$ represents a gross measure of the cellular energy status rather than a specific energy component. In many instances, energy is not necessarily consumed by a process; however, it may be necessary for maintaining enzyme activity.

Table II summarizes the processes in which $\hat{S}_P$ is consumed as well the processes which require $\hat{S}_P$ without its consumption. It is opportune at this stage to clarify the “optimum” resource level $\hat{s}_P^*$ as that which provides for the maximum, instantaneous growth rate $r_{S,J}$ with
Active transport of $S_L$

Synthesis of biomass

Nonspecific maintenance processes

Synthesis of $E_{T,L}$ and $E_{R,L}$

Synthesis of $E_{G,B}$ and $E_{G,GA}$

Synthesis of $E_{M,P}$

Synthesis of $E_{G,PA}$

Synthesis of $E_{G,PA}$

Synthesis of $E_{X}$

$E_{T,L}$ transformation to $E_{T,L}$

$\hat{S}_F$ requirement

<table>
<thead>
<tr>
<th>Process</th>
<th>$\hat{S}_F$ requirement</th>
<th>Change in $\hat{S}_F^*$</th>
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<tr>
<td>$\hat{S}_F$ is a cosubstrate of the enzyme</td>
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<tr>
<td>$\hat{S}_F$ maintains enzyme activity</td>
<td>0</td>
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<tr>
<td>$\hat{S}_F$ maintains enzyme activity</td>
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<tr>
<td>$\hat{S}_F$ maintains process activity</td>
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<tr>
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<tr>
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<td>+</td>
<td></td>
</tr>
<tr>
<td>$\hat{S}_F$ is a cosubstrate of the enzyme</td>
<td>-</td>
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*The notation $+, -, 0$ implies that $\hat{S}_F$ is produced by the process, consumed by the process, or unaltered by the process, respectively.

The allocation of carbon source away from growth and towards maintenance processes as the specific growth rate decreases from the maximum value has been well established within the literature and described from a cybernetic perspective. The consumption of glucose and galactose for maintenance processes is also incorporated here and catalyzed by the key enzyme $\hat{E}_{M,J}$ where

$$\hat{S}_j + B = \frac{\hat{E}_{M,J}}{\hat{S}_j} B; \quad j = Gl, Ga$$

The maintenance rate expression is given by

$$r_{M,j} = \frac{\mu_{M,j} \hat{E}_{M,J} \hat{S}_F \hat{S}_j}{K_{M,j} + \hat{S}_j}; \quad j = gl, ga$$

where $\mu_{M,j}$ is the rate constant and $K_{M,j}$ is the saturation constant for the maintenance process; the concentration of $\hat{E}_{M,J}$ is denoted by $\hat{e}_{M,J}$. As with the growth process, the maintenance process is assumed to require the presence of $\hat{S}_F$ without its direct consumption. Following Turner et al., the key enzyme for maintenance is assumed to be synthesized at a rate equivalent to the rate of synthesis of key enzymes for growth; therefore, $\hat{E}_{M,J}$ and $\hat{E}_{G,J}$ are present within the biotic phase at equal concentrations which implies

$$\hat{e}_{M,J} = \hat{e}_{G,J}; \quad j = gl, ga$$

yet their activities may be regulated in different manners.

Key resource $\hat{S}_F$ is synthesized from $\hat{S}_{Gl}$ and $\hat{S}_{Ga}$. However, we also assume that the efficiency of carbon utilization for resource synthesis is not constant but varies with the availability of usable carbon source. Although the regulation of energy metabolism has not been thoroughly established in the literature its variable efficiency has. A variable efficiency of $\hat{S}_F$ synthesis is therefore incorporated by proposing the synthesis of a by-product of energy metabolism, $\hat{S}_X$. When carbon source is available in sufficient quantities, the efficiency of resource synthesis is reduced and $\hat{S}_X$ is produced as described by eq. (20). As the level of carbon source is reduced, we assume it is conserved and allocated primarily towards maintenance processes and resource synthesis. The control of $\hat{S}_X$ synthesis is discussed in more detail later in the section on metabolic regulation.

Maintenance processes were presented earlier while $\hat{S}_F$ synthesis is described by eq. (19):

$$\hat{S}_j + \hat{S}_F + B = \frac{\hat{E}_{M,P}}{\hat{S}_j} 2\hat{S}_F + B; \quad j = Gl, Ga$$

$$Y_{EP}\hat{S}_j + Y_{PN}\hat{S}_F + B = \frac{\hat{E}_{M,P}}{\hat{S}_X + \hat{S}_F} \hat{S}_X + B; \quad j = Gl, Ga$$

Although we do not consider explicitly the identity of $\hat{S}_X$, eq. (20) may represent one of several possible processes: oxidation of carbon to $CO_2$ that is uncoupled from energy generation, synthesis of storage compounds, or the synthesis of fermentation products. In this development, $\hat{S}_X$ is not required for growth and the quantity of carbon that is converted to $\hat{S}_X$ is not available for further utilization. The rate expressions for producing $\hat{S}_F$ and $\hat{S}_X$ are given by

$$r_{R,j} = \frac{\mu_{R,j} \hat{E}_{M,P} \hat{S}_F \hat{S}_j}{K_{R,j} + \hat{S}_j}; \quad j = gl, ga$$

$$r_{X,j} = \frac{\mu_{X,j} \hat{E}_{M,P} \hat{S}_F \hat{S}_j}{K_{X,j} + \hat{S}_j}; \quad j = gl, ga$$

where $\mu_{R,j}$ and $\mu_{X,j}$ are the rate constants for resource and by-product synthesis, respectively; $K_{R,j}$ and $K_{X,j}$ are the saturation constants for resource and by-product synthesis, respectively; and the concentration of $\hat{E}_{M,P}$ is denoted by $\hat{e}_{M,P}$.

Since the key enzyme $\hat{E}_{M,P}$ is a regulatory enzyme, its synthesis should not be entirely nutrient specific in contrast to, for example, $\hat{E}_{G,P}$. With regards to resource $\hat{S}_F$, the absence rather than the presence of $\hat{S}_F$ signals the need for an increased level of $\hat{E}_{M,P}$ synthesis; therefore, $\hat{S}_F$ limitation is assumed to be one inducing agent:

$$B \xrightarrow{\hat{S}_F} B^- + \hat{E}_{M,P}$$

As a feedback mechanism, eq. (23) describes a process which does not respond until $\hat{S}_F$ has been consumed. In order to respond more efficiently to not only the lack of $\hat{S}_F$ but also to agents which result in the reduction of this resource, additional mechanisms, eqs. (24) and
Since $\hat{E}_{T,L}$ directly consumes $\hat{S}_p$, eq. (24) states that the organism incorporates a feed-forward control action and synthesizes $\hat{E}_{M,P}$ in the presence of high levels of $\hat{E}_{T,L}$. At high levels of $\hat{S}_T$, where $\hat{S}_p$ consumption is reduced due to the higher activity of the slip transport process, a relatively high concentration of $\hat{E}_{M,P}$ is already available as $\hat{S}_p$ is consumed to a concentration at which the active transport process is dominant and the $\hat{S}_p$ consumption rate increases greatly. This mechanism for enzyme synthesis, therefore, prepares the biotic phase during a "feast" environment (i.e., high $\hat{S}_T$) for a future "famine" environment (i.e., low $\hat{S}_T$) which must occur as the abiotic lactose is consumed. Equation (25) assumes that, in the event resource levels remain low, the accumulation of $\hat{E}_{M,P}$ will begin to induce its own synthesis. Enzyme synthesis which is promoted by the presence of the same enzyme (termed autogenous regulation) or by the presence of a different though related protein has been exhibited by established regulatory systems.

With regard to the rate expressions for $\hat{E}_{M,P}$ synthesis, two forms are assumed. Previous work on enzyme induction due to phosphate limitation\textsuperscript{26} indicates that noncompetitive inhibition kinetics may be appropriate for induction due to a $\hat{S}_p$ limitation. Therefore,

$$r_{E,P} = \frac{\alpha_{E,P} K_{E,P}}{K_{E,P} + \hat{S}_p}$$

(26)

where $\alpha_{E,P}$ is the rate constant and $K_{E,P}$ is the inhibition constant for enzyme synthesis. When $\hat{E}_{M,P}$ is induced in the presence of $\hat{E}_{M,P}$ or $\hat{E}_{T,L}$, the rate expression is reduced to a proportionality between the synthesis rate and the inducing agent in order to simplify the development:

$$r'_{E,P} = \mu_{E,P} \hat{E}_{M,P}$$

(27)

$$r''_{E,P} = \mu_{E,L} \hat{E}_{T,L}$$

(28)

The specific rate constants are given by $\mu_{E,P}$ and $\mu_{E,L}$, respectively.

**Metabolic Regulation**

This completes the identification of the pertinent concentration variables, process equations, and process kinetics. The process rates and key enzymes are summarized in Tables III and IV. The incorporation of transport, growth, and maintenance processes becomes complete only when the appropriate metabolic regulation is coupled to the interactions of these processes. Cybernetic or control variables are therefore identified, in addition to the previously identified concentration variables, for the control of enzyme synthesis and activity. Before we discuss the incorporation of metabolic regulation, it is helpful to state several fundamental postulates which identify the basic modeling assumptions regarding our current view of regulatory processes. These postulates are not non-negotiable; further revisions are likely as our data base of information and our view of regulation continue to expand.

**Postulates**

The following postulates identify the basic modeling assumptions:

1. The primary goal of microorganisms is to maximize growth rate.
2. There exists an optimal biotic phase state, independent of growth rate and described by a set of key resources, necessary for maintaining maximal cellular vitality.
3. A resource synthesis system manifests at biotic states removed from the optimal and becomes active at nonoptimal resource levels.
4. Maintenance metabolism manifests at growth rates less than the maximum growth rate and becomes active at lower growth rates.
5. The activities of maintenance and resource synthesis are such that the total rate of substrate con-

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<th>Table III. Key enzyme summary.</th>
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<tr>
<td>Enzyme Definition Equations</td>
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<tr>
<td>$\hat{E}_{T,L}$ Key enzyme for active and slip transport of $S_T$</td>
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<tr>
<td>$\hat{E}_{T,L}$ Key enzyme for leak transport of $S_L$</td>
</tr>
<tr>
<td>$\hat{E}_{G,L}$ Key enzyme for hydrolysis of $\hat{S}_G$</td>
</tr>
<tr>
<td>$\hat{E}_{G,L}$ Key enzyme for growth supported by $\hat{S}_G$ or $\hat{S}_G\alpha$</td>
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<tr>
<td>$\hat{E}_{M,P}$ Key enzyme for maintenance supported by $\hat{S}_G\alpha$ or $\hat{S}_G\alpha_0$</td>
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<tr>
<td>$\hat{E}_{M,P}$ Key enzyme for slip maintenance</td>
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<tr>
<td>$\hat{E}<em>{T,L}$ Concentration variable for $E</em>{T,L}$</td>
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sumption for growth, maintenance, and resource synthesis is maximized.

6. As the specific growth rate decreases to zero the efficiency of carbon utilization increases to a maximum value.

Postulates 1, 4, and 5 have been stated previously in the same or similar form by Turner et al. and are restated here for completeness while Postulates 2, 3, and 6 have been added based upon data and information obtained while incorporating the effects of nutrient transport on the biotic phase.

Postulate 1 implies essentially that growth rate is of paramount interest to a microorganism. It assumes that the microorganism will keep growth metabolism fully active with respect to carbon utilization by processes other than growth under all conditions.

Postulate 2 tries to account for the vitality of the microorganism in a tangible manner by creating a set of resources which describe the status of the biotic phase. We define cellular vitality as a measure of the organism's ability to respond to the prevailing environment. When these resources are at their optimal levels, we call such a state "the state of maximal vitality." When cellular vitality is reduced from its maximal state the activity of key enzymes for growth are not reduced due to the activity of non-growth-related enzymes in order to respond quickly to the environment once conditions become more favorable. Therefore, the primary importance of growth is retained and Postulate 2 does not conflict with Postulate 1. However, the absolute activity of any key enzyme may be reduced due to the absence of key resources necessary for maintaining the vitality of the organism.

In physical terms, the status of the biotic and abiotic phases is assumed to depend upon pH, osmotic conditions, temperature, etc. The ability of a microorganism to attain the optimal state is dictated by the status of the abiotic environment. Since the active transport process requires an energy resource, which is also tightly coupled through ion gradients to the internal environment of the cell, the resource \( \hat{S}_p \) must also be included in the set of resources that are proposed to describe the status of the biotic phase. Though originally intended solely as an energy resource, the relationship between ion gradients and the status of the biotic phase dictates this broader usage of \( \hat{S}_p \).

Postulate 3 states that resource synthesis requirements are satisfied by growth processes automatically when no interaction between the abiotic and biotic phases occurs which disrupts the status of the biotic phase. In the presence of such an interaction additional enzyme systems are synthesized and become active at resource levels removed from the optimal. In the absence of such an interaction the resource levels are assumed to be at their optimum.

Postulate 4 takes a position against a constant maintenance activity. It assumes that maintenance requirements are satisfied by growth processes automatically when they occur at the maximum growth rate thus not necessitating a separate maintenance metabolism. However, when growth occurs at less than the maximum rate a separate maintenance metabolism is activated.

With regard to Postulate 5, the substrate consumption rate can be maximized by maintaining the activities of all enzymes which catalyze substrate consuming processes at their maximum value. According to Postulate 1, the activities of growth enzymes are assumed to be unity, i.e., maximal, when compared to processes other than growth which consume substrate. However, it is inefficient to promote the activities of resource and maintenance enzymes to their maximum value of unity if the resource and maintenance requirements are not as great. Therefore, we assume that the resource synthesis activity is linearly proportional to the difference between the optimal and the prevailing resource levels. Likewise, we assume that the maintenance activity is linearly proportional to the difference between the maximum and the prevailing specific growth rates. Note that these assumptions are consistent with

<table>
<thead>
<tr>
<th>Rate</th>
<th>Definition</th>
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<tbody>
<tr>
<td>( r_{TA,i} )</td>
<td>Rate of ( S_i ) active transport</td>
<td>3, 9</td>
</tr>
<tr>
<td>( r_{TA,i} )</td>
<td>Rate of ( S_i ) active transport with feedback inhibition</td>
<td>9, 29–33, 56, 57, 65</td>
</tr>
<tr>
<td>( r_{TA,i} )</td>
<td>Rate of ( S_i ) slip transport</td>
<td>4, 9</td>
</tr>
<tr>
<td>( r_{TA,i} )</td>
<td>Rate of ( S_i ) leak transport</td>
<td>7, 9</td>
</tr>
<tr>
<td>( r_{TA,i} )</td>
<td>Rate of ( S_i ) leak transport with feedback inhibition</td>
<td>9, 31, 32, 56, 57</td>
</tr>
<tr>
<td>( r_{T_{L,E}} )</td>
<td>Rate of ( E_{T,L} ) transformation to ( E_{T,L} )</td>
<td>8, 60, 61</td>
</tr>
<tr>
<td>( r_{T_i} )</td>
<td>Rate of ( S_i ) hydrolysis</td>
<td>11, 58, 59</td>
</tr>
<tr>
<td>( r_{G_j} )</td>
<td>Rate of growth supported by ( \hat{S}<em>G ) or ( \hat{S}</em>{Ga} )</td>
<td>15, 34, 35, 37, 56, 59, 66</td>
</tr>
<tr>
<td>( r_{M_j} )</td>
<td>Rate of maintenance supported by ( \hat{S}<em>G ) or ( \hat{S}</em>{Ga} )</td>
<td>17, 38, 59</td>
</tr>
<tr>
<td>( r_{E_j} )</td>
<td>Rate of ( \hat{S}_P ) synthesis supported by ( \hat{S}<em>G ) or ( \hat{S}</em>{Ga} )</td>
<td>21, 40, 44, 59, 65</td>
</tr>
<tr>
<td>( r_{E_j} )</td>
<td>Rate of ( \hat{S}_P ) synthesis supported by ( \hat{S}<em>G ) or ( \hat{S}</em>{Ga} )</td>
<td>22, 44, 59, 65</td>
</tr>
<tr>
<td>( r_{E_j} )</td>
<td>Rate of ( E_{T,L} ) and ( E_{T,L} ) synthesis</td>
<td>13, 55, 60, 62</td>
</tr>
<tr>
<td>( r_{E_j} )</td>
<td>Rate of ( E_{G,ci} ) or ( E_{G,ci} ) synthesis</td>
<td>36, 53, 63</td>
</tr>
<tr>
<td>( r_{E_{M,E}} )</td>
<td>Rate of ( E_{M,E} ) synthesis in response to ( \hat{S}_P )</td>
<td>26, 54, 64</td>
</tr>
<tr>
<td>( r_{E_{M,E}} )</td>
<td>Rate of ( E_{M,E} ) synthesis in response to ( E_{M,E} )</td>
<td>27, 48, 54, 64</td>
</tr>
<tr>
<td>( r_{E_{M,E}} )</td>
<td>Rate of ( E_{M,E} ) synthesis in response to ( E_{T,L} )</td>
<td>28, 48, 54, 64</td>
</tr>
</tbody>
</table>
Postulates 3 and 4 since no resource synthesis and no maintenance activity are required at the optimal resource level and the maximum growth rate, respectively. When the resource level and the growth rate fall to zero, the resource and maintenance requirements are at their maximum and we provide for the activities of these enzymes to be at their maximum value.

Postulate 6 simply states that all nonessential carbon utilization decreases to zero as the specific growth rate decreases to zero. This ensures that a sufficient level of carbon source is available for essential processes, e.g., resource synthesis and maintenance, as the availability of carbon source decreases.

By stating Postulate 1 and assuming that the slip transport process operates at a high maximum rate, it follows that the microorganism would promote the slip transport process at high lactose concentrations so that limited energy resources can be available for biomass producing processes rather than transport. At reduced lactose concentrations the high-affinity nature of active transport ensures this process will be the preferred uptake mechanism even though it operates at a reduced maximum rate. The choice of transport mechanism catalyzed by $E_{T,L}$ is regulated by a cybernetic variable $u_{Tk,i}$ which modifies the active and slip transport rates as

$$r'_{Tk,i}u_{Tk,i}; 0 \leq u_{Tk,i} \leq 1 \text{ and } k = A, S$$ (29)

where

$$u_{Tk,i} = \frac{r'_{Tk,i}}{\sum_k r'_{Tk,i}}; k = A, S$$ (30)

Equation (30) is equivalent to the matching law formulation used by Kompala et al. to distribute or invest key cellular resources among competing key enzyme synthesis systems. This formulation invests the resources among the alternatives in a manner which maximizes the fractional returns from each investment. We therefore assume that there exists a key resource which is invested in the active and slip transport processes and the returns from this investment are given by the respective transport rates.

In the presence of competing enzymes, the activation and inhibition of enzyme activity plays an important role in metabolic regulation. This form of regulation has been described from a cybernetic perspective during growth on substitutable substrates and extended here to include not only the regulation of the activities of the two growth enzymes, $E_{G,Gl}$ and $E_{G,Ga}$, but also the activities of the two transport enzymes, $E_{T,L}$ and $E_{T,L}$. Both systems, transport and growth, contain two competing enzymes. The transport rate expressions are therefore modified as

$$r'_{Tk,i}v_{Tk,i}; r'_{Tk,i}v_{Tk,i}; r'_{Tk,i}v_{Tk,i}; 0 \leq v_{Tk,i} \leq 1$$

and

$$k = A, S, L$$ (31)

where

$$v_{Tk,i} = v_{Tk,i} = \frac{r'_{Tk,i}}{\max(r'_{Tk,i}, r'_{Tk,i})}$$ and

$$v_{Tk,i} = \frac{r'_{Tk,i}}{\max(r'_{Tk,i}, r'_{Tk,i})}$$ (32)

with

$$r'_{Tk,i} = \sum_k r'_{Tk,i}u_{Tk,i}; k = A, S$$ (33)

Likewise, the growth rate expressions are modified as

$$r_{Gi,j}v_{Gi,j}; 0 \leq v_{Gi,j} \leq 1 \text{ and } j = gl, ga$$ (34)

where

$$v_{Gi,j} = \frac{r_{Gi,j}}{\max(r_{Gi,j})}; k = gl, ga$$ (35)

In the presence of competing enzymes, the cybernetic variables regulating enzyme activity promote the process supporting the fastest rate to the greatest extent in accord with Postulate 1.

The regulatory processes of induction and repression of key enzymes $E_{G,Gl}$ and $E_{G,Ga}$ are also incorporated by modifying $r_{E,i}$ with the cybernetic variable $u_{Gi,j}$, where

$$r_{E,i}v_{Gi,j}; 0 \leq u_{Gi,j} \leq 1 \text{ and } j = gl, ga$$ (36)

and

$$u_{Gi,j} = \frac{r_{Gi,j}}{\sum_k r_{Gi,k}}; j = gl, ga$$ (37)

Cellular resources are therefore also allocated among the competing growth enzymes in a manner which maximizes the fractional return from the investment.

At specific growth rates reduced from the maximum value the distribution of carbon source away from growth and towards maintenance functions has been observed in continuous as well as fed-batch cultures. Typically, in batch culture the specific growth rate is very close to its maximum value throughout the growth phase, and maintenance effects are generally not observed. However, batch growth on lactose has exhibited specific growth rates ranging between 0.15 and 0.76 h$^{-1}$. Therefore, to account for an increased level of maintenance at reduced specific growth rates the activity of $E_{M,i}$ is modified by the cybernetic variable $v_{M}$ as

$$r_{M,i}v_{M}; 0 \leq v_{M} \leq 1$$ (38)

From Postulate 1, the activity of growth processes when compared with maintenance and resource synthesis processes is always maximal. However, with regards to maintenance processes, enzyme activity as modified by $v_{M}$ is assumed to be proportional to the difference between the maximum and the prevailing specific growth rate or $(\mu_{\text{max}} - \mu)$. Turner et al. has derived the ex-
pression for \(v_M\) in detail; therefore, we do not repeat the derivation here except in its final form as
\[
v_M = 1 - \frac{\mu}{\mu_{\text{max}}} \tag{39}\]
in accord with Postulate 4.

Under purely carbon source limitations it has been assumed in the past\(^{22,23}\) that the variation in cellular yield could be accounted for by reallocating carbon source away from growth and towards maintenance functions at specific growth rates reduced from the maximum. However, when the concentration of a biotic phase resource is reduced the available carbon source must be further allocated for the maintenance of the key resource. The regulation of \(\hat{S}_p\) synthesis is therefore accounted for by modifying its synthesis rate as
\[
r_{p, 1}v_F; \quad 0 \leq v_F \leq 1 \quad \text{and} \quad j = g_l, g_a \tag{40}\]
According to our assumption concerning the activity of the resource synthesis enzyme we can write
\[
v_F = \lambda (\hat{S}_p^* - \hat{S}_p) \tag{41}\]
where \(\lambda\) is the proportionality constant. The constraints on \(v_F\) imply \(0 \leq \lambda \leq 1 / (\hat{S}_p^* - \hat{S}_p)\). From a physical perspective, we have identified \(\hat{S}_p^*\) as the optimal resource level; therefore, there may exist environments in which the value of \(\hat{S}_p\) may be less than or greater than the optimal value. However, if we assume from a modeling perspective that there are no abiotic sources of resource and that the organism will not purposely produce resource levels exceeding \(\hat{S}_p^*\) then \(\hat{S}_p\) will never exceed \(\hat{S}_p^*\). Under these conditions the quantity \((\hat{S}_p^* - \hat{S}_p)\) will always be non-negative and \(\lambda \leq 1 / \hat{S}_p^*\). At a zero resource level we must have the maximum resource synthesis activity, i.e., \(v_F = 1\), which follows from Postulate 5, and
\[
v_F = \lambda \hat{S}_p^* = 1 \tag{42}\]
from which \(\lambda = 1 / \hat{S}_p^*\) and \(v_F\) can be expressed as
\[
v_F = 1 - \frac{\hat{S}_p}{\hat{S}_p^*} \tag{43}\]
in agreement with Postulate 3. If we recall that \(\hat{S}_p\) is associated with the specific growth rate, \(\mu = \mu (\ldots \hat{S}_p)\), and that \(\hat{S}_p^*\) is associated with the maximum, instantaneous specific growth rate, \(\mu^* = \mu^* (\ldots \hat{S}_p^*)\), then the expression for \(v_F\) is completely analogous in form to \(v_M\) and carbon source is diverted away from growth and towards resource synthesis at specific growth rates reduced from the maximum, instantaneous value. Since \(\hat{S}_p^*\) is assumed to be independent of the growth rate, it becomes an input to the model. This simplification does not change the qualitative nature of the model dynamics but only alters the time scale of the response of the resource system to perturbations.

Any attempt to incorporate cellular energetics must consider the efficiency of the processes described. With this in mind the total rate of carbon consumption for \(\hat{S}_p\) synthesis is expressed as
\[
(r_{p, j} + r_{x, j})v_F; \quad 0 \leq v_F \leq 1 \quad \text{and} \quad j = g_l, g_a \tag{44}\]
where \(v_F\) is a cybernetic variable which regulates the efficiency of carbon conversion to \(\hat{S}_p\). As the efficiency of carbon utilization varies more or less by-product \(\hat{S}_p\) is produced. From Postulate 6, it follows that \(v_F\) should be proportional to the specific growth rate \(\mu\) or
\[
v_F = \lambda \mu \tag{45}\]
The constraints on \(v_F\) imply \(0 \leq \lambda \leq 1 / \mu_{\text{max}}\); therefore, \(\lambda \geq 1 / \mu_{\text{max}}\). At the maximum specific growth rate, it follows from Postulate 6 that we must have the minimum \(\hat{S}_p\) synthesis efficiency, i.e., \(v_F = 1\); therefore,
\[
v_F = \frac{\mu}{\mu_{\text{max}}} \tag{46}\]
from which \(\lambda = 1 / \mu_{\text{max}}\) and \(v_F\) can be expressed as
\[
v_F = \frac{\mu}{\mu_{\text{max}}} \tag{47}\]
We see from the multiplication of \(v_F\) and \(v_F\) in eq. (44) that even though the efficiency of carbon conversion to resource decreases as the specific growth rate increases the net activity of \(\hat{E}_{M, p}\) must decrease to zero since \(\hat{S}_p\) approaches \(\hat{S}_p^*\) as \(\mu\) approaches \(\mu_{\text{max}}\) and carbon source is conserved for growth maximization in accord with Postulate 1.

With the imposition of environmental stress upon a growing culture, the ability of the microorganism to rapidly increase the level of regulatory enzymes in order to maintain a suitable biotic phase environment is accounted for by proposing eqs. (24) and (25). For this model development, we assume that such behavior is regulated by modifying the activity of these enzymes as
\[
r_{E, p}v_E + r_{E, p}v_E; \quad 0 \leq v_E \leq 1 \tag{48}\]
where \(v_E\) is a cybernetic variable which regulates the activity of these alternative synthesis mechanisms. Maintaining the analogy between observed regulatory enzyme systems\(^{24}\) and the key enzyme \(\hat{E}_{M, p}\) suggests that the form for \(v_E\) should reflect an autogenous form of regulation where the level of \(\hat{E}_{M, p}\) regulates its own synthesis; therefore, assuming that the level of regulation is proportional to the difference between the maximum and the prevailing levels of \(\hat{E}_{M, p}\), i.e.,
\[
v_E = \lambda (\hat{E}_{M, p}^\text{max} - \hat{E}_{M, p}) \tag{49}\]
results in an expression for \(v_E\) of the form
\[
v_E = 1 - \frac{\hat{E}_{M, p}}{\hat{E}_{M, p}^\text{max}} \tag{50}\]
which is derived in a manner analogous to the derivation of \(v_M, v_F,\) and \(v_R\).
The previous process equations describe in essence two pathways: the synthesis of biomass, $B$, and key resource, $\hat{S}_p$. These pathways are described symbolically as

$$S_L + B \rightarrow (1 + Y_{BL})B$$  \hspace{1cm} (51)

$$S_L + B \rightarrow \hat{S}_P + \hat{S}_X + B$$  \hspace{1cm} (52)

Both pathways require $S_L$ as a carbon and energy source as well as compete for limited cellular resources. These resources therefore must be allocated between the two available pathways towards the synthesis of enzymes present within the two pathways. With this in mind, the rate expressions for the synthesis of growth and maintenance enzymes must be further modified to account for this resource allocation as

$$r_{E,i}u_{C,i}u_{C,i} ; \quad 0 \leq u_{C,i} \leq 1 \quad \text{and} \quad j = gl, ga$$  \hspace{1cm} (53)

and

$$r_{E,P} + r_{E,P}v_E + r_{E,P}v_E u_{P,i} ; \quad 0 \leq u_{P,i} \leq 1$$  \hspace{1cm} (54)

where $u_{C,i}$ and $u_{P,i}$ are cybernetic variables which control the resource allocation between the biomass and resource associated pathways, respectively. Since transport and hydrolysis key enzymes are required for both pathways, the modification of their synthesis rate to account for the additional resource allocation is given as

$$r_{E,i}(u_{C,i} + u_{P,i}) \quad \text{where} \quad u_{C,i} + u_{P,i} = 1$$  \hspace{1cm} (55)

Equation (55) states that regardless of the state of the biotic phase the transport and hydrolysis key enzymes are required for both pathways identified in eqs. (51) and (52).

The expressions for $u_{C,i}$ and $u_{P,i}$ are arrived at by assuming some measure of the returns the microorganism may receive by investing key resources in the two pathways. With regard to biomass synthesis, the return is obviously the total rate of biomass production; however, the measure of returns from investment for resource synthesis is not so apparent. Inspection of the rate expression for $\hat{S}_p$ synthesis implies that the rate of $\hat{S}_p$ synthesis is not an adequate measure of the need for resource allocation towards resource enzyme synthesis. Since at low $\hat{S}_p$ the rate of resource synthesis will be low while the need for $\hat{S}_p$ is actually quite high. However, the expression for $u_{P,i}$ should reflect the need for $\hat{S}_p$ as well as some measure of the microorganism's ability to synthesize $\hat{S}_p$. The expressions for $u_{C,i}$ and $u_{P,i}$ are therefore proposed as

$$u_{C,i} = \frac{\sum r_{G,i}v_{G,i}/Y_{G,i}}{\sum r_{G,i}v_{G,i}/Y_{G,i} + v_P\left(\sum r_{X,i}u_{X,i}v_{X,i} + r_{TL,i}v_{TL,i}\right)/c}$$

and $u_{P,i} = 1 - u_{C,i}$ with $k = A, S$ and $j = gl, ga$. The measure of the returns from $\hat{S}_p$ synthesis is expressed as the combination of $v_P$, which reflects the need for $\hat{S}_p$, and the total substrate uptake rate, which reflects the microorganism's ability to produce $\hat{S}_p$ (i.e., carbon availability). The biomass synthesis rate is converted to a substrate consumption rate for growth by the appropriate cell yield coefficients in order to compare the two processes, growth and transport, on an equivalent basis. All of the cybernetic variables discussed within this section are summarized in Table V.

### Mass Balances and Parameter Estimation

By combining the kinetic rate expressions and the cybernetic variables complete material balances can be derived.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Equations</th>
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<tbody>
<tr>
<td>$u_{C,i}$</td>
<td>Allocates resources for active transport of $S_L$</td>
<td>29, 30, 33, 56, 57, 65</td>
</tr>
<tr>
<td>$u_{X,i}$</td>
<td>Allocates resources for slip transport of $S_L$</td>
<td>29, 30, 33, 56, 57</td>
</tr>
<tr>
<td>$v_{E,i}$</td>
<td>Activates active transport of $S_L$</td>
<td>31, 32, 56, 57, 65</td>
</tr>
<tr>
<td>$v_{M,i}$</td>
<td>Activates transport supported by $\hat{S}_P$ or $\hat{S}_G$</td>
<td>34, 35, 56, 59, 66</td>
</tr>
<tr>
<td>$v_{G,i}$</td>
<td>Allocates resources for growth supported by $\hat{S}_G$ or $\hat{S}_G$</td>
<td>36, 37, 53, 63</td>
</tr>
<tr>
<td>$v_{M}$</td>
<td>Activates nonspecific maintenance processes</td>
<td>38, 39, 59</td>
</tr>
<tr>
<td>$v_P$</td>
<td>Activates synthesis of $\hat{S}_P$</td>
<td>40-44, 56, 59, 60, 61, 65</td>
</tr>
<tr>
<td>$v_M$</td>
<td>Activates synthesis of $\hat{S}_M$</td>
<td>44-47, 59, 65</td>
</tr>
<tr>
<td>$v_G$</td>
<td>Activates synthesis of $\hat{S}_G$</td>
<td>48-50, 64</td>
</tr>
<tr>
<td>$u_{C,i}$</td>
<td>Allocates resources for biomass synthesis</td>
<td>53, 55, 56, 60, 62, 63</td>
</tr>
<tr>
<td>$u_{P,i}$</td>
<td>Allocates resources for $\hat{S}_p$ synthesis</td>
<td>54, 55, 60, 62, 64</td>
</tr>
</tbody>
</table>
written in rate form as

$$\frac{ds_i}{dt} = - \sum_k (r_{k,i} \mu_{k,i} v_{k,i}) - r_{T,i} v_{T,i} \quad k = A, S$$  \hspace{1cm} (57)

$$\frac{ds_i}{dt} = - \frac{1}{cv} \frac{ds_i}{dt} - r_{H,i} - \mu s_i \quad \hspace{1cm} (58)$$

$$\frac{d\hat{s}_i}{dt} = r_{H,i} y_{p,i} - \frac{(r_{G,i})}{y_{G,i}} v_{G,i} - r_{M} v_{M}$$

$$- (r_{P,i} + Y_{PT,X,i} v_{X}) v_{P} - \mu \hat{s}_i \quad j = gl, ga \hspace{1cm} (59)$$

$$\frac{d\hat{G}_{G,i}}{dt} = r_{E,i} (u_{C,i} + u_{P,i}) - r_{T,G} v_{P} - \hat{e}_{T,i} (\mu + \beta_{T,i})$$

$$\frac{d\hat{G}_{M,i}}{dt} = r_{E,i} (u_{C,i} + u_{P,i}) - \hat{e}_{H,i} (\mu + \beta_{H,i})$$

$$\frac{d\hat{G}_{G,i}}{dt} = r_{E,i} (u_{C,i} + u_{P,i}) - \hat{e}_{K,i} (\mu + \beta_{K,i})$$

$$\frac{dG_{M,i}}{dt} = [r_{E,\mu} + (r_{E,\mu} + r_{E,p}) v_{E}] \mu_{\mu} - \hat{e}_{M,i} (\mu + \beta_{M,i})$$

$$\frac{d\hat{s}_P}{dt} = \sum_k (r_{R,k} - Y_{PT,X,k} v_{X}) v_{P} - \frac{(Y_{PT})}{y_{G,k}} r_{TA,i} u_{TA,i} v_{TA,i}$$

$$- (r_{P,i} + Y_{PT,X,i} v_{X}) v_{P} - \mu \hat{s}_P \quad k = gl, ga \hspace{1cm} (65)$$

$$\frac{dc}{dt} = \sum_k r_{G,\nu} v_{G,k} \quad k = gl, ga \hspace{1cm} (66)$$

Enzyme degradation is assumed to be a first-order process\(^{13}\) and equivalent for all key enzymes with a rate constant \(\beta_{i,j}\). The degradation of \(\tilde{s}_P\) is assumed not to be significant. The enzyme synthesis rate constant, \(\alpha_{E,i,j}\), is also assumed to be equivalent for all key enzymes,\(^{13}\) no preferential synthesis is incorporated by arbitrarily increasing the induced rate of enzyme synthesis. With regard to the saturation constants for enzyme synthesis \(K_{E,i} = K_{H,i}, K_{G,i} = K_{G,gl}, K_{E,gl} = K_{G,gl}\), and \(K_{E,p} = \hat{s}_{P}\). Since all biotic phase variables are subject to dilution due to expansion of the biotic phase during growth, their dilution is accounted for by the terms \(\mu s_i\) and \(\mu \hat{s}_i\), in agreement with Fredrickson.\(^{5}\) The calculation of the process rate constants \(\mu_{i,j}\) is given by the relations

$$\mu_{i,j}^{max} = \mu_{i,j} \hat{e}_{i,j}^{max} v$$  \hspace{1cm} (67)

and

$$\mu_{i,j}^{max} = \mu_{i,j} \hat{e}_{i,j}^{max} \hat{s}_P v$$  \hspace{1cm} (68)$$

when the resource \(\hat{s}_P\) is required for maintaining the activity of the key enzyme. The specific cell volume, \(v\), is determined from published cellular composition data.\(^{10}\) The value of \(\hat{s}^{*}\) is set at a level similar to a maximum key enzyme concentration. Maximum key enzyme levels \(\hat{s}^{max}{_T}^{max}{_H}, \hat{s}^{max}{_H}, \) and \(\hat{s}^{max}{_G}\) are determined from pseudo-steady-state simulations which assume that \(\hat{s}_{P} = \hat{s}^{*}\) when \(s_{P}\) is present at a saturating concentration. The maximum value of resource synthesis enzyme, \(\hat{s}^{max}{_P}\) is determined from eq. (64).

Estimates for \(\mu_{i,j}^{max}\) and \(\mu_{i,j}^{max}\) are taken from the literature.\(^{6,7}\) While the existence of a slip transport process is suggested from data\(^{4}\) process kinetics of such a process have not been estimated experimentally; however, since the slip transport is assumed to be a high-capacity system, \(\mu_{i,j}^{max}\) is assumed to be greater than \(\mu_{i,j}^{max}\) but less than \(\mu_{i,j}^{max}\). Lactose hydrolysis is not assumed to be rate limiting; therefore \(\mu_{i,j}^{max}\) is set at a value larger than both the maximum transport rate and the maximum growth rate. Saturation constants for lactose transport \((K_{TA,i}, K_{TS,i}, K_{TS,i},\) and \(K_{TL,i}\)) and lactose hydrolysis \((K_{H,i})\) are estimated from literature values.\(^{8,9}\)

The parameters \(Y_{p,i}\) are determined from hydrolysis stoichiometry which assumes that equivalent amounts of glucose and galactose are produced upon lactose hydrolysis. The parameters \(\mu_{i,j}^{max}, Y_{p,i},\) and \(K_{G,i}\) are determined directly from single-substrate glucose and galactose batch experiments.\(^{13,20}\) The maintenance parameters \(\mu_{M,i}^{max}\) are taken from Turner et al.\(^{22}\) Saturation constants for maintenance, \(K_{M,i}\), and resource synthesis, \(K_{E,i}\), are assumed to be one and two orders of magnitude smaller, respectively, than \(K_{G,i}\) while the saturation constant for by-product synthesis, \(K_{X,i}\), is assumed to be one order of magnitude larger than \(K_{G,i}\). These assumptions identify maintenance processes as having the highest carbon source affinity and by-product processes as having the lowest carbon source affinity.

The inhibition constant, \(K_{I,i}\), which moderates the biotic phase carbon source concentrations, is arbitrarily set at a value that reflects a high concentration that may be observed within the abiotic environment. The stoichiometric coefficients \(Y_{p,i}\) and \(Y_{p,i}\) are assumed to be approximately one and much less than one, respectively. The remaining parameters \(\mu_{i,j}^{max}, \mu_{i,j}^{max}, \mu_{i,j}^{max}, \mu_{i,j}^{max}, K_{TA,i}, Y_{p,i}, K_{TR,i},\) and \(K_{TR,i}\), which are associated with resource metabolism, are determined from some degree of fitting to experimental results with some being more important than others in determining observed dynamics. A complete listing of parameter values is given in Table I.

MATERIALS AND METHODS

The microorganism, Klebsiella oxytoca, obtained from the U.S. Department of Agriculture (Peoria, IL), was used in all experiments. The growth medium, inoculum preparation, fermentor description, and methods of analysis are described elsewhere.\(^{21}\)
RESULTS AND DISCUSSION

In a previous article, the experimental results for aerobic growth on lactose in batch culture in addition to two-substrate batch experiments involving glucose or galactose and lactose were presented. In this work we now compare simulation results of the model equations developed in the previous section to the experimental results presented in the earlier paper for growth on lactose as the only limiting nutrient. Straight et al. reported inhibitory growth behavior at initial lactose concentrations of 1 g/L or less. Such inhibition was apparent in the form of intermittent or oscillatory growth during long lag periods. At higher lactose concentrations, i.e., above 2 g/L, the lag phase was short or nonexistent and the specific growth rate attained a value of 0.76 h\(^{-1}\) at the highest initial lactose concentration of 5 g/L.

Figure 2 presents an overview of the growth behavior observed over the entire range of initial lactose concentrations in addition to the respective model simulations. Considering the range of dynamic behavior observed, the model describes quite well not only the intermittent and rapid growth behavior but the transition region as well.

During growth on lactose, the model simulations indicate that the identity of the limiting enzyme varies as the lactose concentration varies from low to high. In order to maximize cellular growth at reduced lactose concentrations, the preferred lactose uptake mechanism chosen by the model is the active transport process \((u_{TA,1} \rightarrow 1)\). Furthermore, comparison of the parameters for transport with those for growth in Table I indicates that the rate-limiting process in the model at low lactose concentrations is lactose transport. When resource levels are high, a transport limitation results in a reduced availability of biotic phase glucose and galactose since they are rapidly converted to cell mass by the growth process once lactose is hydrolyzed. Because resource is consumed by the active transport process, a resource limitation occurs and the active transport enzyme is converted to the leak or nonactive form to prevent further resource reduction; the growth rate is reduced; and maintenance requirements take priority over those of growth \((v_p \rightarrow 0)\). Glucose and galactose accumulate within the biotic phase and support the necessary increase in resource synthesis and maintenance functions. When the resource level has been reestablished by its maintenance enzyme \((v_p \rightarrow 0)\), cellular growth resumes and active transport remains the preferred lactose uptake mechanism. This preference results in consumption of key resource again and the cycle of resource consumption/maintenance repeats resulting in oscillatory growth behavior until the enzyme system responsible for maintaining the resource level can support the rate of resource consumption. At that time, cellular growth should become nonintermittent in nature.

The intermittent behavior in cell density previously shown in Figure 2 is evident in Figures 3 and 4, which compare experimental and model results and clearly show the cell density exhibiting periods of acceleration and deceleration. Figure 5 shows the oscillatory nature of resource and glucose profiles predicted by the model during the period of intermittent growth on 0.54 g/L lactose. At approximately 15 h the model indicates that the rate of resource synthesis begins to adjust to the

![Figure 2](image-url)  
**Figure 2.** Cell density profiles versus model simulations (-). Initial lactose concentrations are: 5 g/L (○), 2.5 g/L (□), 1.1 g/L (△), 0.54 g/L (○). Cell density was determined from optical density measurements and converted to units of dry weight (ref. 21).

![Figure 3](image-url)  
**Figure 3.** Intermittent growth behavior during first 8 h of batch growth on 0.54 g/L lactose, for cell density (○) versus model simulation (-).
from the reallocation of resources towards the slip transport process no intermittent growth behavior is described by the model. However, inhibition in the form of a reduced resource level, which results in a reduced growth rate, still occurs.

At an initial lactose concentration of 2.5 g/L (Fig. 2), no intermittent growth behavior is observed. Yet both the experimental and the model results indicate the specific growth rate attains a value of only 0.44 h⁻¹, which is significantly reduced from the theoretical maximum value of 1.08 h⁻¹, i.e., the maximum specific growth rate on glucose or galactose in batch culture.¹³,²⁰ By increasing the abiotic concentration lactose to 5 g/L, the model predicts increased levels of biotic phase glucose, galactose, and resource as well as a specific growth rate of 0.67 h⁻¹ as compared with an experimental value of 0.76 h⁻¹ (Fig. 2). The significance of the multiple mechanisms for synthesizing \( \tilde{E}_{M,R} \), eqs. (24) and (25), is readily apparent.

In Figure 6, as the lactose concentration decreases from 5 to 2 g/L at time = 5.5 h the resource concentration begins to decrease greatly, however, since the biotic phase has prepared for this environment a high level of resource enzyme is available when the active transport process becomes the preferred lactose uptake mechanism; the reduction in resource is buffered and the growth rate is reduced but no intermittent or oscillatory growth occurs. Previously, Straight et al.¹¹ interpreted this reduction in specific growth rate as a possible indication of diauxic growth; however, the model indicates that no significant preference for glucose utilization over galactose utilization occurs and the reduction in specific growth rate is due to a reduction in the key energy resource. Both \( v_{G,Gl} \) and \( v_{G,Gl} \) are high and close to or equal to 1 while \( u_{G,Gl} \) and \( u_{G,Gl} \) are approximately equal and close to 0.5 during growth on lactose.

**Figure 4.** Intermittent growth behavior during first 7 h of batch growth on 1.1 g/L lactose, for cell density (○) versus model simulation (—).

**Figure 5.** Predicted oscillatory behavior in biotic phase glucose (—) and resource (—) concentration profiles during simulated growth on 0.54 g/L lactose.

**Figure 6.** Effect of elevated levels of the resource maintenance enzyme (—) on the resource level (—) as the preferred lactose transport mechanism switches from the slip to the active process during simulated growth on 5 g/L lactose.
From the work of Turner et al.\textsuperscript{22,23} the significance of maintenance metabolism at low specific growth rates suggests that the cell yield should decrease monotonically as the initial concentration of lactose decreases since the specific growth rate decreases as the concentration of lactose decreases; however, experimental results indicate a minimum cell yield of 0.35 g dry wt/g lactose at an initial lactose concentration of 2 g/L. As is shown in Figure 7, the model also predicts a minimum cell yield of approximately 0.415 at or near an initial lactose concentration of 2 g/L. Both the experimental results and the model predictions agree and exhibit cell yields approaching 0.45 g dry wt/g lactose as the initial lactose concentration decreases to 0.54 g/L or increases to 5.0 g/L. The prediction of a minimum cell yield is due to the optimal nature of carbon utilization within the model.

In accord with Postulate 6, unnecessary carbon utilization is reduced as the specific growth rate decreases; therefore, at low lactose concentrations where the resource consumption rate is high and the growth rate is very low, carbon utilization is reduced and conserved for maintenance and growth processes; no by-product synthesis occurs ($\nu_X \rightarrow 0$). At high concentrations of lactose, the specific growth rate increases and carbon source is directed toward growth maximization rather than maintenance processes or by-product synthesis in agreement with Postulate 1 ($\nu_M \rightarrow 0$, $\nu_P \rightarrow 0$ therefore $\nu_{xP} \rightarrow 0$). At intermediate lactose concentrations, biotic phase carbon source is available in relative abundance yet not at a level that maximizes the specific growth rate; therefore, a reduced efficiency of carbon utilization in the form of by-product, $\delta_X$, synthesis is permitted and a minimum cell yield is predicted.

The model indicates that the intermittent or oscillatory phenomenon observed during growth on lactose is attributed to the coupling between the key enzyme for active transport and the key energy resource. Since the same key energy resource is also assumed to be required for enzyme synthesis, enzyme levels will oscillate as well during periods of intermittent growth. One example of this behavior is presented in Figure 8, which shows the model prediction of the concentration profile for the lactose hydrolysis key enzyme, $\hat{e}_{h,l}$, during growth on lactose at 0.54 g/L. Several investigators in the literature\textsuperscript{14,16} have reported observing oscillatory behavior in $\beta$-galactosidase activity. The oscillatory phenomenon was attributed either to the interaction between induction by allolactose and repression by glucose at the lac operon\textsuperscript{15} or to the effects of catabolite inhibition exerted by glucose.\textsuperscript{14}

The conspicuously complex nature of the model here with approximately 40 constants may present a perplexing contrast to the argument of simplicity advanced in defense of cybernetic models in the past. It is however important to recognize that the constants are attached to the \textit{kinetic} features of the model which include a dozen components involved in about fifteen or more reaction and transport processes. The regulatory processes, which in actuality must feature complex interactions between several components and many reactions, are simply described by cybernetic variables that contain \textit{no additional constants}. The kinetic complexity arises from the incorporation of important scientific findings about biochemical transport but yet notably within the framework of “lumped species” and “lumped interactions” implicit in the original model of Monod. The price for the simplicity of Monod's model is of course the limitation of its predictions. It will indeed be desirable to measure at least some of the proposed components in the present model beyond those reported here, in particular the enzyme levels. Although such measurements are being investigated currently, they are not always feasible. It seems reasonable to us that phenomena arising from the role of key components in the cell, whether or not they are measurable, cannot be

![Figure 7](image-url)  
**Figure 7.** Comparison between experimental cell yield values (○) and model cell yield predictions (—).

![Figure 8](image-url)  
**Figure 8.** Predicted model concentration profile for the lactose hydrolysis key enzyme during simulated growth on 0.54 g/L lactose.
CONCLUSIONS

In this article, we have addressed the issue of nutrient transport and its effect on the biotic phase. Bacterial growth on lactose in our laboratory has indicated not only the need to include transport processes but alternative transport processes, high- and low-affinity systems, which compete for cellular resources. Within the model, regulation of lactose transport is described from a cybernetic perspective using the same concepts and formulations proposed by Kompala et al. to describe diauxic growth behavior. In the absence of resource consumption by the active transport process, i.e., resource remains at its optimal value, the growth and maintenance aspects of the model reduce to a system identical to one proposed by combining the results of Kompala et al. and Turner et al., thus retaining the predictive capabilities of these earlier models.

Growth inhibition which has been previously attributed to the presence of lactose is accounted for by including cellular energetics in the form of a single lumped key resource that is coupled to the active transport process. Because of this interaction a switching between two cellular objectives, growth maximization and cellular vitality, is described by the model when the active transport process is the preferred lactose uptake mechanism. The result of this switching is an intermittent or oscillatory growth behavior with a frequency that agrees well with experimental results. Identification of vitality as a cellular objective does not conflict with Postulate 1 as previously noted. When active transport is the less preferred uptake mechanism the model describes inhibited growth, which is nonoscillatory in nature, in agreement with experimental results.

Finally, the identification of two distinct pathways, eqs. (51) and (52), within the model indicates that processes which occur in series or parallel compete for limited cellular resources. This viewpoint is in contrast to previous investigators who only considered parallel processes as competing processes. Recent work by us has shown that the regulation of enzyme systems within metabolic pathways that are linear, branched, or cyclic in nature, i.e., composed of both series and parallel processes, may be described from a cybernetic perspective. A subsequent article will address these issues in detail and demonstrate that feedback regulation of biosynthetic processes can also be simply described according to cybernetic principles.

References


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