The internal regulatory processes, which underlie a variety of behavior in microbial growth on multiple substrates, are viewed as a manifestation of an invariant strategy to optimize some goal of the cells. A goal-seeking or cybernetic model is proposed here, with the optimization based on a short-term perspective of response to the environment. The model parameters are determined from the growth data on single substrates. The model predicts the entire range of microbial growth behavior on multiple substrates from simultaneous utilization of all sugars to sequential utilization with pronounced diauxic lags. It is shown to predict the many variations of the diauxic phenomenon in different growth conditions. The transients in continuous culture growth on mixed substrates caused by varying the feed strategies are easily simulated by this model. The framework of this model can be applied to batch or continuous culture growth of many bacteria on different combinations of substrates.

INTRODUCTION

Microbial growth is an efficiently regulated system of thousands of chemical reactions. Some of these regulatory processes become readily apparent when the growth takes place in a multisubstrate environment. Microbial growth on multiple substrates exhibits a variety of behavior ranging from simultaneous utilization of all substrates to sequential utilization of substrates, resulting in multiexponential growth phases. An understanding of the internal regulatory processes causing such varied behavior is important in such processes as the fermentation of cellulose and hemicellulose hydrolysates, the activated sludge systems, and the biodegradation of pollutants.

The diauxic phenomenon, discovered by Monod, is a well-known example of sequential utilization of two carbon substrates, with an intermediate lag phase between the two exponential growth phases. Monod has classified sugars into two categories A and B for a few organisms on the basis that diauxic growth results from a mixture of sugars, one from A and the other from B with the A sugar as the preferred substrate. However, he points out that “. . . the difference in behavior between ‘A’ and ‘B’ substrates is of a quantitative rather than a qualitative nature.” When the inoculum is precultured on the less preferred substrate (maltose) and presented with the mixed substrates (glucose and maltose) the growth behavior is still diauxic. The preformed catabolic enzymes for maltose are inhibited and their synthesis is repressed by the presence of glucose. Only after glucose concentration falls to low levels, the induction and activation of maltose enzymes take place. When the initial substrate (glucose: xylose) concentrations are varied from a ratio of 1:1 to 1:1000, the diauxic lag gets progressively suppressed.

Since the pioneering work of Monod on the diauxie, many researchers have observed the same phenomenon in the growth of a variety of bacteria on multiple substrates. Due to the prevalence of these observations with glucose as the preferred substrate, this phenomenon was called the glucose effect. However, this term is not really appropriate as some cases have been found wherein glucose is the less preferred substrate. To mention a few, Pseudomonas aeruginosa prefers citrate over glucose; Arthrobacter crystallopoietes prefers succinate over glucose; and Propionibacterium shermanii prefers lactate over glucose. In all the observations of the diauxie including those mentioned above, it can be noticed that the first phase of growth is at a higher growth rate than the second growth phase. This clearly points out the inherent capability of the microorganisms to optimize their growth behavior.

The efficacy of the cellular regulatory processes in optimizing the growth on multiple substrates may be brought out by the observations of Lee and co-workers. When P. shermanii was grown in batch cultures with either lactate or glucose as the sole carbon source, the specific growth rate $\mu_{\text{max}}$ was found to be 0.142 h$^{-1}$ on lactate and 0.141 h$^{-1}$ on glucose. When P. shermanii was grown on a mixture of lactate and glucose, the utilization of the substrates was sequential with no intermediate lag. As might be predicted with an optimization objective, lactate was the preferred substrate and glucose remained unutilized until all of lactate was consumed.

Some previous modeling efforts on the microbial growth on multiple substrates have been limited to simple
unstructured models to explain these complex regulatory processes. Some other modeling efforts\textsuperscript{12-14} have included some detailed mechanisms of repression and induction based on the operon theory of Jacob and Monod.\textsuperscript{15} Many of these models require an \textit{a priori} specification of which of the substrates is the preferred. If, for example, glucose is not the preferred substrate, that fact should be given as an input to these models. The preferred substrate is then, assumed to have constitutive catabolic enzymes, unaffected by any regulatory action. Inclusion of the known details of some of the mechanisms involved complicates these models enormously. All the above models are developed to explain a particular set of experimental data and do not take note of the optimal nature of microbial growth on multiple substrates. Hence the predictive capability of such models remains within the bounds of the experiments on which they are based.

**CYBERNETIC MODELING**

As noted above, in the sequential growth on multiple substrates, the cells grow first on the fastest assimilated substrate in the medium. How the cells have acquired such a strong capability to control their regulatory processes to optimize their growth behavior may be as follows: Assume that in a multisubstrate environment, there exist cells with different strategies of responding to the environment. The cells that arbitrarily choose to grow first on the fastest substrate available proliferate much faster than the cells that respond differently. Very quickly all the cells that remain in that environment will be those that have responded in the most optimal manner. Hence, it is reasonable to postulate that over the many years of the evolutionary processes in environments with varying menus of substrates, microbes have acquired the capability to control their regulatory processes to optimize their growth pattern. This concept is not new to the biologists and it falls out as a corollary to the theory of natural selection. Dhurjati et al.\textsuperscript{16} have discussed this cybernetic viewpoint elaborately in the previous part of this article. The basic merit of the cybernetic approach is that it adopts a mathematically simple description of a complex organism but compensates for the over-simplification by assigning an optimal control motive to its response.

**MODEL WITH A LONG-TERM PERSPECTIVE**

Viewing the cell as an optimal control system raises some important issues, which have been discussed elaborately by Ramkrishna.\textsuperscript{17} One key issue is whether the cellular optimization is done over a finite period of time or at every instant. The former represents a long-term perspective and the latter a short-term perspective. Dhurjati et al.\textsuperscript{18} have applied the long-term perspective to model the diauxic growth of \textit{Aerobacter aerogenes} on glucose and xylose. They view the substrate consumption as catalyzed by a “key enzyme,” $E_i$, the enzyme catalyzing the slowest and rate-determining step in the pathway for the assimilation of $S_i$. The synthesis of the right set of key enzymes is controlled by a resource allocation policy $\{u_i\}$ which is chosen by the cellular regulatory processes to optimize the goal of maximizing the cell mass productivity. Employing Pontryagin’s maximum principle to find the optimal policy $u_i(t)$, they find it to be a bang–bang policy for their model equations. This bang–bang policy implies an exclusive production of $E_1$ for some time and an exclusive production of $E_2$ after $S_1$ falls to low levels. They have shown that with the constants obtained from the single-substrate growth experiments, the model is able to describe the diauxic satisfactorily. However, since this model predicts only a bang–bang policy, the simulation of simultaneous utilization becomes difficult, requiring frequent bang–bangs in the policy.

In the extension of this long-term perspective model to perturbed batch simulations, some difficulties arise. As the model determines the policy of substrate utilization for a finite period of time based on some prescribed environmental changes, it is inherently incapable of anticipating any perturbations from the prescription above. In continuous cultures, the concepts of initial and terminal time for the evaluation of policy have to be defined more appropriately. These conceptual difficulties with the long-term perspective model are overcome by the models with short-term perspectives of optimization.

**MODELS WITH A SHORT-TERM PERSPECTIVE**

We have investigated two different strategies of optimization with a short-term perspective, one based on instantaneous maximum biomass productivity and the other based on Herrnstein’s\textsuperscript{18} matching law. After a brief discussion of the model based on instantaneous maximization of biomass productivity, we will concentrate on the matching law model which forms the main thrust of our work.

Let the microbial growth on multiple substrates be simplistically represented by equation:

$$B + S_i \xrightarrow{E_i} (1 + Y_j)B + \cdots \quad (1)$$

The assimilation of the $i$th substrate ($S_i$) by the biomass ($B$) is assumed to be catalyzed by a key enzyme ($E_i$), representing the whole set of enzymes catalyzing the metabolic pathways of growth on $S_i$. The key enzyme required for the utilization of a given substrate must be synthesized before growth can occur on that substrate. The inductive synthesis of $E_i$ in the presence of $S_i$ may be simplistically represented as

$$B \xrightarrow{S_i} E_i + B' \quad (2)$$

where $B'$ is the biomass excluding the key enzyme $E_i$. All the enzymes are also constantly being degenerated to supply the amino acid pool for the synthesis of required enzymes.
Both the reactions (1) and (2) are gross over-simplifications of the actual processes involved, without the inclusion of the regulatory actions like the catabolite repression, feedback inhibition, etc. The rate equation for the biomass production in a through reaction (1) may be written as a modified form of Monod's equation:

\[ r_{b,i} = \mu_i e_i s_i c / (K_i + s_i) \]  

where \( c \) is the biomass concentration; \( s_i \) is the concentration of the \( i \)th substrate; and \( e_i \) is the specific level of the key enzyme \( E_i \). During balanced growth, \( e_i \) remains at a constant level, \( e_{i,\text{max}} \). Hence \( \mu_i e_{i,\text{max}} \) can be combined to give \( \mu_{i,\text{max}} \), the specific growth rate in the Monod form for growth rate during the balanced growth. Before the growth reaches the balanced exponential phase, i.e., during the initial lag phase, the key enzymes \( E_i \) are being synthesized. The specific enzyme level starts from a low initial value \( e_{i,0} \) and attains its maximum \( e_{i,\text{max}} \) during this lag phase. The rate equation for enzyme synthesis through reaction (2) may be written as

\[ r_{E_i} = \alpha_i s_i c / (K_i + s_i) \]  

The form of this rate equation is obtained from the measurement of the relative quantities of DNA, rRNA, tRNA, Protein, mRNA, and tRNA at different growth rates by Maaloe and Kjeldgaard\(^9-21\) and discussed elaborately elsewhere.\(^{22}\)

During microbial growth on multiple substrates, the cellular regulatory processes of repression/induction and inhibition/activation affect the rate equations (4) and (3), respectively. The central idea of our modeling efforts is that these regulatory processes may be considered as the outcome of some strategy of optimization. We note that reactions (1) and (2) cannot proceed without some critical cellular resources (for example, ATP to catalyze key endergonic intermediate steps, transcription of different intermediate steps, etc; more discussion on this will be presented in a later section) which must be optimally allocated for growth on different substrate systems. Then the actual rate of synthesis of enzyme \( E_i \) may be written as

\[ r_{E_i} u_i (0 \leq u_i \leq 1, \sum u_i = 1) \]  

where \( u_i \) is the fractional allocation of a critical resource for the synthesis of \( E_i \). Small values of \( u_i \) will repress the rate of enzyme synthesis and high values of \( u_i \) will induce the enzyme synthesis. The model must choose the values of \( u_i \), henceforth referred to as cybernetic variables to maximize some cellular objective. The first model presented below considers the instantaneous maximization of biomass productivity as a possible cellular objective.

**Instantaneous Maximum Biomass Productivity Model**

This model proposes that, in a multiple substrate environment, at any instant \( t \), the organism chooses to synthesize the enzyme for the substrate which accelerates the biomass growth rate the most at that instant. In other words, the fractional allocation \( u_i \) at any instant is calculated by maximizing \( dE/c/dt \) at that instant. From equations (3), (4), and (5), it can be shown that

\[ u_j = 1 \text{ if } \frac{\mu_j e_{j,\text{max}}}{(K_j + s_j)^2} \text{ is the max of } \left\{ \frac{\mu_i e_{i,\text{max}}}{(K_i + s_i)^2} \right\} \]  

\[ u_j = 0 \text{ otherwise} \]

This result, obtained from simple differentiation of eq. (3) in combination with eqs. (4) and (5), is analogous to the bang-bang policy of the long-term perspective model, obtained from the numerical computations involving the forward integration of the state equations, backward integration of the adjoint equations, and iterations of the above to update the initial guesses of the policy \( u \), until the optimal policy is obtained. However, the instantaneous optimization strategy overcomes one important drawback of the previous model, which is the anticipation of the future perturbations, resulting in a non-optimal situation if the actual perturbations are different from the expected.

With the bang-bang allocation of resources, the present model is able to describe the diauxic batch growth curve. Suppose the medium contains two substrates \( S_1 \) and \( S_2 \). Assume that no \( E_2 \) is present at the beginning of growth in batch culture. Let \( \mu_1 \) be greater than \( \mu_2 \) and \( \alpha_1 \) be greater than \( \alpha_2 \). Then clearly the model will predict the synthesis of \( E_1 \) with the repression of \( E_2 \) synthesis and, consequently, the utilization of only \( S_1 \) at the exclusion of \( S_2 \) as long as the \( S_1 \) concentration is sufficiently larger than \( K_1 \). After \( S_1 \) concentration drops sufficiently below \( K_1 \), the switch in the policy occurs resulting in the synthesis of \( E_2 \) and the consumption of \( S_2 \) in the second exponential growth phase.

This model has some limitations. As the model predicts an all-or-nothing allocation of resources to the enzyme synthesis alternatives, the simulation of simultaneous utilization of multiple substrates becomes constrained to frequent changes in the resource allocation policy. Experimental observations of these frequent switches in policy (provided there is a quick experimental way to recognize the switch) are needed to support this optimization strategy.

Another difficulty with the model is its disregard of the control of the activity of the existing enzymes. In the batch growth on two substrates, suppose as a result of preculturing on \( S_2 \), the initial level of enzyme \( E_2 \) is high. Then even if \( u_2 \) is zero, the growth on \( S_2 \) will proceed unhindered by the presence of \( S_1 \). This is in conflict with the observations\(^5\) of the diauxie even after the preculturing of the inoculum on \( S_2 \). In other words, this model lacks the manifestation of the important regulatory processes like inhibition and activation of the existing enzymes.

The next model incorporates these regulatory actions...
as well and proposes Herrnstein’s matching law as the cellular strategy of optimization.

The Matching Law Model

The matching law is analogous to the law of equal marginal utilities per dollar in microeconomic theory. Suppose a fixed amount of resource \( r \) is to be allocated among \( n \) alternatives. Let \( r_i \) be the resource allocated to the \( i \)th alternative and \( p_i \) be the returns obtained from that alternative. The law of diminishing marginal utility implies that for each \( i \), \( p_i'(r_i) \) (derivative of \( p_i(r_i) \) w.r.t. \( r_i \)) is non-negative and \( p_i''(r_i) \) (the second derivative of \( p_i(r_i) \) w.r.t. \( r_i \)) is nonpositive. The maximum for the total returns \( \sum_i p_i(r_i) \), subject to the constraint \( \sum_i r_i = r \), can be shown to be obtained when \( r_i \)'s are allocated such that

\[
p_i(r_i) = p_2(r_2) = \cdots = p_n(r_n) \quad (7)
\]

Now if additional resources \( 'd'r \) are available for further allocation to the \( n \) alternatives, then an optimal system would distribute this \( 'd'r \) into \( \{d r_i\} \) to obtain \( \{d p_i\} \) such that

\[
\frac{dp_1}{dr_1} = \frac{dp_2}{dr_2} = \cdots = \frac{dp_i}{dr_i} = \cdots = \frac{dp_n}{dr_n} \quad (8)
\]

or

\[
u_i = \frac{dr_i}{\sum_j dr_j} = \frac{dp_i}{\sum_j dp_j} \quad (9)
\]

which is the matching law statement that the fractional allocation must match the fractional return.

To apply this matching law to the modeling of microbial growth, let us first consider some of the resources and returns optimized in the growth processes. As we have noted in the introduction, the cell growth rate is an important objective that is optimized. The resources invested by the cells are in terms of the syntheses of the enzymes for the different substrates present in the environment. The key mechanism for the control of the allocation of these resources is at the level of transcription of the DNA by the RNA polymerase to produce the required set of mRNAs for the synthesis of proteins. The transcription is controlled by highly specific effectors or by less specific catabolite effectors. If we focus on a particular operon, we note that at least three factors are involved in determining the probability that the next act of transcription in the cell takes place in this rather than any other segment of the genome. First, the activity of the operator which we assume depends on the intracellular concentration of the operator-specific and the less specific catabolite effectors; second, the affinity of the promoter site for the transcribing RNA-polymerase enzyme; third, the average gene dose. In a balanced growth situation, each protein, and indeed every cell component, increases its mass at the growth rate \( \mu \) of the culture. The limited number of the polymerase enzyme molecules have to circulate transcribing the genes for each cellular component. The major portion of these genes are for the biosynthetic enzymes, structural proteins, the \( r \)-proteins, etc.; the genes for the catabolic key enzymes form one small part. This major portion of the transcription activity is essential for growth and the time the RNA polymerase spends transcribing these portions remains unaltered by presence or absence of any particular substrate \( S_i \). What is altered of course is the transcription of the genes or the groups of genes corresponding to the key enzymes \( E_i \). The mechanisms—how these alterations are brought about by the various highly specific effectors or the less specific catabolite effectors—are still to be understood precisely. The net result of all the control mechanisms acting together is the distribution of the time of transcription for the catabolic key enzymes, the total time for which is limited by the other required activities of the polymerase.

We propose that this critical resource is optimally allocated according to the matching law strategy to maximize the cellular growth rate at each instant. Equation (3) gives the instantaneous returns in the form of growth rate on each of the substrates. Equations (4) and (5) show how the allocation of the resource (the time of transcription) control the enzyme syntheses. The fractional allocation of resource \( u_i \) can be determined from the matching law equation as:

\[
u_i = \frac{r_{B,i}}{\sum_j r_{B,j}} \quad (10)
\]

Physically, if \( u_i = 0 \), the polymerase does not transcribe the gene for \( E_i \) at all, i.e., that enzyme is fully repressed. If \( u_i \) is close to one, the polymerase spends most of its available time transcribing for the enzyme \( E_i \), i.e., that enzyme is induced and the other enzymes \( E_k (k \neq i) \) are repressed.

The other important cellular regulatory action of inhibition and activation of the already existing enzymes may be introduced into the model equations as follows. Equation (3) represents rate of biomass production through the consumption of substrate \( S_i \), assuming all the key enzymes \( E_i \)’s are fully active. If \( v_i \) represents the fractional allocation of another critical resource required for utilization of the \( i \)th substrate, the actual rate of biomass production may be written as

\[
r_{B,i} v_i \quad (0 \leq v_i \leq 1, \quad \sum_i v_i = 1) \quad (11)
\]

The \( v_i \)'s are the cybernetic variables which incorporate the regulatory actions of inhibition and activation of the existing enzymes. If \( v_i \) is close to zero, then even if \( S_i \) is present in the environment and \( E_i \) is present in the protoplasm, reaction (11) is inhibited. If \( v_i \) is close to one, then the enzyme is activated and reaction proceeds.

The critical resource may be in terms of the ATP needed to catalyze some key thermodynamically unfavoried intermediate steps in the catabolic pathway of each substrate. ATP is produced in the catabolic mechanisms of substrate utilization and it is required in the anabolic synthesis of macromolecules during cell growth.
The allocation of this key resource to the endergonic intermediate steps is controlled through the allosteric regulation of the catalytic enzymes. A plausible objective for this control is the optimization of ATP production rate so that the cells have a high degree of flexibility in their anaerobic reactions. This optimization becomes more obvious in the case of yeast growth.

The metabolism of glucose by yeast takes place in two alternative pathways, namely, the respiratory and the fermentative pathways. During batch growth on glucose, the cells grow only on the fermentative pathway, due to the primary optimization of the growth rate at the transcription level. However, during the continuous culture growth at low dilution rates, when either pathway can sustain the growth rate at levels determined by the dilution rate, the cells choose the respiratory pathway to optimize the energy production capability.

In bacterial systems, such a clear indication of energy production optimization is not readily apparent but it is nevertheless present. For example, whenever the energy production through the tricarboxylic acid cycle gets backed up due to the slow oxidation of NADH through the electron transport system, the substrate level phosphorylation get switched on. This and other switches in the pathways of energy production are optimally controlled by complex allosteric feedback regulation of the existing enzymes.

With this physical basis, let us now incorporate in our model, a second matching law strategy to optimize the fractional allocation of ATP to the different substrate utilization pathways. If \( v_i r_{B,i} \) is the energy production rate from each substrate utilization pathway, then the optimal allocation of ATP to each pathway is given by the matching law as:

\[
v_i = \frac{v_i r_{B,i}}{\sum_j v_j r_{B,j}}
\]

The model development is now complete and the equations are given below:

\[
\frac{dE_i}{dt} = \frac{\alpha_i s_i}{K_i + s_i} u_i - \beta_i e_i - e_i \frac{d\text{inc}}{dt} - \frac{dE_i}{dt}
\]

\[
\frac{dc}{dt} = \sum_i Y_i \left( -\frac{ds_i}{dt} \right) ; \quad u_i = \frac{r_{B,i}}{\sum_j r_{B,j}}
\]

\[
\frac{ds_i}{dt} = -\frac{r_{B,i}}{v_i} v_i ; \quad v_i = \frac{v_i r_{B,i}}{\sum_j v_j r_{B,j}}
\]

The second term of eq. (13) represents a first-order degenerative loss of the active enzyme \( E_i \). The third term represents the dilution of specific enzyme level due to cell growth. The other equations are readily put together from the previous discussions of the model. The cybernetic variables \( u_i \) and \( v_i \) represents the cellular regulatory processes of induction/repression and inhibition/activation, respectively.

Equations (13)-(15) are easily integrated with appropriate parameter values and initial conditions to simulate the entire range of microbial growth behavior on multiple substrates. Figures 1 and 2 show the simulation results for a typical diauxie with all the experimentally observed phases, namely, the initial lag, the first exponential growth, the diauxic lag, and the second exponential growth phases. Let us examine in detail how this model describes the sequential utilization of two substrates [say glucose \( (S_1) \) and xylose \( (S_2) \)] in Figures 1 and 2. The initial values of \( c, s_1 \), and \( s_2 \) are easily measured for each experiment. The maximum specific enzyme levels, \( e_{i,max} \)
can be fixed appropriately by the parameter values of \( \alpha_i \) and \( \beta_i \). The growth rate parameters \( \mu_i \)'s are then determined as \( \mu_i e_{i,max} \) equals the maximum specific growth, \( \mu_{max} \), which is measured in single-substrate growth experiments. The relative enzyme level \( e_{1,0}/e_{1,max} \) can be estimated from the past history of the culture. The usual preculturing of the inoculum in glucose causes \( e_{1,0} \) to be greater than \( e_{2,0} \). From the single-substrate growth experiments, we determine that \( \mu_1 \) is greater than \( \mu_2 \). Therefore \( r_{B,1} \) is greater than \( r_{B,2} \), causing \( u_1 \) and \( v_1 \) to be higher than \( u_2 \) and \( v_2 \). From eq. (13), if \( u_1 \) has a high value, the synthesis of \( E_1 \) proceeds at a high rate, i.e., \( E_1 \) is induced. Since \( u_2 \) has a low value, the synthesis of \( E_2 \) is curtailed even though there is a large amount of \( S_2 \) present in the medium, i.e., \( E_2 \) is repressed. From eq. (15), if \( v_2 \) has a low value, the \( S_2 \) consumption is reduced even if there is some \( E_2 \) already present, i.e., \( E_2 \) is inhibited. Since \( v_1 \) has a high value, \( E_1 \) is activated for the consumption of \( S_1 \). Thus, the induction and activation of \( E_1 \) and repression and inhibition of \( E_2 \) come into effect, thereby increasing the asymmetry of the consumption of the sugars until an almost exclusive utilization of \( S_1 \) occurs. As glucose is exhausted, \( r_{B,1} \) and \( v_1 r_{B,1} \) drop to zero; this triggers the induction and activation of \( E_2 \) and after a lag period, during which time \( E_2 \) rises to the balanced growth levels, the \( S_1 \) consumption begins. The outcome of this whole sequence is the typical diauxie phenomenon. The model thus brings out the internal regulatory processes to be a manifestation of an invariant strategy to optimize the cellular objectives. We have summarized the regulatory properties of the cybernetic variables \( u_i \) and \( v_i \) in Table 1.

Figure 3 shows the model simulation of the effect of preculturing the inoculum in the less preferred substrate. The difference in the simulations of the "no preculturing" and the "precultured" curves is the assumption of a higher initial value of \( e_2 \) for the second case. The difference between the curves is only at the intermediate lag phase. Whereas the "no preculturing" curve shows a noticeable lag phase, during which there is no cell growth, the "precultured" curve shows no such cessation of growth after the first growth phase. These simulation results are in agreement with Monod's experimental observations. The exclusive utilization of \( S_1 \), even when there is preformed \( E_2 \) is due to the catabolite inhibition, which is incorporated into the model through the cybernetic variable \( v_i \).

These diauxic curves show small intermediate lag phases between the two growth phases. There are situations such as in the growth of Klebsiella aerogenes on glucose-lactose mixtures in which the lag phase is very pronounced. Figure 4 shows the data for the growth of K. oxytoca on arabinose-lactose mixture and the model's simulation of the same system. The very low initial assumed values for \( e_2 \) due to the stringent control of the lac operon cause \( r_{B,2} \) to be very small even long after \( S_1 \) falls to low levels. This prevents the allocation of the resources for the \( E_2 \) synthesis. Thus the autocatalytic nature of enzyme formation, pointed out by Spiegelman and

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Regulatory process</th>
</tr>
</thead>
<tbody>
<tr>
<td>( u_1 )</td>
<td>high</td>
<td>induction of enzyme synthesis</td>
</tr>
<tr>
<td>( u_2 )</td>
<td>low</td>
<td>repression of enzyme synthesis</td>
</tr>
<tr>
<td>( v_1 )</td>
<td>low</td>
<td>inhibition of enzyme activity</td>
</tr>
<tr>
<td>( v_2 )</td>
<td>high</td>
<td>activation of enzyme activity</td>
</tr>
</tbody>
</table>

Figure 4. Diauxic curve with pronounced intermediate lag. Data for the growth of Klebsiella oxytoca on arabinose and lactose are from Kompala (ref. 25).
Cohnberg\textsuperscript{26} and Monod\textsuperscript{5} is inherent in our model formulation.

Figure 5 shows the variations in the diauxie phenomenon due to increasing concentrations of \(S_2\). Experiments of this kind were first done by Monod\textsuperscript{5} on the growth of \textit{Escherichia coli} on mixtures glucose and xylose. When \(S_2\) is low, there is a diauxic lag phase after \(S_1\) is consumed. This lag period decreases gradually as the initial \(S_2\) is increased. The intermediate lag is the period during which \(E_2\) is depressed and its level builds up gradually until the growth on \(S_2\) reaches its maximum rate. For small values of \(S_2\), resources are not allocated to the growth on \(S_2\) or the synthesis of \(E_2\) until \(S_1\) has dropped to very small values. At higher concentrations of \(S_2\), however, the resources are allocated sooner, thus reducing the diauxic lag.

Monod observed that the growth of \textit{E. coli} H1 on a mixture of glucose, sorbitol, and glycerol produced a triauxic growth with glucose as the substrate utilized first, sorbitol second, and glycerol as the last. This destroys the concept of inhibition as a prerogative of the substrate belonging to class A. Sorbitol belongs to class B but still inhibits the growth of \textit{E. coli} H1 on glycerol. Therefore, the classification of sugars into classes A and B is not of a qualitative nature but rather of a quantitative nature. The simulation of Monod's triauxic growth curve by the matching law model, shown in Figure 6, was accomplished with parameter values of \(\mu_i\)'s assumed such that \(\mu_{\text{glucose}} > \mu_{\text{sorbitol}} > \mu_{\text{glycerol}}\). Indeed, the model does not need an \textit{a priori} specification of the order in which the sugars are consumed. It is capable of determining that from the given parameter values, which can be estimated from the single-substrate growth experiments.

The framework developed here identifies an organism-substrate combination by a set of parameter values. The growth behavior of this organism on a mixture of substrates is then predicted by the interplay of the corresponding sets of parameter values within the cybernetic scheme. The model is capable of predicting both the sequential as well as the simultaneous utilization of mixed substrates. The predictions of the so-called glucose effect (the diauxie with glucose as the preferred substrate) and the exceptions\textsuperscript{6-8} to this effect are equally possible with this model.

**CONTINUOUS GROWTH ON MULTIPLE SUBSTRATES**

When a mixture of sugars that are consumed sequentially by an organism in batch cultures is fed to a continuous culture of the same organism at low dilution rates (i.e., low growth rates), both the sugars are consumed simultaneously. This seemingly "anomalous" behavior is easily explained with the framework of our model. In batch cultures when the growth is unconstrained by the dilution rate, the organism optimizes its growth such that it grows first on the substrate supporting the faster growth and then on the slower substrate. In continuous cultures, however, the growth rate is held at the experimentally imposed dilution rate. At low dilution rates, when either substrate can support the growth at such levels, the need for preferential utilization of the faster substrate does not exist. As the dilution rate is increased, this need is gradually felt and the simultaneous utilization changes gradually to a preferential utilization of the faster assimilated sugar.

Baidya and co-workers\textsuperscript{24} worked with a strain of \textit{Klebsiella aerogenes} grown on a mixture of glucose and lactose in a simple salts medium. In batch cultures, this system gives a prolonged diauxic lag of ca. 15 h. In
continuous cultures, when lactose was introduced for the first time, it was utilized only after a lag of 40 h. This lag period increased with dilution rate and at high dilution rates, lactose was not consumed at all. Also, Harte and Webb26 have studied the growth of *K. aerogenes* in a continuous fermentor under varying substrate feed strategies for different dilution rates. Their feed strategies consisted of switching the feed from glucose as the sole carbon source, to a mixed feed of glucose and maltose at equal concentrations. To determine the extent of utilization of maltose in the presence of glucose, they switched the feed back to glucose alone and then doubled its concentration and compared the steady-state cell mass concentrations during the feed of mixed sugars and the feed of doubled concentration of glucose. Their results can be summarized as follows. At low dilution rates, maltose was consumed just as completely and as quickly as glucose. At moderate dilution rates, there was a time lag before the consumption of maltose began, and much of maltose remained unutilized. At high dilution rates, there was no consumption of maltose at all.

The cybernetic model for bacterial growth that we have developed accounts for the same cellular regulatory processes that in batch cultures produce sequential and simultaneous utilization patterns and in continuous cultures produce the preferential and simultaneous utilization patterns. We have used our model equations to simulate growth in a chemostat when the cells are presented with varying single and mixed sugar feed strategies. The results are shown in Figures 7, 8, and 9 for increasing dilution rates of 0.15, 0.45, and 0.60 h⁻¹, respectively. In these simulations, the cells initially growing on a sugar feed of only glucose at a concentration of 0.1 g/L, are presented with a mixed feed of glucose and xylose, each at a concentration of 0.1 g/L (total sugar concentration = 0.2 g/L) at 50 h. After the steady state is reached on this mixed feed, at 150 h, the feed is switched back to only glucose of concentration 0.1 g/L. At 200 h, the glucose concentration is doubled to 0.2 g/L.

At 50 h, when the feed is switched from glucose to the mixture of glucose and xylose, the xylose concentration builds up in the culture before it gets consumed. The time lag before xylose is utilized increases with higher dilution rates.

The cell mass concentration during the mixed feed of total sugar concentration of 0.2 g/L may be compared with the same curve during the feed of only glucose at the concentration of 0.2 g/L. Comparing these levels at different dilution rates shows that the simultaneous utilization of xylose and glucose at low dilution rates changes gradually to a preferential utilization of glucose at higher dilution rates. These simulation results are in good qualitative agreement with the observations of Harte and Webb.26

Mateles and co-workers27,28 have collected steady state data from the continuous cultures of *Pseudomonas fluorescens* and *E. coli* b6 on a mixed substrate feed (of the kind that produces the diauxie phenomenon in batch growth).
cultures). At low dilution rates, both the sugars are consumed simultaneously, while at high dilution rates, only one sugar is preferentially utilized. For the growth of \textit{P. fluorescens} on glucose and fructose, the utilization of fructose gets gradually reduced due to the presence of glucose at dilution rates much lower than the maximum dilution rate on fructose alone. Figure 10 shows the fitting of this data of Mateles and co-workers\textsuperscript{27} by the simulations of the matching law model. The data for the growth of \textit{E. coli} b6 on glucose and lactose shows simultaneous utilization of both sugars over a wide range of dilution rates and a sudden change to preferential utilization of glucose at a higher dilution rate. Figure 11 shows how this data of Silver and Mateles\textsuperscript{28} may be fitted by the simulations of the matching law model.

CONCLUDING REMARKS

The matching law model has been shown to be very promising in explaining all the experimentally observed phenomena in the microbial growth on multiple substrates. This model does not need an \textit{a priori} specification of the order in which the substrates are consumed. Thus, both the glucose effect and its exceptions are predicted by this model. We are able to reconcile the "conflicting" behaviors in batch and continuous with respect to sequential versus simultaneous utilization of substrate mixtures. It reproduces the various manifestations of the diauxie phenomena in batch and continuous cultures qualitatively. Quantitative fits are excellent where they were attempted.

This model provides a powerful framework for predicting all types of bacterial growth on multiple substrates. Inputs for the model are just the parameter values obtained from the single substrate growth experiments and no further information is needed for the prediction of growth behavior on multiple substrates. The available experimental literature on the multiple substrate growth does not extensively cover all the manifestations of the diauxie phenomena on any single systems of substrates and organism. Thus a complete evaluation of the model's potential to predict the behavior of any single system under different experimental conditions awaits further experimentation.

**NOMENCLATURE**

- \( B \) biomass
- \( c \) biomass concentration
- \( e_i \) specific enzyme level
- \( E_i \) \( i \)th key enzyme
- \( K_i \) Michaelis constant
- \( p_i \) returns
- \( r_i \) resources
- \( r_{B_i} \) rate of biomass production through consumption of \( S_i \)
- \( r_{E_i} \) rate of \( E_i \) synthesis
- \( s_i \) substrate concentration
- \( S_i \) \( i \)th substrate
- \( t \) time
- \( u_{ij}, v_i \) cybernetic variables
- \( Y_i \) yield coefficient
- \( \alpha_i \) enzyme synthesis rate constant
- \( \beta_i \) enzyme decay rate constant
- \( \mu_i \) growth coefficient
- \( \mu_{\text{max}} \) maximum specific growth rate
- \( r_i \) energy production coefficient

**Subscripts**

- 0 initial
- max maximum
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References

25. D. S. Kompala, Ph.D. thesis, Purdue University, Lafayette, IN, under investigation.