Cybernetic Modeling of Growth in Mixed, Substitutable Substrate Environments: Preferential and Simultaneous Utilization

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Growth of microorganisms on substitutable substrate mixtures display diverse growth dynamics characterized by simultaneous or preferential uptake of carbon sources. This article shows that cybernetic modeling concepts which were successful in predicting diauxic growth patterns can be extended to describe simultaneous consumption of substrates. Thus the growth of *Escherichia coli* on mixtures of glucose and organic acids such as pyruvate, fumarate, and succinate has been described successfully by the cybernetic model presented here showing both diauxic and simultaneous uptake when observed. The model also describes the changes in utilization patterns that occur under changing dilution rates, substrate concentrations, and models of preculturing. The model recognizes the importance of the synthesis of biosynthetic precursors in cell growth through a kinetic structure that is quite general for any mixture of carbon-energy sources. © 1996 John Wiley & Sons, Inc.

Key words: cybernetic modeling • biosynthetic precursors • metabolic regulation • enzyme synthesis

INTRODUCTION

The performance of most biochemical processes mediated by microorganisms, such as fermentations and biodegradation, is influenced by the dynamics of microbial growth in multiple nutrient-limited environments. The nutrient limitation may include sets of substitutable substrates that is, those fulfilling identical growth requirements, or complementary ones where each substrate fulfills an essential growth requirement. More generally, both types of substrate combinations may be present. The vast majority of studies of growth in mixed substitutable substrate cultures have been with carbon-energy sources and reveal a spectrum of growth dynamics and substrate utilization patterns. These situations are frequently encountered in fermentations that use complex media and in the biodegradation of pollutants.

Mathematical models which possess predictive capabilities to describe these dynamics are invaluable in the rational design of bioprocesses. In the literature, there are several modeling efforts that attempt to describe mixed-substrate growth dynamics. These models are only able to predict diauxic growth and cannot describe simultaneous utilization of the substrates.

Unstructured models based on modified forms of the Monod equation, such as the model of Yoon et al. (1977), are inadequate for describing varied growth patterns that arise from complex regulatory processes. The models of van Dedem and Moo-Young (1975) and Nikolajsen et al. (1991), which are based on structured representations of a cell and do include some aspects of metabolic regulation, however incorporate specific biochemical features that constrain their application to the system under consideration. However, the cybernetic model of Kompala et al. (1986), also based on a structured representation of the cell, represents the control of the important biochemical processes incorporating no new parameters through the recognition of the optimal nature of microbial growth on mixed substrates. This kinetic structure and description of regulation is general to any combination of substitutable substrates and describes mixed-substrate growth with parameters determined from single-substrate experiments. The model, discussed in more detail under Cybernetic Modeling, is however unable to describe simultaneous utilization of substrates during mixed-substrate growth, due to the rigidity of the kinetic structure.

We have developed a model with an expanded kinetic structure that uses the same cybernetic principles as the model of Kompala and considers the formation of key biosynthetic precursors and enzymes to describe a more complete spectrum of mixed-substrate growth dynamics observed in the literature. The model describes diauxic growth as well as simultaneous substrate uptake and...
also describes situations where the pattern of substrate uptake switches from sequential to simultaneous, or vice versa. These include preculturing effects and changing substrate levels in batch growth and varying dilution rates in continuous cultures.

**MICROBIAL GROWTH ON MIXED SUBSTRATES**

The growth of microorganisms in multiple nutrient-limiting conditions has been the subject of numerous studies. We focus our discussion on relevant studies dealing with microbial growth on mixtures of substitutable substrates, that is, substrates fulfilling identical growth requirements, particularly carbon-energy sources such as sugars, hydrocarbons, and organic acids.

Monod (1942) provides examples of the two general classes of substrate utilization in his work on the growth of bacteria on mixtures of sugars. Both sequential and simultaneous utilization was observed with most of the sequential utilization cases characterized by an intermediate lag phase or diauxic lag phase.

A recent study by Narang (1994) reveals many interesting features of simultaneous utilization for the growth of *Escherichia coli* on organic acids under substrate-sufficient batch growth, that is, batch growth where substrate concentrations are sufficiently high that the maximum specific growth rate is maintained during exponential growth. The mixed-substrate experiments involved substrate pairs comprised of either L-lactate or pyruvate and succinate, fumarate, or α-ketoglutarate, all of which are central metabolic intermediates. The mixed-substrate growth rates were in all cases significantly higher than the single-substrate growth rate on either substrate and were in the range of 0.5–0.52 h⁻¹ for all the organic acid pairs except pyruvate–α-ketoglutarate where the growth rate was 0.45 h⁻¹. Succinate supported the highest single substrate growth rate, 0.44 h⁻¹. In addition, the specific uptake rate of the two substrates is less than their specific uptake rates during single-substrate growth.

Diauxic growth was observed during mixed-substrate growth in all cases where an organic acid was paired with glucose (\(\mu_{max} = 0.72\) h⁻¹), except for growth on glucose–pyruvate mixtures. Simultaneous utilization with a growth rate (0.56 h⁻¹) intermediate to that on glucose or pyruvate (0.27 h⁻¹) was observed when the inoculum was precultured on pyruvate alone and diauxic growth when the preculturing was on glucose or glucose–pyruvate. This preculturing effect was not observed in the other cases. This effect has also been observed with the growth of *Pseudomonas oxalicus* on oxalate and formate which exhibits simultaneous utilization when precultured on oxalate alone (Dijkhuizen et al., 1980).

Such transitions between the two general utilization patterns are generally observed in continuous cultures with changing dilution rates in systems including acetate–oxalate (Harder and Dijkhuizen, 1982), glucose–xylose (Baloo and Ramkrishna, 1991a) glycerol–xylose (Brinkmann and Babel, 1992), and glucose–methanol (Egli et al., 1986) where the first substrate is always preferred in batch growth. In all these situations the two substrates are simultaneously utilized at low dilution rates while the less preferred substrate is washed out at higher dilution rates. An interesting experiment by Wanner (Wanner and Egli, 1990) shows that simultaneous and sequential utilization of substrates are both possible in batch growth depending on the concentration of the carbon sources. The growth of *E. coli* ML30 on a mixture of glucose and galactose exhibits diauxic growth patterns when the concentrations are in the 100-mg/L range, but in the 5-mg/L range both carbon sources are utilized simultaneously.

To summarize, sequential and simultaneous utilization is possible for particular substrate pairs depending on the substrate concentrations, preculturing procedures, and cultivation modes (batch or continuous) used. Under substrate-sufficient conditions, simultaneous utilization is generally observed with substrate mixtures where the individual substrates support low to intermediate growth rates compared to the growth rates possible on the faster substrate. Simultaneous utilization in these cases is characterized by the achievement of a higher growth rate by the microbial culture and reduced substrate uptake rates when compared to single-substrate growth.

**PHYSIOLOGY OF MIXED SUBSTRATE GROWTH**

The synthesis of a bacterial cell involves thousands of distinct metabolic reactions which may be classified as fueling, biosynthetic, polymerization, and assembly reactions (Neidhardt et al., 1990). The fueling reactions produce precursor metabolites and energy in the form of reduced pyridine nucleotides [e.g., (NADH)] and adenosine triphosphate (ATP) or some other source of high-energy bonds. There are 12 essential precursors, some of which are formed from fueling reactions, and these replenish the remaining precursor pools drained by biosynthesis, through anapleurotic reactions. Microorganisms show remarkable diversity in fueling pathways, while the biosynthetic steps are more or less the same for all species.

As discussed earlier, bacterial species exhibit different growth rates on different substrates. Much research has focused on the influence of energetics, particularly the production of ATP on growth rates and yields (Stouthamer, 1979). However, the hypothesis that the concentration or the rate of ATP production determines the growth rate in *E. coli* is disputed by Marr (1991). Marr conjectures that "the kinetic connection between fueling reactions and macromolecular synthesis is the concentration of precursor metabolites and monomers..."
derived from these metabolites and that the concentrations of these monomers are sensed by and control the machinery of macromolecular synthesis,” as hypothesized by Jensen and Pedersen (1990). Russell and Cook (1995) observe that this hypothesis is consistent with the supposition that a common feature of growth with an excess of energy is the presence of energy-spilling reactions.

The 12 precursors may be classified as a set of complementary resources since they are all essential for biosynthesis. The necessary synthesis of these precursors as possibly the key factor in effecting differences in growth of bacteria on different carbon-energy substrates is an important aspect in the development of the model proposed to describe the general patterns of mixed-substrate utilization and is discussed in the next section.

MODEL DEVELOPMENT

Cybernetic Modeling

The cybernetic perspective of microbial growth (Ramkrishna, 1982) recognizes that metabolic regulation of biochemical processes is mediated through the control of enzyme synthesis and enzyme activity. The microorganism is viewed as an optimal system in which the regulatory processes implement optimal strategies, and the outcome of these strategies modifies the intrinsic process kinetics through cybernetic or control variables. The cybernetic model developed by Kompala et al. (1986) describes the dynamics associated with diauxic growth, or the sequential utilization of substitutable substrates with the preferential utilization of the substrate supporting the higher growth rate. The model includes the synthesis of two key enzymes, $E_1$ and $E_2$, responsible for biomass synthesis from $S_1$ and $S_2$, respectively:

$$S_i \rightarrow E_i, B$$

These enzymes are induced by the substrates that they act on. Enzyme synthesis is controlled by modifying the enzyme synthesis rate $r_{E_i}$ as $r_{E_i} \mu_i$, where $u_i$ is the cybernetic variable controlling enzyme synthesis. The form for the variable $u_i$ is based on the matching law (Herrnstein, 1974), according to which the optimal allocation of resources to an alternative is proportional to the amount of returns obtained from that alternative. Therefore, the fractional allocation of limited resources to enzyme synthesis, $u_i$, to the $i$th alternative, that optimizes the total returns is given by

$$u_i = \frac{r_i}{\sum_j r_j}$$

where $r_i$ are the returns from the $i$th alternative. The return is considered to be the growth rate on the substrate $S_i$ mediated by $E_i$. The proportional law for the formulation of $v_i$ to account for the control of enzyme activity modifies the rate of the $i$th growth process, as $r_i v_i$, where $v_i$ is given by

$$v_i = \frac{r_i}{\max_j r_j}$$

This model has been extremely successful in describing the growth of Klebsiella oxytoca on sugars. The model parameters were estimated from single-substrate growth, and the description of regulation introduced no additional variables or parameters.

Narang's (1994) analysis of the cybernetic model proposed by Kompala reveals that the rigidity of the structure is unable to accommodate a description of simultaneous utilization. In addition, the assumption in the cybernetic model formulation which states that the growth rate on the mixture is never greater than the maximum of the growth rates on individual substrates is not true for the case of simultaneous utilization where the growth rate on the mixture is in fact higher than either growth rate. The structure of Kompala's model considers the formation of biomass from substrate as a single step. This viewpoint ignores the role of fueling reactions and precursor formation in biosynthesis, discussed in earlier sections. In the next section, an expanded kinetic structure derived from the basic aspects of microbial physiology and incorporating the complementary nature of precursors is discussed. This kinetic view, while using the same cybernetic principles for describing regulation, has the potential to describe both sequential and simultaneous utilization of carbon energy substrates.

Expanded Kinetic Structure for Multiple-Substrate Growth

As mentioned earlier, during growth on a carbon-energy source, initial catabolism leads to the formation of one or two precursor metabolites from which the remaining are synthesized through appropriate anapleurotic reactions. If a substrate, say $S_1$, forms a precursor or constituents of a lumped precursor pool, which we refer to as $M_1$, then the other precursors, designated as $M_2$, must be formed from $M_1$. On the other hand, the synthesis of $M_2$ and $M_1$ can occur through the uptake of a substrate, say $S_2$, where $S_2$ initially forms $M_2$, from which $M_1$ is synthesized. This scheme is shown in Figure 1. Each of the reactions is mediated by a key enzyme, and biomass formation depends on the levels of both precursor pools. This scheme represents the complementary nature of the precursors with respect to the biosynthetic process. However, there are substitutable alternatives for the formation of the precursor pools during mixed-substrate growth, as can be seen in Figure 1. For instance, the formation of $M_2$ can be through...
uptake of either $S_2$ or $M_1$ present intracellularly. The interconversion of the precursor pools $M_1$ and $M_2$ is a simplified representation of anapleurotic reactions and under mixed-substrate growth compete with catabolic sequences in generating the precursors. This is a very important feature since it reveals the ability of the kinetic structure to accommodate a description of both sequential and simultaneous utilization, provided regulation is appropriately incorporated.

If $S_1 \rightarrow M_1$ and $M_1 \rightarrow M_2$ are the preferred alternatives for the synthesis of the precursors, then the scenario is preferential utilization of $S_1$ over $S_2$ or diauxic growth. However, if the preferred alternative for the synthesis of $M_2$ is $S_2 \rightarrow M_2$, with $M_1$ being synthesized from $S_1$, then it leads to the case of simultaneous utilization. If the objective of the cell is to maximize growth, then the growth rates supported by $S_2$ and $S_1$ are an important aspect of regulation, and the influence of either substrate on growth is their ability to saturate these precursor pools. Under sequential growth, it can be inferred that $S_1$ would saturate these precursor pools faster, eliminating the need for $S_2$. However, under simultaneous utilization, the uptake of both substrates is perhaps a better choice for synthesizing precursor pools over the anapleurotic reactions, thus alleviating precursor limitations and enabling the cell to achieve higher growth rates than those possible during single-substrate growth.

Since simultaneous utilization is observed with substrates supporting low to moderate growth rates and diauxic growth occurs during growth on substrate mixtures where one substrate generally supports a superior growth rate, there is a rational basis for this teleological viewpoint. The rates of processes involved in precursor formation and growth have therefore a very important role in deciding the regulatory strategy for substrate uptake and growth. The calculation of the cybernetic variables using the rates of the growth processes in the model for diauxic growth reflects this importance. The development of the cybernetic framework using the same principles in the model by Kompala to extend its application to more structured kinetic representations was accomplished by Straight and Ramkrishna (1994).

**Regulation of Substitutable Processes**

Straight and Ramkrishna (1994) have analyzed metabolic pathways by considering the topology of metabolism. Metabolic pathways are assumed to be constructed from four basic structures: linear segments, convergent branch points, divergent branch points, and cycles. Appropriate metabolic regulation for the synthesis and activity of the key enzymes is derived considering each structure to be an independent unit. An objective is proposed for each structure from which appropriate cybernetic variables are derived, with the contention that regulatory strategies implemented by microorganisms have outcomes consistent with a desired goal. The objectives for these structures have been stated by Straight and Ramkrishna, and we shall list here the pertinent postulates for substitutable processes:

1. The objective of promoting any irreversible branched pathway is the maximization of the mathematical product of the end products by one or more branch points.
2. In the presence of competing processes, whether substitutable or complementary in nature, the enzyme catalyzing the process that provides for the greatest return will be activated to the greatest extent. The appropriate measure of the returns received from promoting a given process is dependent upon the process type, e.g., substitutable or complementary.

In each of the individual structural units, each process is catalyzed by a key enzyme and each key enzyme represents an alternative in which material and temporal resources have been invested by the microorganism. The convergent branch point represents a substitutable process since there exist multiple alternatives for achieving the same goal.

The form of the cybernetic variable for the regulation of enzyme synthesis is derived as

$$u_{i,j} = \frac{r_{i,j}}{\sum_{k=1}^{n} r_{i,k}}$$  \hspace{1cm} (3)

where $r_{i,j}$ is the rate of producing the $i$th product from the $j$th substrate. The cybernetic variable for the regulation of enzyme activity is determined from the second postulate as

$$v_{i,j} = \frac{r_{i,j}}{\max_k (r_{i,k})}$$  \hspace{1cm} (4)

These are identical in form to the expression proposed by Kompala et al. in the model for diauxic growth. While the cybernetic variables in Kompala's model regulated competing growth processes, the formulation by
Straight and Ramkrishna (1994) extends the concept of the matching and proportional laws to any competing set of processes.

Therefore, the substitutable alternatives for the synthesis of \( M_1 \) and \( M_2 \) are two sets of competing processes whose regulation can be described in the above manner.

**Rate Expressions for the Expanded Model**

The principal rates involve the syntheses of the precursors and biomass and the syntheses of four key enzymes involved in precursor synthesis. The rate expressions for all cellular processes are assumed to follow saturation kinetics. The level of the key enzyme which catalyzes the particular process affects the process rate, and the rate expression is modified to account for this fact. Therefore the rate of synthesis of \( M_1 \) from \( S_1 \) is considered as

\[
r_{s_1,m_1} = r_{s_1,m_1}^\text{max} \left( \frac{s_1}{s_1 + K_{s_1,m_1}} \right) \left( \frac{e_{s_1,m_1}}{e_{s_1,m_1}^\text{max}} \right)
\]

and the synthesis rate for the enzyme \( E_{s_1,m_1} \) is

\[
r_{s_1,m_1}^E = r_{s_1,m_1}^{E\text{max}} \left( \frac{s_1}{s_1 + K_{s_1,m_1}^{E\text{max}}} \right)
\]

The rate expression applies only to the inducible synthesis of \( E_{s_1,m_1} \), and the overall rate of enzyme synthesis will include a constitutive rate of synthesis, \( r_{s_1,m_1}^{E\text{const}} \).

The rate expressions for the other processes involved in the synthesis of \( M_1 \) and \( M_2 \) and the appropriate key enzymes are similarly formulated. The growth process

\[
M_1 + M_2 \rightarrow B
\]

is assumed to follow a multiple saturation form of growth, to account for the dependence on both precursors, and is given by

\[
r_G = r_G^{\text{max}} \left( \frac{m_1}{K_{G,m_1} + m_1} \right) \left( \frac{m_2}{K_{G,m_2} + m_2} \right)
\]

The synthesis of a key enzyme for the growth step is not considered in the model. The assumption is that the biosynthetic apparatus is induced at sufficiently high levels that the level of a key growth enzyme \( E_G \) will always be equal to \( e_G^{\text{max}} \), its maximum level. Such an assumption is consistent with the viewpoint of Jensen and Pedersen (1990) that the machinery for macromolecular synthesis is subsaturated by precursors.

**Regulation and Model Equations**

The cybernetic variables that describe the regulation of the substitutable processes for the synthesis of \( M_1 \) and \( M_2 \) are formulated as derived by Straight and Ramkrishna (1994). For the synthesis of \( M_1 \), they are

\[
u_{s_1,m_1} = \frac{Y_{s_1,m_1} r_{s_1,m_1}}{Y_{s_1,m_1} r_{s_1,m_1} + r_{m_2,m_1}}
\]

\[
u_{m_2,m_1} = \frac{r_{m_2,m_1}}{Y_{s_1,m_1} r_{s_1,m_1} + r_{m_2,m_1}}
\]

\[
u_{s_1,m_1} = \frac{Y_{s_1,m_1} r_{s_1,m_1}}{\max(Y_{s_1,m_1} r_{s_1,m_1}, r_{m_2,m_1})}
\]

\[
u_{m_2,m_1} = \frac{r_{m_2,m_1}}{\max(Y_{s_1,m_1} r_{s_1,m_1}, r_{m_2,m_1})}
\]

The cybernetic variables are similarly formulated for the two substitutable processes synthesizing \( M_2 \).

The balance equations for the substrates, biomass, enzymes, and precursors are as follows:

\[
ds_1 = -\frac{1}{Y_{s_1,c}} r_{s_1,m_1} v_{s_1,m_1} \rho
\]

\[
ds_2 = -\frac{1}{Y_{s_2,c}} r_{s_2,m_2} v_{s_2,m_2} \rho
\]

\[
dm_1 = Y_{s_1,m_1} r_{s_1,m_1} v_{s_1,m_1} + r_{m_2,m_1} v_{m_2,m_1} - \frac{1}{Y_{m_1,c}} r_G - r_{m_1,m_2} v_{m_1,m_2} - r_{G,m_1}
\]

\[
dm_2 = Y_{s_2,m_2} r_{s_2,m_2} v_{s_2,m_2} + r_{m_1,m_2} v_{m_1,m_2} - \frac{1}{Y_{m_2,c}} r_G - r_{m_2,m_1} v_{m_2,m_1} - r_{G,m_2}
\]

\[
dc = r_{c,G}
\]

\[
de_{s_1,m_1} = r_{E_{s_1,m_1}} + r_{E_{s_1,m_1}} - \beta_{s_1,m_1} e_{s_1,m_1}
\]

\[
de_{s_2,m_2} = r_{E_{s_2,m_2}} + r_{E_{s_2,m_2}} - \beta_{s_2,m_2} e_{s_2,m_2}
\]

\[
de_{m_1,m_1} = r_{E_{m_1,m_1}} + r_{E_{m_1,m_1}} - \beta_{m_1,m_1} e_{m_1,m_1}
\]

\[
de_{m_2,m_2} = r_{E_{m_2,m_2}} + r_{E_{m_2,m_2}} - \beta_{m_2,m_2} e_{m_2,m_2}
\]

The term \( r_{c,G} e_{m_1,m_2} \) represents the dilution in the concentration of \( E_{m_1,m_2} \) through cell growth and \( \beta_{m_1,m_2} e_{m_1,m_2} \), the degradation of the enzyme. The precursor pools are also diluted through expansion of the biomass. The control of the enzyme synthesis and precursor
Table I. Parameter values used in the model general to all substrates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_{i_1}^{\text{max}} )</td>
<td>0.001 g/g dw·h</td>
<td>( r_{i_2}^{\text{max}} )</td>
<td>0.001 g/g dw·h</td>
</tr>
<tr>
<td>( r_{i_1}^{\epsilon} )</td>
<td>( 1 \times 10^{-5} ) g/g dw·h</td>
<td>( r_{i_2}^{\epsilon} )</td>
<td>( 1 \times 10^{-5} ) g/g dw·h</td>
</tr>
<tr>
<td>( r_{m_1}^{\text{max}} )</td>
<td>0.001 g/g dw·h</td>
<td>( r_{m_2}^{\text{max}} )</td>
<td>0.001 g/g dw·h</td>
</tr>
<tr>
<td>( r_{m_1}^{\epsilon} )</td>
<td>( 1 \times 10^{-5} ) g/g dw·h</td>
<td>( r_{m_2}^{\epsilon} )</td>
<td>( 1 \times 10^{-5} ) g/g dw·h</td>
</tr>
<tr>
<td>( K_{i_1,m_1} )</td>
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<td>( K_{i_2,m_2} )</td>
<td>0.001 g/L</td>
</tr>
<tr>
<td>( K_{m_1,m_1} )</td>
<td>0.001 g/g dw</td>
<td>( K_{m_2,m_2} )</td>
<td>0.001 g/g dw</td>
</tr>
<tr>
<td>( K_{G,m_1} )</td>
<td>( 5 \times 10^{-4} ) g/g dw</td>
<td>( K_{G,m_2} )</td>
<td>( 5 \times 10^{-4} ) g/g dw</td>
</tr>
<tr>
<td>( K_{X,m_1} )</td>
<td>( 1 \times 10^{-4} ) g/L</td>
<td>( K_{X,m_2} )</td>
<td>( 1 \times 10^{-4} ) g/L</td>
</tr>
<tr>
<td>( K_{Y,m_1} )</td>
<td>( 1 \times 10^{-4} ) g/g dw</td>
<td>( K_{Y,m_2} )</td>
<td>( 1 \times 10^{-4} ) g/g dw</td>
</tr>
<tr>
<td>( \beta_{i_1,m_1} )</td>
<td>0.05 h^{-1}</td>
<td>( \beta_{i_2,m_2} )</td>
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</tr>
<tr>
<td>( \beta_{m_1,m_1} )</td>
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<td>( \beta_{m_2,m_2} )</td>
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<tr>
<td>( e_{i_1}^{\max} )</td>
<td>0.002 g/g dw</td>
<td>( e_{i_2}^{\max} )</td>
<td>0.002 g/g dw</td>
</tr>
<tr>
<td>( e_{m_1}^{\max} )</td>
<td>0.002 g/g dw</td>
<td>( e_{m_2}^{\max} )</td>
<td>0.002 g/g dw</td>
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</tbody>
</table>

For glucose–fumarate, glucose–pyruvate, succinate–pyruvate, and fumarate–pyruvate, the first substrate was \( S_1 \) and the second \( S_2 \).

\( r_{G}^{\text{max}} = 0.75 \text{ h}^{-1} \).

formation processes is achieved by multiplying the intrinsic rate with the appropriate cybernetic variable.

Computational Methods and Parameter Estimation

The model equations were solved using the Livermore solver for ordinary differential equations (LSODE) subroutine, which solves the initial-value problem for stiff or nonstiff systems of first-order ordinary differential equations. SIMUSOLV, a modeling and simulation software developed by Dow Chemical Company, was used in parameter estimation from single-substrate growth data.

Parameters were estimated from experimental data, literature studies, previous cybernetic modeling efforts, and order-of-magnitude estimations. The value for \( r_{G}^{\text{max}} \) was set at 0.75 h^{-1}, the maximum growth rate observed on glucose for \( E. coli \) K12 (Narang, 1994). The saturation constants \( K_{G,m} \) and \( K_{G,m,*} \) were chosen arbitrarily to be an order of magnitude lower than the size of the intracellular intermediate pools, i.e., 0.25–0.5% of biomass dry weight (Cooney et al., 1976; Mandelstam, 1958). The saturation constants for the different processes were also set at values reflecting the intracellular concentrations of the precursors.

The maximum specific rates for the formation of \( M_1 \) and \( M_2 \) were estimated from the single-substrate batch data. The values for \( r_{i_1,m_1}^{\max} \), \( Y_{i_1,c} \), \( r_{m_1,m_1}^{\max} \), and \( Y_{m_1,m_1} \) were estimated from the experimental data for growth on \( S_1 \), and the parameter values for growth on \( S_2 \) were similarly evaluated from data for \( S_2 \) using SIMUSOLV. The values for the parameters involved in enzyme syntheses were set to values utilized in previous cybernetic models, and the constitutive synthesis was set at 1% of the respective \( r_{G}^{\text{max}} \), the maximum synthesis rate of inducible enzyme synthesis. The complete set of parameters is listed in Tables I and II. Table I lists the parameters that were held the same in all the simulations. Table II lists the parameters that depended on the substrate pairs considered and were calculated from the single-substrate experimental data.

RESULTS AND DISCUSSION

The model simulations were compared with experiments for three different batch growth situations, fumarate–pyruvate, succinate–pyruvate, and glucose–fumarate (Narang, 1994). Figure 2 shows the model simulations and experimental data for biomass growth on fumarate and pyruvate alone and a mixture of both. The increased growth rate on the mixture is well predicted by the model. The model predictions for the consumption of fumarate and pyruvate under mixed-substrate and single-substrate conditions are indicated in Figures 3 and 4. The model predictions for growth on succinate–pyruvate are shown in Figures 5–7. The model slightly underpredicts the growth rate on succinate alone. The predictive capabilities of the model can be further refined with the aid of additional experiments, especially in continuous cultures, as carried out by Straight and Ramkrishna (1994) for growth under carbon and/or nitrogen limitations.

The capability of the model to simulate diauxic behavior is demonstrated, considering data during growth on a mixture of glucose and fumarate. While the characteristic biphasic nature of diauxic growth is clearly observed (Fig. 8), the agreement with data...
Table II. Parameter values used in the model specific to a particular substrate.

<table>
<thead>
<tr>
<th></th>
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<tbody>
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<td>$r_{m_1}$</td>
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<td>2.0 h⁻¹</td>
<td>0.69 h⁻¹</td>
<td>0.58 h⁻¹</td>
</tr>
<tr>
<td>$r_{m_2}$</td>
<td>0.58 h⁻¹</td>
<td>0.44 h⁻¹</td>
<td>0.44 h⁻¹</td>
<td>0.44 h⁻¹</td>
</tr>
<tr>
<td>$m_{m_1}$</td>
<td>1.165 g/g dw⁻¹</td>
<td>1.165 g/g dw⁻¹</td>
<td>1.165 g/g dw⁻¹</td>
<td>0.784 g/g dw⁻¹</td>
</tr>
<tr>
<td>$m_{m_2}$</td>
<td>0.784 g/g dw⁻¹</td>
<td>1.047 g/g dw⁻¹</td>
<td>1.047 g/g dw⁻¹</td>
<td>1.047 g/g dw⁻¹</td>
</tr>
<tr>
<td>$Y_{x_{m_1}}$</td>
<td>0.8 g dw⁻¹</td>
<td>0.8 g dw⁻¹</td>
<td>0.31 g dw⁻¹</td>
<td>0.31 g dw⁻¹</td>
</tr>
<tr>
<td>$Y_{x_{m_2}}$</td>
<td>0.31 g dw⁻¹</td>
<td>0.23 g dw⁻¹</td>
<td>0.23 g dw⁻¹</td>
<td>0.23 g dw⁻¹</td>
</tr>
<tr>
<td>$Y_{s_{m_1}}$</td>
<td>1.165 g/g</td>
<td>1.165 g/g</td>
<td>1.165 g/g</td>
<td>1.204 g/g</td>
</tr>
<tr>
<td>$Y_{s_{m_2}}$</td>
<td>1.204 g/g</td>
<td>1.189 g/g</td>
<td>1.189 g/g</td>
<td>1.189 g/g</td>
</tr>
</tbody>
</table>

For glucose–fumarate, glucose–pyruvate, succinate–pyruvate, and fumarate–pyruvate, the first substrate was $S_1$ and the second $S_2$.

**Figure 2.** Model simulations compared to experimental data for biomass concentrations during growth of E. coli K12 on fumarate and pyruvate.

**Figure 4.** Model simulations compared to experimental data for pyruvate utilization during growth of E. coli K12 on fumarate alone and fumarate–pyruvate mixtures.

**Figure 3.** Model simulations compared to experimental data for fumarate utilization during growth of E. coli K12 on fumarate alone and fumarate–pyruvate mixtures.

**Figure 5.** Model simulations compared to experimental data for biomass concentrations during growth of E. coli K12 on succinate–pyruvate mixtures.
is more qualitative for growth on fumarate due to insufficient data. Since the initial concentration of fumarate is low and the cell density high at the end of the first growth phase, the growth phase on fumarate is short. The utilization of fumarate and glucose is seen to be sequential (Fig. 9). A simulation with a higher concentration of fumarate (Fig. 10) illustrates more clearly the diauxic nature of growth on glucose–fumarate mixtures.

The ability of the model to display the very interesting transition from sequential to simultaneous substrate utilization at very low substrate concentrations, observed by Wanner and Egli (1990), is shown in Figure 11. The initial concentrations for the glucose–fumarate system were reduced to the 10 mg/L range from the 1 g/L range.

The lowered substrate concentrations result in the preferred substrate's inability to satisfy the precursor requirements alone, leading therefore to simultaneous utilization.

The characteristic pattern of growth under continuous cultures with simultaneous utilization at low dilution rates and the transition to growth on the preferred substrate and eventual washout are reproduced by the model (Fig. 12). The washout, however, occurs at a dilution rate less than $\mu_{\text{max}}$ on the preferred substrate, which is generally not the case. The model does not consider maintenance processes that are particularly important in chemostat growth and their regulation. Baloo and Ramkrishna (1991a,b) have developed cybernetic models that describe metabolic regulation in chemostat

![Figure 6. Model simulations compared to experimental data for succinate utilization during growth of E. coli K12 on succinate alone and succinate–pyruvate mixtures.](image)

![Figure 7. Model simulations compared to experimental data for pyruvate utilization during growth of E. coli K12 on pyruvate alone and succinate–pyruvate mixtures.](image)

![Figure 8. Model simulations compared to experimental data for biomass concentrations during growth of E. coli K12 on glucose–fumarate mixtures.](image)

![Figure 9. Model simulations compared to experimental data for substrate concentrations during growth of E. coli K12 on glucose–fumarate mixtures.](image)
cultures, and these features must be included to obtain better quantitative description.

The model is also able to predict the effect of preculturing on the growth of *E. coli* on glucose–pyruvate mixtures (Fig. 13). The growth on the mixture after preculturing on pyruvate alone was simulated with lower initial levels of \( E_{s1,m1} \) and \( E_{m1,m2} \) than the case where the cells are precultured on glucose and pyruvate. While the exact physiological rationale for the preculturing effect is as yet unclear, it is apparent that growth on pyruvate does not allow the cells to adapt for optimal metabolism of glucose. Therefore it is reasonable to assume that preculturing on pyruvate results in lowered levels of those enzymes specific to glucose metabolism. As seen from the substrate consumption simulations (Fig. 14), preculturing on pyruvate alone results in simultaneous utilization of pyruvate and a lowered rate of glucose utilization. The initial enzyme levels used in the simulation for growth after preculturing on glucose and pyruvate was \( 1 \times 10^{-3} \) g/g dw (dry weight) for all four key enzymes. When the preculturing was on pyruvate alone, the levels of \( E_{s1,m1} \) and \( E_{m1,m2} \) were set at \( 1 \times 10^{-4} \) g/g dw.

Since these enzymes catalyze processes that would be active during preferential utilization of glucose, the lowered enzyme levels result in decreased rates of \( r_{s1,m1} \) and \( r_{m1,m2} \). Therefore glucose is not recognized as a substrate that can support a much higher growth rate than pyruvate. The uptake of pyruvate is therefore activated to a certain degree as an alternative for synthesizing the precursor, \( M_2 \), leading to simultaneous uptake

Figure 10. Diauxic growth on glucose–fumarate mixtures. Model simulations with a higher fumarate concentration.

Figure 11. Model simulations of simultaneous utilization of glucose and fumarate at low initial substrate concentration, 15 mg/L (0.015 g/L).

Figure 12. Model simulations of the characteristic biomass and substrate concentration profiles of mixed substrate growth in a chemostat with increasing dilution rates.

Figure 13. Effect of preculturing on biomass growth rates for the growth of *E. coli* K12 on glucose–pyruvate mixtures: (A) precultured on a mixture of glucose and pyruvate; (B) precultured on pyruvate.
of both substrates. The uptake of glucose is reduced, as seen by the substrate consumption curves (Fig. 14). Hence the growth rate observed on the mixture is lower than that observed on glucose alone and higher than that on pyruvate. The influence of initial enzyme levels is therefore quite critical in determining the growth characteristics on mixed-substrate growth. If glucose had been the sole carbon source, the only effect of lower initial enzyme levels would have been to extend the lag phase and the growth rate would have been unchanged. In the absence of a second substrate, the processes involved in glucose uptake will continue to be promoted. This result emphasizes the importance of proper inoculation in biochemical process involving mixed-substrate microbial growth.

CONCLUSIONS

In this article we have shown that cybernetic modeling concepts which were successful in predicting diauxic growth patterns can be extended to describe simultaneous consumption of substrates. A key feature of the model was the incorporation of precursor synthesis and their regulation. The growth of *Escherichia coli* on mixtures of glucose and organic acids such as pyruvate, fumarate, and succinate have been described successfully by the cybernetic models presented here, showing both diauxic and simultaneous uptake when observed. In addition, the model successfully describes transitions from one utilization pattern to the other under changes in dilution rates, substrate concentrations, and preculturing. The microbial degradation of aromatics in the presence of auxiliary substrates exhibits growth dynamics similar to the cases shown here. The models are currently being applied to evaluate strategies that use these auxiliary substrates as nutrient supplements to boost growth rates and specific degradation rates. The models which describe intrinsic biological kinetics can be used in conjunction with engineering models of transport of pollutants and supplementary nutrients in porous media for in-depth analysis of processes such as soil remediation.

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References


