Microbial Growth on Substitutable Substrates: Characterizing the Consumer–Resource Relationship

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Abstract: In this article, we consider the growth of microorganisms on mixtures of carbon sources and characterize the consumer–resource relationship for this system. The characteristic features observed for the growth of a single microorganism on a pair of carbon sources allow a representation of this relationship based on a general paradigm for resource classification. This representation is verified using a comprehensive model for microbial growth on carbon sources. The results show that for the same pair of carbon sources the qualitative nature of the consumer–resource relationship changes with changing specific growth rate, and therefore a change in the identity of the rate-limiting substrate(s). © 1997 John Wiley & Sons, Inc. Biotechnol Bioeng 54: 77–90, 1997.

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INTRODUCTION

All forms of life require a number of essential nutrients to ensure their survival and rely on a variety of resources that range from chemical entities to other living organisms to satisfy these requirements. The growth rate of individual species and the outcome of competition among species utilizing the same set of growth limiting resources is dependent on the availability of these resources. Each consumable resource for a particular species fulfills some part of the overall nutritional requirement and, hence, classifying resources based on their nutritional roles provides a systematic framework to elucidate the relationship between resource availability and the population dynamics of species. A general paradigm for resource classification, inspired from microeconomic theory, exists where representations for different resource pairs based on their effect on the growth of the species, or consumer, have been developed. Many of the efforts in developing such “consumer–resource” relationships have addressed inter-species competition in a traditional ecological sense. However, in many industrial processes, the dependence of the growth of a single species on the availability of multiple resources is itself, an issue of considerable importance. The number of resources, or substrates, as more commonly used in the microbiological parlance, that satisfy a particular nutritional requirement is usually vast. Therefore, a particularly important question is the identity of the rate-limiting substrate or substrates during mixed substrate growth.

For processes that involve growth on multiple nutrients, the identity as well as the number of rate-limiting substrates can change and, consequently, affect the growth dynamics of interest. In order to design processes to minimize excessive use of costly substrates or to avoid inefficient operating conditions, it is necessary to qualitatively and quantitatively characterize mixed substrate growth. An important and often encountered situation featuring mixed substrate growth is the presence of multiple carbon-energy sources. There have been a vast number of studies on the dynamics of microbial growth on multiple carbon-energy sources, beginning with the pioneering work of Monod (1942). These studies involve both batch and continuous cultures and establish certain general features about the nature of growth and substrate utilization.

In this article, the consumer–resource relationship for microbial growth on carbon sources is characterized for substrate pairs that exhibit qualitatively different interactions using the concepts of resource classification. We begin by discussing the different schemes for resource classification proposed in the literature, particularly the concept of a “constant growth isocline.” These isoclines provide a means for representing the interactions between substrates under multiple-nutrient growth and to identify the different domains of substrate concentrations where the qualitative nature of the interaction changes, thereby changing the set of rate-limiting substrates. Applying the knowledge gained from several batch and chemostat experiments, the nature of isoclines that a particular substrate pair would exhibit is then deduced. Ramakrishna et al. (1996) have developed a cybernetic model for microbial growth which is able to describe the diverse patterns of substrate utilization on carbon mixtures. This model is used to simulate steady state
growth patterns in a chemostat and construct isoclines that clarify the deduced consumer-resource relationship. The results highlight the changing nature of substrate interactions over the spectrum of attainable growth rates and consequently the change in the identity of the rate-limiting substrate(s) at different combinations of substrate concentrations, as well as different growth rates during mixed substrate growth.

CLASSIFICATION OF RESOURCES

The concept of *indifference curves* was borrowed from microeconomic theory initially by Rapport (1971) and Covich (1972) to elucidate the consumer-resource relationship for a single species, viz., the consumer, whose growth is limited by two resources. An indifference curve represents different combinations of resources that are equally preferred by the consumer and is represented on a graph with the resource concentrations on the axes. For the same set of resources a map of indifference curves, each corresponding to some constant level of benefit derived from resource consumption can be constructed to represent the consumer-resource interactions and the shape of the indifference curve reflects the preference of the consumer for the two resources. For biological systems, this benefit from resource consumption, has generally been considered to be the growth rate of a population of a particular species.

Tilman (1980, 1982) extended these ideas to develop classifications for the different interactions between resource pairs. The dependence of population growth on the joint availability of two resources is the basis for a pairwise classification of resources. The mathematical model for describing the growth of *n* species on *k* resources is as follows:

\[
\frac{dX_i}{dt} = f_i(R_1, \ldots, R_k) - m_i \tag{1}
\]

\[
\frac{dR_j}{dt} = g_j(R_j) - \sum_{i=1}^{n} X_i f_i(R_1, \ldots, R_k) h_{ij}(R_1, \ldots, R_k) \tag{2}
\]

\(X_i\) is the population density of species *i* and *m* the mortality rate; \(R_j\) is the availability of resource *j*; \(f_i\) is the functional dependence of the specific growth rate on the resource availabilities; \(g_j\) is the resource supply rate and \(h_{ij}\) describes the fraction of resource *j* required to form each new individual of species *i*. The equations assume that species interact only through the use of their resources and that resources are not interactive, i.e., that

\[
\left(\frac{\delta g_j}{\delta R_j}\right)^* = 0 \text{ for } i \neq j,
\]
evaluated at equilibrium. That is, the uptake of a particular resource is not explicitly dependent on the availability of the other resources. The indifference curves plotted for constant \(f_i\)'s are called zero net growth isoclines or ZNGIs. The growth isoclines for different resource classes as proposed by Tilman (1980, 1982), relevant to later discussion, have been shown in Figure 1.

In Figure 1A, 1B and 1C the isoclines intersect both the \(R_1\) and \(R_2\) axes, implying that a given growth rate can be

![Image of Figure 1](image_url)

*Figure 1.* Isoclines representing consumer-resource relationships for different resource types according to Tilman. Adapted from Tilman (1982).
maintained in the absence of either resource. When the isoclines are straight lines, one resource can be substituted in direct proportion to the other and, hence, $R_1$ and $R_2$ are termed to be perfectly substitutable resources with respect to each other. When the isoclines are bowed in towards the origin, the amount of $R_1$ and $R_2$ required to sustain a certain growth rate is less than that needed if they were perfectly substitutable resources. This is indicative of a synergistic effect in the consumption of $R_1$ and $R_2$ and hence, Tilman’s definition of these resources as a “complementary” set. In Figure 1C, the resources interact in a manner where only one substrate at any time is limiting, except for medium composition corresponding to the corner. This is an extreme case of the isocline for two resources that show an antagonistic effect, that is, when both resources are present the amount of $R_1$ and $R_2$ required to maintain the growth rate is higher than the perfectly substitutable case. Figures 1D and 1E, where the isoclines do not intersect either axes, represent resources that are both required for growth to be possible. The isoclines in Figure 1D are for resources where there is no substitutability between $R_1$ and $R_2$ and growth is always limited by one or the other. Therefore, it is limiting along the horizontal part of isocline $R_2$ and along the vertical, $R_1$. When the resources can partially substitute for one another, then the isoclines show a curved corner, indicating that $R_1$ and $R_2$ exhibit some substitutability. Nevertheless, in the absence of either resource, no growth would be possible. The resources in Figure 1D are termed essential and those in 1E, interactive essential. Figure 1F shows isoclines where growth is possible on $R_2$ alone, and that $R_1$ may partially substitute for $R_2$. Therefore, the isoclines intersect $R_2$ axis and run parallel to the $R_1$ axis and Tilman refers to these resources as “hemi-essential.”

Baltzis and Fredrickson (1988) have used a set theoretic scheme for classifying nutrient pairs that considers four cases, depending on the intersection of sets $S_1$ and $S_2$. A set represents the chemical requirements that are satisfied by the nutrient, $S$, and the resultant intersection of the two sets indicate the interaction between the substrates. The four cases considered are shown in Figure 2. The difference in terminology used by Tilman and by Baltzis and Fredrickson is unfortunately, a potential source for confusion and we shall clarify the semantics in the following paragraphs. In the first case (Figure 2A), where the two sets satisfy completely different requirements, $S_1$ and $S_2$ are considered complementary to each other, while Tilman refers to these as essential. Case 2B is analogous to the interactive essential case considered by Tilman, where there is a certain amount of substitutability between $S_1$ and $S_2$. The term, substitutable, has been used in the same manner by both authors (Figure 2C), although Tilman has various sub-classes for substitutable substrates. The final case of Baltzis and Fredrickson (Figure 2D) is analogous to the hemi-essential case as considered by Tilman. These set–theoretic ideas have been used by Pavlou and Fredrickson (1989) to focus on microbial growth and develop concepts to identify choices that arise when growth is limited by multiple nutrients. Their analysis for growth on a pair of substitutable substrates is reviewed later in this article.

**Microbial Growth on Carbon Sources**

Investigations on the growth of microorganisms on mixtures of substitutable substrates have almost exclusively dealt with carbon—energy sources. Carbon-energy sources such as sugars, hydrocarbons and organic acids fulfill the growth requirements for carbon and energy for microbial growth. Based on the earlier discussion, carbon-energy sources are classified as substitutable since each by itself can satisfy the nutritional requirement for carbon and energy.

**Batch and Chemostat Growth Patterns**

There are two general patterns of substrate utilization that manifest themselves during growth on carbon mixtures in batch growth where substrate concentrations are sufficiently high to maintain the maximum specific growth rate. Monod (1942), in his work on the growth of bacteria on mixtures of sugars, observed sequential and simultaneous utilization of substrates. In diauxic growth or sequential utilization of carbon sources, the preferred substrate is usually one which supports a higher specific growth rate. Narang (1994) observed, in a systematic study of the growth of *Escherichia coli* on organic acids, several interesting features of simultaneous utilization of carbon sources. The specific growth rate on pairs of organic acids was significantly higher than the maximum specific growth rate ($\mu$ max) on either substrate alone. In addition, the specific substrate uptake rate was lower in the mixed substrate case compared to single substrate uptake rates. Simultaneous utilization is generally observed with substrates that support significantly lower growth rates compared to the highest growth rate observed for the particular microbial species.

Many studies with continuous cultures (Baloo and Ramkrishna, 1991; Egli et al., 1986; Harder and Dijkhuizen, 1982) reveal a characteristic trend with changing dilution rates for substrates that show diauxic growth in batch cultures. The substrates are consumed simultaneously at low
rates higher than growth both substrates continue to be utilized at dilution rates by Brinkmann and Babel (1992). In mixed-substrate cultures by Brinkmann and Babel (1992), Ramkrishna et al. (1996) have developed a model based on the same cybernetic principles, to describe a spectrum of growth patterns observed with growth on mixtures of carbon—energy sources.

The model considers an expanded kinetic structure featuring the formation of key biosynthetic precursors and enzymes and the regulation of these processes. 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 $S_2$ which initially forms $M_2$ from which $M_1$ is synthesized. This scheme is shown in Figure 3. 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 3. For instance, the formation of $M_2$ can either be through uptake of $S_2$ 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 $S_2 \rightarrow M_1$ 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 $M_1$ is $S_2 \rightarrow M_2$, with $M_1$ being synthesized from $S_1$, then it leads to the case of simultaneous utilization.

**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 \( E \) from \( S \) is considered as
\[
\dot{r}_{s_{m_{1}}E} = \frac{e_{s_{m_{1}}E}}{s_{1} + K_{s_{m_{1}}E}} r_{s_{m_{1}}E} \tag{3}
\]
and the synthesis rate for the enzyme \( E_{s} \) is
\[
\dot{r}_{E_{s}} = \frac{r_{E,\text{max}}}{s_{1}} \tag{4}
\]
The rate expression applies only to the inducible synthesis of \( E_{s} \), and the overall rate of enzyme synthesis will include a constitutive rate of synthesis,
\[
\dot{r}_{E,\text{const}}
\]
The rate expressions for the other processes involved in the synthesis of \( M \) and \( E \) 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, to account for the dependence on both precursors, of growth and is given by
\[
r_{G} = r_{G,\text{max}} \left( \frac{m_{1}}{K_{G,m_{1}} + m_{1}} \right) \left( \frac{m_{2}}{K_{G,m_{2}} + m_{2}} \right) \tag{5}
\]
The synthesis of a key enzyme for the growth step is not considered in the model. The assumption 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 viewpoints of Jensen and Pedersen (1990) that the machinery for macromolecular synthesis is subordinated 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:
\[
u_{s_{m_{1}}} = \frac{Y_{s_{m_{1}}/m} r_{s_{m_{1}}E}}{Y_{s_{m_{1}}/m} r_{s_{m_{1}}E} + r_{m_{1}m_{2}}} \tag{6}
\]
\[
u_{m_{1}} = \frac{r_{m_{2}m_{1}}}{Y_{s_{m_{1}}/m} r_{s_{m_{1}}E} + r_{m_{1}m_{2}}} \tag{7}
\]
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 for batch growth, while the terms in square brackets constitute those to be added to obtain the continuous culture model:
\[
\frac{ds_{1}}{dt} = - \frac{1}{Y_{s_{m_{1}}} m_{1}} r_{s_{m_{1}}E} v_{s_{m_{1}}} c \left[ + D(s_{1} - s_{1}) \right] \tag{8}
\]
\[
\frac{ds_{2}}{dt} = - \frac{1}{Y_{s_{m_{1}}} m_{2}} r_{s_{m_{1}}E} v_{s_{m_{1}}} c \left[ + D(s_{1} - s_{1}) \right] \tag{9}
\]
\[
\frac{dm_{1}}{dt} = Y_{s_{m_{1}}/m} r_{s_{m_{1}}E} v_{s_{m_{1}}} + r_{m_{1}m_{2}} v_{m_{2}} - \frac{1}{Y_{m_{1}}} r_{G} Y_{G,m_{1}} - r_{G} m_{1} \tag{10}
\]
\[
\frac{dm_{2}}{dt} = Y_{s_{m_{1}}/m} r_{s_{m_{1}}E} v_{s_{m_{1}}} + r_{m_{1}m_{2}} v_{m_{2}} - \frac{1}{Y_{m_{1}}} r_{G} Y_{G,m_{1}} - r_{G} m_{2} \tag{11}
\]
\[
\frac{dc}{dt} = r_{G} c \left[ - D c \right] \tag{12}
\]
\[
\frac{de_{s_{m_{1}}}}{dt} = r_{E_{s_{m_{1}}}} + r_{E_{s_{m_{1}}}} u_{s_{m_{1}}} - r_{G} e_{s_{m_{2}}} - \beta_{s_{m_{1}} e_{s_{m_{1}}}} \tag{13}
\]
\[
\frac{de_{s_{m_{2}}}}{dt} = r_{E_{s_{m_{2}}}} + r_{E_{s_{m_{2}}}} u_{s_{m_{2}}} - r_{G} e_{s_{m_{2}}} - \beta_{s_{m_{2}} e_{s_{m_{2}}}} \tag{14}
\]
\[
\frac{de_{m_{1}m_{1}}}{dt} = r_{E_{m_{1}m_{1}}} + r_{E_{m_{1}m_{1}}} u_{m_{1}m_{2}} - r_{G} e_{m_{1}m_{2}} - \beta_{m_{1}m_{1} e_{m_{1}m_{1}}} \tag{15}
\]
\[
\frac{de_{m_{2}m_{1}}}{dt} = r_{E_{m_{2}m_{1}}} + r_{E_{m_{2}m_{1}}} u_{m_{1}m_{2}} - r_{G} e_{m_{1}m_{2}} - \beta_{m_{2}m_{1} e_{m_{1}m_{2}}} \tag{16}
\]
The term, \( r_{G} e_{m_{1}m_{2}} \), 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 formation processes is achieved by multiplying the intrinsic rate with the appropriate cybernetic variable.

**GROWTH ISOCLEINES FOR CARBON-ENERGY SOURCES**

Pavlou and Fredrickson (1989) have used cybernetic ideas to identify the diverse interactions possible under multiple nutrient limitations. A brief discussion of their analysis for growth on substitutable substrates is presented here. Subsequently, we present our deduction of the possible interactions during growth on substitutable substrates, using the information on the growth patterns observed during growth on carbon-energy sources, gathered from various studies.
**Possible Isoclines from Different Nutrient Use Patterns**

Growth on substitutable nutrients $N_1$ and $N_2$ is assumed to offer four possible choices for satisfying the microorganism’s carbon and energy requirements. These choices are represented through “nutrient use pattern vectors,” or $U_i$. There are four possible nutrient use vectors, $U_1$ where both carbon and energy requirements are satisfied by the uptake of $N_1$, $U_2$ where $N_2$ satisfies both requirements, $U_3$ where $N_1$ is taken up as a carbon source alone and $N_2$ fulfills energy requirements and finally, $U_4$ where $N_2$ is used as the carbon source and $N_1$ as the energy source. They assume the nutrient pattern vector chosen is based on cybernetic ideas and that vector is one which supports the highest growth rate at the prevailing substrates concentration in the medium. For each $U_i$ there is an associated $\mu_i(s)$ and the $U_i$ chosen is

$$\mu(s) = \max_i [\mu_i(s)], \quad i = \ldots, 4$$

Pavlou and Fredrickson consider steady state growth such as in a chemostat and develop the possible isoclines for two differing situations. For each nutrient use vector, when all other vectors are hypothesized to be inactive, the isoclines for a fixed specific growth rate, arbitrarily considered to be 1 unit, are either straight lines parallel to the ordinate ($U_1$), parallel to the $N_1$ axis ($U_2$) or a curve characteristic of the perfectly essential case considered by Tilman (1982) (Figure 1C) for $U_3$ and $U_4$. Each isocline represents the dependence of growth rate on the prevailing medium concentrations of $N_1$ and $N_2$ for the corresponding nutrient vector. When all choices are available to the microorganism, the pattern vector chosen for the fixed growth rate over a range of substrate levels can be represented on the isocline map as a portion of the isocline corresponding to the $U_i$. Figure 4 represents two possible scenarios, one where only $U_1$ and $U_2$ are preferred and the other where $U_1$, $U_2$ and $U_3$ are preferred. The latter case represents a scenario where the cell chooses the uptake of $N_1$ and $N_2$ as the optimal strategy for growth and hence, the portion of the isocline represented by $U_3^{(1)}$ and $U_3^{(2)}$ reflects the complementary (or essential) role of the two substrates. The first scenario is analogous to the switching case proposed by Tilman (1982).

This analysis reveals the diversity of interactions possible during growth on two substitutable substrates, since the aforementioned scenarios are not the only ones possible. However, the number of cases where a microorganism uses one substrate exclusively for carbon and the other for energy is quite limited. The use of formate as an energy source for fixing carbon dioxide (Dijkhuizen et al., 1980) is one situation where this may be true. For the majority of substrates, it is not possible to distinguish whether a substrate is exclusively satisfying carbon or energy requirements once each substrate enters the central metabolic pathways and is converted to one of many carbon precursors.

**Substrates Exhibiting Diauxic Growth**

For single substrate growth in chemostats, the dilution rate at which washout occurs, $D^{co}$, is equal to the maximum growth rate, $\mu^{max}$ on that substrate. In continuous cultures involving two substrates, $S_1$ and $S_2$, where the former is the preferred substrate supporting a higher growth rate, $S_2$ is not utilized beyond a dilution rate slightly higher than $\mu_2^{max}$, say $\mu_2^{max}$, the $D^{co}$, at which $S_1$ ceases to be utilized is the same $D^{co}$ as in single substrate growth, i.e., $\mu_1^{max}$. Consider then, the division of the range of dilution rates up to $\mu_1^{max}$ into the subranges, $D < \mu_2^{max}$, $\mu_2^{max} < D < \mu_2^{max}$ and $\mu_2^{max} < D < \mu_1^{max}$. In the first range, it is possible to sustain growth in the presence of $S_1$ or $S_2$ alone; therefore, for some fixed $D$ in this range all possible combinations of $S_1$ and $S_2$ will form an isocline which intersects both $S_1$ and $S_2$ axes.

For any dilution rate in the next range, the presence of $S_1$ is necessary but the presence of $S_2$ is not required to sustain
growth and so the resultant isocline will resemble the hemi-essential case proposed by Tilman (Figure 1F) or case D in Figure 2. At dilution rates higher than \( \mu_{max}^{mix} \), the situation is one of just single substrate growth on \( S_1 \) and therefore, the isocline will be parallel to the \( S_2 \) axis.

**Substrates Exhibiting Simultaneous Utilization**

For substrates that are used simultaneously in batch cultures, there are again three ranges of specific growth rates that can be considered. If \( \mu_{max}^1 \) is greater than \( \mu_{max}^2 \), then at dilution rates less than \( \mu_{max}^2 \), \( S_1 \) and \( S_2 \) interact in a substitutable manner since \( S_1 \) or \( S_2 \) alone will support growth at that particular dilution rate. However at dilution rates between \( \mu_{max}^1 \) and \( \mu_{max}^2 \), the presence of \( S_2 \) alone will not support growth at that particular dilution rate. Therefore, the isocline will indicate the hemi-essential nature of interaction between \( S_1 \) and \( S_2 \). At a dilution rate higher than \( \mu_{max}^1 \), unless both substrates are present, no growth is possible. \( S_1 \) and \( S_2 \) will therefore interact as complementary substrates (or essential as defined by Tilman, 1982).

**MODEL SIMULATIONS**

The cybernetic model detailed earlier has previously described successfully (Ramakrishna et al., 1996), experimental data for batch growth of *Escherichia coli* on mixtures of carbon sources including glucose and organic acids such as fumarate, pyruvate and succinate (Narang, 1994). Thus, the model predicted simultaneous utilization of substrates during growth on fumarate–pyruvate and succinate–pyruvate mixtures and diauxic growth on glucose–fumarate mixtures with preferred uptake of glucose. The same parameter values have been used in the simulations here. The model is modified for a continuous culture system by incorporating the appropriate feed and washout terms and was applied to generate isoclines for the situations described previously. The 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 (Hindmarsh, 1983).

Two situations for which batch growth data has been successfully described and are representative of the two general cases of substrate utilization discussed here are considered. The fumarate–pyruvate system is an example of a class of substrate pairs that lead to simultaneous utilization in batch growth. Glucose–fumarate is used as an example for systems that show diauxic growth in batch cultures where glucose is the preferred substrate and would therefore exhibit chemostat patterns leading to exclusive utilization of glucose at high dilution rates.

Figures 5 and 6 show the residual steady state substrate concentrations with changing dilution rates for the fumarate–pyruvate and the glucose–fumarate systems respectively. The model predicts the expected trends for mixed substrate growth versus single substrate growth. As seen in Figure 5, fumarate and pyruvate are simultaneously utilized up to a dilution rate higher than the critical dilution rates for either substrate alone. The critical dilution rate \( (D^{cr}) \) is the dilution rate at which washout of biomass occurs during single or mixed substrate growth. \( D^{cr} \) corresponds to the \( \mu_{max} \) on that particular system. For glucose and fumarate (Figure 6), fumarate is simultaneously utilized up to a dilution rate slightly higher than the \( D^{cr} \) observed with single

![Figure 5](image-url)  
**Figure 5.** Residual steady state substrate concentrations with changing dilution rates for growth on fumarate and pyruvate.
substrate growth on fumarate alone. The $D^c_f$ at which wash-out occurs is, as expected, the same as the $D^c_g$ for glucose alone, since glucose is preferentially consumed at higher dilution rates in mixed substrate growth. At lower dilution rates, the glucose concentration is very low; thereby the enzymes for the uptake of fumarate are not repressed consequently leading to simultaneous utilization. At higher dilution rates, as the concentration of glucose within the chemostat increases, the uptake of fumarate ceases due to catabolite repression of the fumarate metabolizing enzymes. The enzymatic composition of the microorganisms are dependent on the dilution rate of the culture. Hence, the substrate concentration profiles shown in Figure 6 for glucose in mixed as well as single substrate growth are almost iden-

Figure 6. Residual steady state substrate concentrations with changing dilution rates for growth on glucose and fumarate.

Figure 7. The isocline for growth on fumarate and pyruvate at a dilution rate of 0.15 h$^{-1}$. 
tical. These simulations are in agreement with the growth patterns discussed earlier, so we can proceed with the necessary calculations for generating the various isoclines.

For the fumarate-pyruvate system three dilution rates are chosen: (a) below the $D^{cr}$ for pyruvate in single substrate growth, (b) between the $D^{cr}$ for pyruvate and the $D^{cr}$ for fumarate in single substrate growth, and (c) between the $D^{cr}$ for fumarate and the mixed substrate $D^{cr}$. For chemostat growth on glucose and fumarate, there are again three domains of dilution rates to be considered: (a) below the $D^{cr}$

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**Figure 8.** The isocline for growth on fumarate and pyruvate at a dilution rate of 0.26 h$^{-1}$.

**Figure 9.** The isocline for growth on fumarate and pyruvate at a dilution rate of 0.35 h$^{-1}$.
for fumarate in single substrate growth, (b) between the $D^{f}$ for fumarate in single substrate and the dilution rate at which fumarate ceases to be utilized during mixed substrate growth ($D^{f,\text{mix}}$), and (c) between $D^{f,\text{mix}}$ and the $D^{g}$ for the mixture which is the same as the $D^{g}$ during growth on glucose alone. A representative dilution rate in each range is chosen, and for a range of feed concentrations, the steady state residual substrate concentrations during mixed substrate growth are calculated and the resultant isocline is plotted.
RESULTS

Figures 7–9 show the isoclines at three different dilution rates for the fumarate–pyruvate system. These isoclines are obtained by plotting one residual steady state substrate concentration versus the other. The isocline for $D = 0.15 \text{ h}^{-1}$ (Figure 7) intersects both axes and is indicative of the substitutable nature of substrate uptake. The isocline is seen to be composed of three distinct segments where the curved portion represents the "interactive substitutable" nature of the substrates, $S_1$ (fumarate) and $S_2$ (pyruvate) over a certain domain of substrate comparison. At high $S_2$ and low $S_1$

![Graph](image1.png)

**Figure 12.** The isocline for growth on glucose and fumarate at a dilution rate of 0.5 h$^{-1}$.

**Figure 13.** Rate of substrate uptake versus dilution rates for growth on fumarate-pyruvate mixtures compared to single substrate growth on fumarate and pyruvate.
concentrations, the curve runs parallel to the $S_1$ axis, since at these substrate levels, $S_2$ alone is limiting and any change in $S_1$ will not lead to an increase in growth rate. However, at low $S_2$ and high $S_1$ compositions, $S_1$ alone is limiting; this is evident from the segment of the curve parallel to the $S_2$ axis. This isocline is somewhat similar to the one proposed by Pavlou and Fredrickson (1989) (Figure 4b), where there are three distinct nutrient uptake choices, one of which is preferred depending on the substrate compositions. The residual steady state concentrations of either substrate during growth on the mixture is always lower than those observed during single substrate growth in the interactive substitutable domain of substrate concentrations, and is consistent with the observations of Lendenmann et al. (1996).

For $D = 0.26 \, \text{h}^{-1}$, the resultant isocline (Figure 8) reflects the essential role of fumarate and the non-essential role of pyruvate, since the curve only intersects the $S_1$ axis as no growth is possible when pyruvate is the sole carbon source. There are two domains of substrate composition, one where $S_1$ alone is limiting and the other where both substrates are limiting. At this dilution rate, the substrates interact in a manner qualitatively similar to the hemi-essential case proposed by Tilman (Figure 1F). When the dilution rate is increased to a level higher than the $D^{cr}$ for either substrate in single substrate growth, the isocline (Figure 9) does not intersect either axis, since both substrates are always required for growth. As expected, the isocline reflects the essential (Tilman, 1982) or complementary (Baltzis and Fredrickson, 1988) nature of $S_1$ and $S_2$. Since the isocline is not sharply right-angled, it is rather interactive-essential or interactive-complementary.

In the case of glucose ($S_1$) and fumarate ($S_2$), at a dilution rate of $0.2 \, \text{h}^{-1}$, which is below the $D^{cr}$ for fumarate in single substrate growth, the isocline (Figure 10) is similar in form to the first case in the previous system, showing the substitutable nature of either substrate. When the isocline at $D = 0.3 \, \text{h}^{-1}$ is considered (Figure 11), it is seen that the isocline does not intersect the $S_2$ (fumarate) axis, since sustenance of growth requires the presence of glucose. For a dilution rate in the range where glucose alone is preferentially consumed, the isocline as expected, is just a straight line parallel to the ordinate (Figure 12).

**DISCUSSION**

The isoclines for the two cases, each representing a distinctive substrate uptake pattern, are in agreement with the deduced consumer-resource relationship. It is clear that the identity of the rate-limiting substrate(s) changes with changes in feed composition at the same dilution rate. In addition, changes in dilution rates bring about qualitative changes in substrate interactions.

The optimal nature of microbial growth is evident, particularly in the specific rate of substrate consumption over the range of dilution rates. As shown in Figure 13, the uptake rate of both fumarate and pyruvate in mixed substrate growth is lower than the single substrate uptake rates. During growth on glucose and fumarate, the substrate uptake rate for both substrates at dilution rates where simultaneous growth is observed is lower than the single substrate uptake rate (Figure 14). However as expected, when glucose is preferentially utilized the uptake rate is the same as in single substrate growth. These simulations are consistent with the experimental observations for growth on xylose—
glycerol and glucose–xylose mixtures (Brinkmann and Babel, 1992). Cybernetic models consider enzyme synthesis to be regulated based on the optimal allocation of key resources which are limited in availability. Therefore, in mixed substrate growth, the reduction of specific substrate uptake rates during simultaneous utilization may be attributed to the cell implementing an optimal strategy for growth by attenuating the substrate uptake rates through the control of enzyme levels and activities. In addition, when the uptake of an inferior substrate is infeasible beyond a certain growth rate, the faster substrate is preferentially utilized.

An important application of the ideas of Tilman and others is in the area of population ecology, where questions dealing with dominance of species, the effects of resource competition on the diversity of a biotic community among others abound. The stress of course, is on inter-species competition and Tilman (1982) has used a theory that is a graphical approach towards analyzing the equilibrium structure of communities and their resources. The isoclines shown in Figure 1 are important in this analysis. However, the qualitative variation in isoclines as demonstrated here over the range of growth rates is not recognized, and there is an implicit assumption that a particular system will exhibit only one kind of isocline. Even recent applications of this theory (Smith, 1993) persist in assuming that once a resource pair is classified as substitutable or complementary, the isoclines are of a fixed nature. In fact, a recent model for the analysis of growth kinetics on multiple nutrients (Haas, 1994) is based in part on the supposedly characteristic isoclines that particular pairs of substrates would exhibit.

It is clear however that while two substrates may be perfectly substitutable in a qualitative sense, i.e., in their ability to sustain growth, a diversity of interactions during mixed substrate growth is observed depending on the specific growth rate (dilution rate) imposed on a microbial growth system. Approaches such as the ratio theory used when studying population interactions must recognize that the growth rate that a species seeks to achieve is pivotal to deciding how the species interacts with its resources and determining the characteristic isoclines or indifference curves. Cybernetic models which have well demonstrated capabilities for describing these diverse interactions are ideally suited to extending the applications of such equilibrium analyses to the more natural case of transient growth situations.

CONCLUSIONS

Hypothesized indifference curves or zero net growth isoclines for microbial growth on carbon sources were developed. The analysis reveals a diversity in the nature of the isoclines over the range of growth rates for a pair of substrates that are completely substitutable for each other in their nutritional roles. A cybernetic model previously developed for describing microbial growth dynamics on mixtures of carbon sources, was adapted to simulate chemostat growth on carbon mixtures. The isoclines at different constant dilution rates generated through model simulations were in agreement with the isoclines deduced from experimental results involving batch and chemostat growth. The work reveals that substrates satisfying identical nutritional requirements can interact in substitutable and complementary manners. In particular two qualitatively different isocline maps exist for substrate pairs that either exhibit preferential or simultaneous utilization in batch cultures. These results indicate that approaches that implicitly assume a constancy in the nature of the isocline map for a given resource composition to analyze species-resource interactions are subject to serious errors.

References


