Microbial growth requires the presence of several complementary nutrients within the growth medium. Each of these nutrients supplies a different nutritional requirement, e.g., carbon or nitrogen. Furthermore, nutrients that supply the same nutritional requirement, i.e., substitutable nutrients or (substrates), may also be present. As the limiting substrate is altered, a microorganism's internal structure is also altered by preferentially invoking different metabolic pathways for the utilization of the limiting substrate. Transitions between different pathways are moderated by processes associated with metabolic regulation. The present paper describes an objective-oriented approach to account for such regulatory processes. Ramkrishna and co-workers [Kompala, D. S.; et al. Biotechnol. Bioeng. 1986, 28, 1044–1055; Ramkrishna, D.; et al. Biotechnol. Prog. 1987, 3, 121–126; Turner, B. G.; et al. Biotechnol. Bioeng. 1989, 34, 252–261] have developed a cybernetic or goal-seeking modeling perspective that accounts for metabolic regulation in a simple manner by utilizing the optimal behavior of microorganisms when presented with a set of substitutable carbon sources. A single, consistent framework that addresses both substitutable and complementary nutrients is presented. Rather than consider all possible substrate combinations, a topological analysis of metabolic pathways is discussed that suggests that pathways may be derived from three structural types: linear processes, branch points, and cycles. Optimal objectives, from which appropriate metabolic regulation in the form of control variables can be derived, are stated for each of these structures. A complete analysis, however, is presented for only two branch point structures. We derive regulation for convergent branch points or substitutable processes, which is equivalent to the original cybernetic framework, as well as regulation for divergent branch points or complementary processes. The present approach greatly reduces the number of alternatives that must be considered since a process perspective can be easily abstracted. The regulation of substrates that are present in excess is also developed. Finally, the expanded cybernetic modeling framework is applied to a simple, model microbial system developed for the utilization of two complementary substrates under steady-state conditions. Within the model system, structural components such as biosynthetic intermediates and biopolymers are incorporated. The combined polymer concentration represents the biomass concentration. Only processes that ultimately result in polymer synthesis are considered, i.e., no maintenance processes are incorporated. Without defining the two complementary substrates as interactive or noninteractive, the proposed model is capable of exhibiting interactive, noninteractive, and intermediate behavior between these two extremes. Results indicate that the incorporated regulatory structures, i.e., cybernetic or control variables, preferentially activate the synthesis of the biosynthetic intermediate, which limits the specific growth rate. The proposed model is also capable of describing variable cell yields as the status of each of the two complementary substrates changes from limiting to nonlimiting. The resulting variable cell yields are in quantitative agreement with experimental data previously reported in the literature. Furthermore, the degree of interaction between two complementary substrates is predicted to be a function of the medium composition. The presence of biosynthetic intermediates in the model is shown to be capable of converting an interactive system to a noninteractive system; however, conditions for the reverse transition, i.e., a noninteractive to an interactive system, have not been identified at this time.
sensitivity of the flux with respect to a fractional change in enzyme concentration. Likewise, each concentration control coefficient provides a measure of the sensitivity of a metabolite in response to a fractional change in enzyme concentration. The magnitudes of these coefficients subsequently give the extent of control exerted by each step of the overall system. However, application of MCT requires complete knowledge of the in vivo control structure, as well as experimental measurements of intracellular conditions and metabolite concentrations. Furthermore, in the absence of models that describe the internal interactions of the system and provide for the variation of control coefficients, the predictive ability of MCT remains in question (Crabtree and Newsholme, 1985). More recently, MCT has been extended to calculating the flux and concentration control coefficients for branched (Cascante et al., 1989b) or more complex pathways (Cascante et al., 1989b; Hofmeyer et al., 1986). Besides MCT, biochemical system theory (Savegeau, 1972) and flux-oriented theory (Crabtree and Newsholme, 1985) (FOT) are two related approaches that have been proposed for the analysis of control structures in biochemical pathways.

All three approaches are based, either explicitly (Crabtree and Newsholme, 1985; Savageau, 1972) or implicitly, (Kacser and Burns, 1973; Kacser and Porteous, 1987), on a power-law formulation of the governing rate equations. The similarities and differences between these theories have been discussed (Cornish-Bowden, 1989; Savageau et al., 1987a).

The cybernetic framework (Ramkrishna et al., 1987) now offers an alternative method of identifying the control structure of a complex biochemical reaction network in a simple manner. The development of the cybernetic framework in the past has been focused primarily on the microbial response when limited by one or more carbon sources and subjected to high or low specific growth rates (Baloo and Ramkrishna, 1991; Kompala et al., 1986; Turner et al., 1989). Past work that has dealt with the utilization of complementary substrates (Alexander and Ramkrishna, 1991) has focused upon the problem of product or metabolite production rather than the issues that have been encountered under carbon-sufficient and carbon-limiting conditions (Holme, 1957; Neijssel and Tempest, 1975; Neijssel et al., 1984; Preiss, 1989). The objective of the current paper therefore is to propose a perspective of microbial growth that arrives at a set of general principles for the utilization and regulation of complementary as well as substitutable substrates. This expansion of the cybernetic framework is accomplished not by immediately incorporating any additional complexity of the medium, i.e., the presence of limiting complementary and substitutable substrates, but rather by reconsidering and expanding the structured model for growth on a single limiting substrate:

\[
S_j + B \xrightarrow{E_{Gj}} (1 + Y_{Bj})B
\]

where \(E_{Gj}\) is a key growth enzyme that catalyzes the synthesis of biomass \(B\) from substrate \(S_j\). The amount of biomass produced per unit substrate is given by the yield coefficient \(Y_{Bj}\). Any expansion of eq 1 therefore indicates that the presence of metabolic pathways must be considered and recognized.

The organization of this paper is as follows. It broadly comprises two sections. In the first, we present our treatment of metabolic pathways and the extended application of cybernetic principles as we have formulated them in the past (Kompala et al., 1986). This lays down the framework for the treatment of growth in the presence of limiting complementary nutrients, which is discussed in the second section. We have thus included a Conclusions subsection for both sections.

**Metabolic Pathways**

**Expanded View of Metabolism.** Within the original cybernetic framework (Kompala et al., 1986), growth on a single limiting carbon source was viewed as being void of alternatives from which a microorganism must choose in order to maximize its growth rate. Such a viewpoint is grossly in error and arises because the synthesis of biomass in eq 1 is lumped into a single process. The processes that are lumped in eq 1 do represent alternatives to the microorganism. Ignoring such alternatives may not alter the modeling results under many conditions; however, as the nature of the limitation changes, e.g., carbon- or nitrogen-limited, a serious departure between model predictions and experimental observations may arise if the model does not account for the significance of the processes lumped in eq 1. At this point, the view of metabolism must therefore be expanded to recognize not just a single key enzyme but potentially multiple key enzymes acting coordinately in one or more metabolic pathways. Our working definition of a metabolic pathway is therefore given as follows.

**Definition:** A metabolic pathway is a set of processes, each catalyzed by a key enzyme, that directs the flux of substrates through a set of intermediates toward one or more required products.

The presence of multiple products acknowledges that metabolic pathways are, in general, highly branched and cyclical in nature, thus serving as a source for many products and not necessarily a single end product. In eq 1, the presence of metabolic pathways was incorporated implicitly by lumping the outcome of all intermediate processes into a single final outcome. To recognize the potential significance of the intermediate processes under transient conditions, eq 1 must be "unlumped" into \(n\) distinct processes. This expanded view of growth may simply be represented by

\[
S_j + B \xrightarrow{E_1} E_2 \xrightarrow{E_n} (1 + Y_{Bj})B
\]

where \(E_1, E_2, \ldots, E_n\) represent a set of key enzymes that may act in series and/or in parallel to produce the final product, biomass. However, while not shown explicitly in eq 2, the presence of multiple key enzymes for the utilization of a single substrate necessarily implies the existence of one or more key intermediates from which measured biomass is synthesized. Equation 2 thus recognizes the presence of \(n\) alternatives to which the microorganism must allocate key cellular resources for the synthesis of \(n\) key enzymes.

Protein synthesis and therefore enzyme synthesis require material, energy, and temporal resources. With regard to material and energy resources, protein synthesis requires the presence of a number of cellular components, such as ribosomes, mRNA, tRNA, ATP, and polymerase enzyme molecules, among others. Furthermore, the amount of time that a polymerase molecule spends transcribing the genes for each cellular protein can also be considered as a resource (Kompala et al., 1986). In order to fulfill the requirements of cellular growth, structural proteins, r-proteins, biosynthetic proteins, catabolic proteins, etc., must be produced; there-
tion and the objective of a structure are intimately related, i.e., form implies function. Proposed objectives are therefore an implicit assumption that the configuration of the structure is most easily obtained without explicitly identifying them. In line with the contention that microorganisms implement regulatory strategies with outcomes consistent with a desired goal, an objective is proposed for each structure from which kinetic detail.

**Basic Postulates**

1. The primary goal of microorganisms is to maximize growth rate.
2. The objective of promoting any irreversible linear pathway is the maximization of that pathway's end product.
3. All end products produced at a branch point are essential for growth; therefore, cellular growth stops when any one end product is absent.
4. The objective of promoting any irreversible branched pathway is the maximization of the mathematical product of the end products produced by one or more branch points.
5. The objective of promoting any irreversible cyclic pathway is the maximization of the mathematical product of all the intermediate products of the cycle whether or not they were withdrawn for further utilization in order to maintain the integrity of the cycle.
6. 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.
7. Metabolic regulation is present in a hierarchical structure comprising both local and global levels.
8. As the status of a substrate is altered from limiting to nonlimiting, the excess substrate is available for the synthesis of additional structural and energetic resources.

**Topology of Metabolic Pathways**. A more fruitful method of analyzing metabolic pathways is to consider the topology of metabolism. This investigation therefore makes use of the geometric configuration of metabolism and assumes that any pathway may be constructed from four basic structures: linear segments, convergent branch points, divergent branch points, and cycles. Furthermore, each structure is considered as an independent unit from which appropriate metabolic regulation is derived for the synthesis and activity of the key enzymes contained within each structure. Simple examples of each of these structures are presented in Figure 1. This approach is therefore local rather than global in nature since it is assumed that results for individual structures may be combined in order to reconstruct any particular pathway that is pertinent to the current system of interest. In this way, all pathways may be accounted for without explicitly identifying them. In line with the contention that microorganisms implement regulatory strategies with outcomes consistent with a desired goal, an objective is proposed for each structure from which appropriate cybernetic or control variables are derived. Therefore, an implicit assumption is that the configuration and the objective of a structure are intimately related, i.e., form implies function. Proposed objectives for the structures described in Figure 1 are most easily stated in the form of the following postulates, which identify the basic modeling assumptions regarding the incorporation of metabolic regulation.

![Figure 1](image-url)
two types of branch points that comprise complementary and substitutable processes. This analysis assumes that by identifying the appropriate regulation for specific process types, the regulation of specific substrate types directly follows. By converting the problem from a comparison of substrates to a comparison of processes, the commonalities of all substrates, i.e., intermediates, are identified. Therefore, the scope of the problem is greatly reduced since the number of key intermediates required for microbial growth is considerably less in comparison to the vast array of substrates that may provide the nutrient requirements. A similar analysis of linear and cyclic structures has led to results that are equivalent to the results for convergent and divergent branch points, respectively (Straight, 1991).

This development of the cybernetic framework has concentrated upon the internal characteristics of metabolism and metabolic pathways; however, a broader understanding of the framework becomes apparent from the overview presented in Figure 2, which identifies the key components of the biophase, the environment, and their interactions. The biophase is composed of key enzymes, key resources, intermediates, and the remaining biomass. In addition, the environment may now contain potentially multiple limiting nutrients, identified as carbon, C, nitrogen, N, etc., which are converted into biomass by a set of processes commonly referred to as metabolism. The cybernetic perspective of cellular growth employs the assumption that a microorganism generally acts in an optimal manner; therefore, regulation of enzyme synthesis and activity is also included in the form of the cybernetic variables u and v. Superscripts s and c identify the regulation of substitutable and complementary processes, respectively. The expressions for these variables remain to be derived. In contrast to the original cybernetic perspective, Figure 2 acknowledges the complexity of the medium and the corresponding regulatory variables.

In past investigations (Kompala et al., 1986) the view has been that microbial behavior can be predicted from an economic perspective in much the same way that the behavior of a consumer can, in theory, be predicted. In other words, when given a set of n alternatives from which to choose on an equivalent basis, a consumer will invest available resources in such a manner to maximize the returns from his investment (Kamerschen and Valentine, 1977). The investment of resources to synthesize new enzymes is the biological equivalent. A second aspect of microeconomics is the utilization of previously invested resources. An example of this situation is the availability of preexisting equipment, i.e., new investment of resources is unnecessary. According to economic theory, when faced with this choice, a consumer will preferentially utilize the technology or equipment that maximizes the return from investment (Harris, 1979). The regulation of enzyme activity or enzymes already available is the biological equivalent. When presented with competing alternatives, the optimal usage of cellular resources, whether invested in the present or past, is assumed to be determined by the processes of metabolic regulation. Regardless of the microbial system, this philosophy has been present consistently within the past as well as the current development of the cybernetic framework.

**Substitutable Processes.** To demonstrate that the current analysis of metabolic pathways leads to results equivalent to the cybernetic variables derived earlier by Kompala et al. (1986), the analysis of substitutable processes is presented first. Within Figure 1, both the linear structure and the convergent branch point contain substitutable processes. Straight (1991) has demonstrated that the regulation of enzymes associated with linear and convergent branch point structures is functionally equivalent; therefore, only the results for convergent branch points are presented here.

When given a set of n alternatives, such as the synthesis of n key enzymes, with a fixed quantity of resource R to invest, let \( R_{ij} \) be the quantity of resource allocated to the jth method for producing the ith alternative and \( P_i \) be the returns obtained from the investment of \( R_j \). For the convergent branch point in Figure 1b consisting of two converging processes, each capable of producing \( P_i \), the total return is expressed as \( \sum_{j=1}^{n} P_{ij} \) (\( R_{ij} \)). According to postulate 4, the objective of any convergent branch point consisting of n converging processes that compete to produce a single product \( P_{ij} \) may be stated as

\[
\max_{R_{1}, R_{2}, ..., R_{n}} \left[ \sum_{j=1}^{n} P_{ij}(R_{ij}) \right]
\]

which is subject to the constraint \( \sum_{j=1}^{n} R_{ij} = R \). As a constrained maximization problem, the maximum for the total return is obtained when the \( R_{ij} \)'s are allocated such that

\[
\frac{dP_{i1}}{dR_{i1}} = \frac{dP_{i2}}{dR_{i2}} = ... = \frac{dP_{in}}{dR_{in}}
\]

Equation 4 can be rewritten to determine the general expression for the resource allocation policy:

\[
u_{ij} = dR_{ij} \sum_{k=1}^{n} dP_{ik} = dP_{ik} \sum_{k=1}^{n} dP_{ik}
\]

Equation 5 is equivalent in form to the matching law proposed by Herrnstein (1970) and the principle of equal marginal utilities per dollar found in microeconomics.
which states that the fractional allocation of resources must equal the fractional returns. If it is assumed that the allocation policy is implemented at every instant and the investment \( dR_{ij} \) is made in time \( dt \), then eq 5 may be rewritten as

\[
u_{ij}^* = r_{ij}/h \sum_{k=1}^{n} r_{i,k} \tag{6}
\]

where \( r_{ij} \) is the rate of producing the \( i \)th product from the \( j \)th substrate. The form of the cybernetic variable for the regulation of enzyme activity is determined from postulate 6 as

\[
u_{ij}^* = r_{ij}^{max}(r_{i,k}) \tag{7}
\]

Kompala et al. (1986) originally proposed a heuristic optimal strategy for the formulation of \( v_i \) [notation of Kompala et al. (1986)]. Since additive growth typically is not observed in the presence of substitutable carbon sources, Kompala et al. (1986) recognized the presence of a constraint on the utilization of a less preferred carbon source when a preferred carbon source is available. The result of this constraint was modeled as \( v_i \propto r_i \), the growth rate on the \( i \)th carbon source. Constraint of the activity variable to \( 0 \leq v_i \leq 1 \) leads to a formulation equivalent to eq 7; therefore, postulate 6 is a generalization of their result for different process types. Equations 6 and 7 are equivalent to the original cybernetic variables proposed by Kompala et al. (1986) to regulate the utilization of substitutable carbon sources; however, a specific process type has not been assumed here, and the results in eqs 6 and 7 are applicable to substitutable processes in general. Furthermore, the following results for divergent branch points indicate that the results derived by Kompala et al. (1986) are a subset of a more general problem.

While it has been stated that both the linear and convergent branch point structures contain substitutable processes, such a conclusion may not be as obvious for the linear process in Figure 1a. At first glance, the processes within the series structure appear to be alternatives for producing the intermediates rather than the end product. By assuming an irreversible linear pathway, the synthesis of any of the intermediates will ultimately result in the synthesis of the end product; therefore, each alternative is actually a substitutable alternative for producing the desired end product. However, every alternative may not be equivalent since at any given instant it may be more beneficial to promote an upstream process because downstream intermediates may not have accumulated; therefore, the microorganism must optimize its resource usage by the appropriate metabolic regulation, which is functionally analogous to eqs 6 and 7 (Straight, 1991).

**Complementary Processes.** In order to complete the current expansion of the cybernetic framework, the problem of complementary processes is now addressed. Within Figure 1, both the divergent branch point and the cyclic structure contain complementary processes. Earlier, the necessity of incorporating metabolic regulation when alternative choices are present was proposed. In the case of substitutable processes, both alternatives compete to produce the same product; however, in the case of complementary processes, both alternatives compete to utilize the same substrate.

For divergent branch points, the expression for the total return cannot be expressed as a summation, as in the case of convergent branch points, since such a form would imply that the microorganism could attain a nonzero objective even in the absence of one or more of the products produced by the branch point. This situation clearly violates postulate 3. Therefore, the total return for the divergent branch point in Figure 1c is assumed to be given by the product expression \( \prod_{i=1}^{n} P_{ij} \), where \( P_{ij} \) is the \( i \)th product produced from the \( j \)th substrate. The objective for a divergent branch point, consisting of \( n \) diverging processes that compete for a common substrate to produce \( n \) products, can thus be expressed using postulate 4:

\[
\max_{R_{ij}} \left[ \prod_{i=1}^{n} P_{ij}(R_{ij}) \right] \tag{8}
\]

which is subject to the constraint \( \sum_{i=1}^{n} R_{ij} = R. \) The objective function in eq 8 mimics the interaction of complementary substrates. In the absence of any one product produced at a divergent branch point, the objective function is zero. Likewise, in the absence of any one complementary substrate primary metabolic processes will stop. In contrast to eq 5, Heinrich et al. (1987) have proposed that optimization of the flux rate is the underlying objective of the evolutionary process. As before, the maximum for the total returns is obtained when the \( R_{ij} \)'s are allocated such that

\[
\frac{1}{P_{ij}} \frac{\partial P_{ij}}{\partial R_{ij}} = \frac{1}{P_{2j}} \frac{\partial P_{2j}}{\partial R_{2j}} = \ldots = \frac{1}{P_{n_j}} \frac{\partial P_{n_j}}{\partial R_{n_j}} = \frac{\partial P_j}{\partial R_j} \tag{9}
\]

Equation 9 can be rewritten as

\[
\frac{\partial (\ln P_{ij})}{\partial R_{ij}} = \frac{\partial (\ln P_{ij})}{\partial R_{ij}} \tag{10}
\]

and the general resource allocation policy can now be expressed as

\[
u_{ij}^* = \frac{\partial R_{ij}}{\partial R_{ij}} \tag{11}
\]

As in eq 5, if we assume that the allocation policy is implemented at every instant and the investment \( dR_{ij} \) is made in time \( dt \), then eq 11 may be rewritten as

\[
u_{ij}^* = \frac{r_{ij}/P_{ij}}{\sum_{k=1}^{n} r_{ik}/P_{kj}} \tag{12}
\]

where \( r_{ij} \) is the rate of producing the \( i \)th product from the \( j \)th substrate. In contrast to eq 6, the allocation policy for divergent branch points now accounts for the product levels as well as the process rates. Equation 11 thus incorporates feedback repression within the cybernetic framework without specifically identifying a kinetic mechanism to implement feedback control. Feedback repression of enzyme synthesis has been well established in biosynthetic processes (Jensen, 1969; Sanwal et al., 1971). The form of the cybernetic variable for the regulation of enzyme activity is determined from postulate 6 as
which is equivalent to feedback inhibition. From eqs 12 and 13 it is apparent that the resource allocation to the ith branch, as well as the activity of the key enzyme catalyzing the ith branch of a divergent branch point, is enhanced when the level of ith product is small. Therefore, from a substrate perspective rather than a process perspective, the regulation of substitutable processes promotes the utilization of the substrate that is least limiting, while the regulation of complementary processes promotes the utilization of the substrate that is most limiting. This result is consistent with the noninteractive model of complementary substrates that is so often invoked when complementary substrates are limiting. As will be shown in a subsequent paper, however, the prior identification of two complementary substrates as interactive or noninteractive is unnecessary when an appropriate regulatory structure is incorporated into the proposed modeling efforts.

While it has not been explicitly stated, it is interesting to note that the regulation of enzyme synthesis and activity is equivalent, regardless of process type, when expressed as a function of the process returns. Therefore, the general forms of the previously identified cybernetic variables may be expressed as

\[
\begin{align*}
\nu_i^c &= \frac{r_{ij}P_{ij}}{\max_k(r_{kj}P_{kj})} \quad (13) \\
\end{align*}
\]

where the returns have been identified as \(r_{ij}\) and \(r_{kj}P_{kj}\), for substitutable and complementary processes, respectively. In general, the cybernetic laws for computing the appropriate control variables for regulating the synthesis and activity of key enzymes have remained unchanged.

The analysis presented within this paper therefore indicates that the regulatory strategies in eq 14 appear to be more general than originally anticipated (Kompala et al., 1986). The application of regulatory strategies, which may be identified in future investigations, may therefore depend in large part on whether the appropriate returns can be identified for the appropriate process type.

Pathway Excess. The focus of this paper is devoted to presenting a simple alternative for describing the regulation of potentially complex metabolic networks. One of the key assumptions of this problem is that the resources available to a particular pathway are optimally distributed between the available alternative pathways. Therefore, given two alternative pathways, the one that is not limiting will contain excess resources and/or substrates that may be effectively utilized for other aspects of metabolism.

It is generally acknowledged that the efficiency of substrate, e.g., carbon source, utilization depends considerably on whether or not the substrate is limiting (Neijssel and Tempest, 1975). The classic example is overflow metabolism, which consumes the available carbon/energy source apparently as a means of disposing of energy that is unusable, i.e., excess energy. In addition, the overproduction of biosynthetic intermediates generally is observed. An objective-oriented view of overflow metabolism has been taken by Neijssel and Tempest (1975, 1984), who have proposed that a microorganism, when faced with energy-sufficient conditions, employs ancillary processes to produce the appropriate excess biosynthetic intermediates, so that the limiting substrate can be metabolized as efficiently as possible without additional limitations occurring. With regard to nitrogen limitation, the appropriate intermediates are pyruvate, 2-oxoglutarate, NADPH, and ATP. Therefore, the maintenance of high concentrations of biosynthetic intermediates implies that carbon uptake should not be stringently controlled. In short, overflow metabolism guarantees that the efficiency with which the growth-limiting substrate can be taken into the cell and metabolized is not further impeded by generating key intermediary metabolites at a high rate and excreting reactants that cannot be utilized.

From a cybernetic perspective, overflow metabolism generates excess resources from the availability of excess substrates and gives the organism an opportunity to reallocate such resources to processes that otherwise may have received a reduced level. An ability to reallocate critical resources would provide the organism a means of optimizing the utilization of the nonlimiting substrate, thereby ultimately optimizing its biomass productivity under the prevailing limitation. In order to account for the significance of overflow metabolism as the microorganism becomes carbon/energy-sufficient, a concept of pathway excess, which is equivalent to postulate 8, is proposed. Key biosynthetic intermediates are identified as structural resources, while ATP and the additional reducing equivalents that are generated during the synthesis of the biosynthetic intermediates are identified as energetic resources.

In general, it is possible to associate intermediates and/or pathways to a particular substrate or limitation (Neijssel and Tempest, 1975). Therefore, we assume that the amount of excess within a pathway is proportional to the difference between the potential specific growth rate supported by the characteristic intermediate of a pathway, \(\mu_{m_j}\), and the observed specific growth rate \(\mu\), i.e., \(\mu_{m_j} - \mu\). From this assumption, an appropriate cybernetic variable \(u_{x,m}\) can be expressed as

\[
\nu_{x,m} = \lambda(\mu_{m_j} - \mu) 
\]

(15)

Constraint of the value of \(u_{x,m}\) such that \(0 \leq u_{x,m} \leq 1\) implies that \(0 \leq \lambda \leq 1/(\mu_{m_j} - \mu)\). Therefore, \(\lambda = 1/\max(\mu_{m_j} - \mu)\) and \(u_{x,m}\) can be expressed as

\[
u_{x,m} = \frac{(\mu_{m_j} - \mu)}{\max(\mu_{m_j} - \mu)}
\]

(16)

As the characteristic intermediate becomes the limiting intermediate, \(\mu_{m_j}\) approaches \(\mu\) and no excess is available within the respective pathway. Therefore, \(u_{x,m}\) essentially acts as an on-off control signal that turns processes associated with the presence of excess substrate on, \(u_{x,m} = 1\), or off, \(u_{x,m} = 0\).

Equation 16 only addresses the issue of regulating enzyme activity for the utilization of excess substrate. As identified for enzymes that catalyze both substitutable and complementary processes, the regulation of enzyme synthesis and enzyme activity is necessary for a complete regulatory structure. From eq 16, an appropriate measure of the return from enzyme synthesis for the production of storage compounds might be expressed as the difference \((\mu_{m_j} - \mu)\). However, the synthesis of enzymes for the metabolism of storage compounds appears to
respond to the specific growth rate rather than the availability of excess substrate (Preiss, 1989); therefore, the cybernetic variable that has been proposed for the regulation of maintenance processes (Turner et al., 1989) has been utilized in conjunction with eq 6 to regulate the synthesis of enzymes associated with excess carbon utilization (Straight, 1991). This discrepancy does not invalidate the tenets of the cybernetic framework. It typically has been assumed (Kompala et al., 1986) that the returns for the two regulatory strategies in eq 14 are equivalent; however, there is no requirement incorporated within the cybernetic framework that states such an equivalence.

The preceding analysis assumes that just as excess carbon may be utilized to produce additional resources, under a non-carbon limitation there would be an analogous utilization of non-carbon substrates, which would be promoted for the ultimate synthesis of additional resources under a carbon limitation. This assumption is supported by the synthesis of polyphosphates and the storage of phosphate in cell wall constituents under conditions of excess phosphate, as well as the storage of nitrogen by cyanobacteria under conditions of nitrogen excess (Preiss, 1989). Just as there is an economic basis for the cybernetic regulation of enzyme synthesis and activity, there is also an economic basis for the synthesis and regulation of storage compounds. The economic analogy is the accumulation of inventory, which serves to decouple series processes and ultimately to maximize the final output productivity (Lewis, 1975).

**Conclusions.** This paper has addressed so far several of the aspects associated with describing and regulating metabolic pathways from a cybernetic perspective. The cybernetic framework in the past has been and in the future will continue to be an evolving entity; therefore, the current results are not intended to describe a finalized theory. The results obtained for the cybernetic variables do demonstrate that simple representations of regulatory mechanisms obtained earlier for substitutable processes are retained in the case of complementary processes and pathway excess. Furthermore, by identifying metabolic pathways that are composed of simple structural types, the original cybernetic variables (Kompala et al., 1986) are retained as well, thus demonstrating that an optimal perspective of metabolic regulation is sufficient to derive established regulatory strategies. Therefore, the expanded cybernetic framework is capable of addressing complex limitations that involve complementary and/or substitutable substrates, as well as substrates that fulfill both of these roles, e.g., amino acids.

Currently, and in the past, Baloo and Ramkrishna (1991), Kompala et al. (1986), and Turner et al. (1989) have been associated primarily with the RNA components of the protein-synthesizing system; however, future endeavors are aimed at including energetic resources in the cybernetic framework, which will require a generalized matching law to account for multiple resources. Furthermore, situations may arise in which resources are not limiting; therefore, objectives may not be constrained as in eqs 3 and 8, but rather objectives may be constrained by the availability of sufficient precursor molecules. An example of such a situation may be microbial growth in the presence of biosynthetic intermediates, e.g., pyruvate. In contrast to control coefficients proposed by MCT (Kaesz and Burns, 1973), cybernetic variables are not stated as definitions, but rather they are derived from specific process objectives. Subsequently, potential control sites can be quickly identified as well as understood from fundamental principles. Application of the expanded cybernetic framework is straightforward. When one is presented with a complex reaction network, a preliminary control strategy can be easily proposed by identifying the pertinent structures within the network and applying the appropriate cybernetic variables to regulate the synthesis and activity of the incorporated key enzymes. The expanded cybernetic framework, as proposed here, has recently been applied to quantitatively predict a continuous culture microbial system when limited by carbon or nitrogen under singly-limiting conditions, as well as when limited by carbon and nitrogen under dual-limited conditions (Straight, 1991). Both steady-state and transient conditions were investigated experimentally. These results are presented elsewhere (Straight and Ramkrishna, 1994).

**Growth on Complementary Nutrients**

We will now consider the ideas developed in the preceding section for growth on complementary nutrients. Previous efforts in modeling the utilization of limiting complementary substrates have relied on an unstructured modeling approach and classified substrates as either interactive or noninteractive in order to determine the specific growth rate (Bader, 1982; Lee et al., 1984; Baltzis and Fredrickson, 1988). [Lee et al. (1984), however, also used an elaborately structured model capable of predicting growth rate as a function of glucose and ammonium concentrations.] Although this distinction may implicitly recognize the presence of metabolic regulation, such classifications do not permit any possibility of a gradual transition between the two extremes. Furthermore, to identify substrates as interactive or noninteractive requires a priori knowledge about the system; such knowledge is seldom available. In contrast, the cybernetic framework (Ramkrishna et al., 1987) is intended to remove this limitation by identifying the significance of metabolic regulation, as well as incorporating the decision-making processes associated with regulation into the proposed models.

Baltzis and Fredrickson (1988) previously posed the question of whether cybernetic principles could be applied to modeling the utilization of complementary substrates. The response here is therefore intended to be in the affirmative. Pavlou and Fredrickson (1989) have also recognized the advantages of the cybernetic framework by accepting, as a working hypothesis, that free-living microorganisms utilize the resources of their environment to grow as fast as possible. Therefore, Pavlou and Fredrickson (1989) extended the postulates of cybernetic modeling to apply not only to choices between substitutable substrates but also to choices between nutrient use pattern vectors. Each vector contains a set of dynamically significant substrates that are available in the medium at concentrations that are potentially limiting. Since Pavlou and Fredrickson (1989) address issues associated with nonminimal media, nutrients that are substitutable or complementary, as well as nutrients that can fulfill multiple requirements, e.g., amino acids, were considered in their modeling efforts.

While the discussion presented by Pavlou and Fredrickson (1989) offers many interesting ideas, the perspective of their proposals is still external in nature, i.e., it does not offer insight into the control structures present within a microorganism. Therefore, Pavlou and Fredrickson (1989) still propose the standard method of determining the specific growth rate in the case when the dynamically significant substrates are complementary to each other, i.e., eq 22. The objective of this paper is to take an internal perspective and utilize the control
strategies proposed here in order to address, from a cybernetic modeling perspective, some of the phenomena that have been observed or may be observed when microbial growth is limited by complementary substrates. The final result is the ability of the cybernetic framework to represent the full range of behavior between and including interactive and noninteractive.

**Model System.** To demonstrate the significant effects of metabolic regulation on the degree of interaction exhibited between two complementary substrates, a simple though realistic metabolic network is proposed in Figure 3b. According to the method proposed in prior sections, this model system may be decomposed into both linear and divergent branch point structures; however, the current paper only considers the regulation of divergent branch points in order to test the applicability of the results to the regulation of complementary processes without additional influences from other competing regulatory strategies. Due to the complexity of microbial growth, model identification and simplification are extremely important. While models that incorporate many kinetic details of metabolism (Shuler and Domach, 1983; Domach et al., 1984) are useful for biochemical studies and possibly engineering applications, e.g., bioreactor design, problems associated with parameter estimation and implementation make such models unattractive for applications such as online process control. The problem, therefore, is incorporating the aspects of metabolism that are necessary for describing the observed data and, hopefully, for predicting results beyond the realm of experimental observations without overburdening the model.

Bader (1978) has suggested that the system given in Figure 3a would be necessary to observe noninteractive behavior between the two substrates S₁ and S₂; however, since the utilization of S₁ does not appear to affect the utilization of S₂ or vice versa, it is not clear that the same system would also be capable of exhibiting interactive behavior as well. In reality, some level of interaction exists between all of the available substrates that are converted into biomass. Therefore, the system in Figure 3a would be representative of only those systems in which the degree of interaction between S₁ and S₂ is weak. In order to resolve this limitation, the model system in Figure 3b is proposed. In the proposed model system, S₁, S₂, or both may be at a concentration that is potentially limiting. From these two substrates, three monomers M₁, M₂, and M₃ are produced. Monomer M₁ is produced from S₁, monomer M₂ is produced from S₂, and monomer M₃ is produced as a result of the interaction between S₁ and S₂. In the absence of either S₁ or S₂, cellular growth must stop because of the coupling between the two substrates to produce M₃; therefore, S₁ and S₂ are complementary, i.e., each substrate provides a nutrient that is unavailable from the other. Once M₁, M₂, and M₃ are available, they serve as building blocks to produce two biopolymers, P₁ and P₂. Polymer P₁ is produced from M₁ and M₃, while P₂ is produced from M₂ and M₃. It is assumed that all monomer synthesis and P₁ synthesis is catalyzed by P₂ while P₂ synthesis is catalyzed by P₁. Therefore, the polymer P₂ may be viewed as a lumped protein component, and P₁ may be viewed as a lumped information polymer comprising RNA and DNA.

Key enzymes, rather than P₂, could also have been identified as the catalysts involved in monomer synthesis; however, in order to reduce the level of structure, the simpler viewpoint has been followed. Incorporation of key enzyme components should, at best, improve the final results or, at worst, not alter the final results since two levels of regulation (i.e., enzyme synthesis and activity) rather than just one level of regulation (i.e., P₂ or protein activity) would be incorporated. The following results are intended for steady-state conditions only. The complete model equations for the model in Figure 3b are given by eqs 17-19 for continuous culture conditions; upper case letters have denoted process variables, while lower case letters now denote their respective concentration variables. Monomer synthesis rates are denoted by rₘ, while polymer synthesis rates are denoted by rₚ.

\[
\frac{ds_j}{dt} = D(s_{j,F} - s_j) - \sum_{k=1}^{3} Y_{s,m_k} r_{m_k} v_{m_k}^e \quad j = 1, 2 \quad (17)
\]

\[
\frac{dm_i}{dt} = D(m_{i,F} - m_i) + r_{m_i} v_{m_i}^e - \sum_{k=1}^{2} Y_{m_p} r_{p_k} \quad i = 1, 2, 3 \quad (18)
\]

\[
\frac{dp_k}{dt} = r_{p_k} - Dp_k \quad k = 1, 2 \quad (19)
\]

where

\[
r_{m_1} = \frac{\mu_{m_1}^\text{max} P_{p_1} s_1}{K_{m_1} + s_1}, \quad r_{m_2} = \frac{\mu_{m_2}^\text{max} P_{p_2} s_1 s_2}{(K_{m_2} + s_1)(K_{m_2} + s_2)}
\]

and

\[
r_{m_3} = \frac{\mu_{m_3}^\text{max} P_{p_2} s_2}{K_{m_3} + s_2}
\]
The dilution rate is given as $D$, while the appropriate stoichiometric coefficients are given by $Y_{m,s}$ and $Y_{m,s'}$. Finally, the cybernetic variable $v^*_m$ (eq 13) is reproduced here as eq 20 in a form appropriate for the previous model equations:

$$v^*_m = \frac{r_m/m_i}{\max (r_m/m_i)} \quad j = 1, 2, 3$$  \hspace{1cm} (20)

It is apparent from Figure 3b that the synthesis of $P_1$ and $P_2$ from the three monomers may also be drawn as a divergent branch point; however, the regulation of polymer synthesis according to the cybernetic variables derived for divergent branch points has not been clearly established in the literature. Therefore, regulation analogous to eq 20 is not applied to the synthesis of $P_1$ and $P_2$.

The above model assumes that the dilution rate is sufficiently high that maintenance effects are not significant. Furthermore, a more complex medium may be accounted for by eqs 16–19 since the model permits the presence of $M_1$, $M_2$, and $M_3$ within the feed. A simple example of such a situation is growth in a complete medium containing intermediates such as amino and nucleic acids. The model equations also assume Monod kinetics to apply, and when necessary, an interactive form is utilized. However, as the results demonstrate in the next section, the chosen kinetic forms do not restrict the range of behavior.

**Results and Discussion**

Steady-state simulations of eqs 16–19 are presented in Figures 4–6. The chosen constants listed in Table 1 are not entirely arbitrary. Some of the parameters were chosen to reflect values that were utilized to describe microbial growth on glucose and NH$_4^+$ as limiting substrates in continuous cultures (Straight and Ramkrishna, 1994). For the results in Figure 4, the concentration of $S_1$, $P_1$ was set at approximately 1.5 g/L and the concentration of $S_2$, $P_2$ was increased in a stepwise manner. Initially, the monomers $M_1$, $M_2$, and $M_3$ are not available in the feed; therefore, the medium is simple in nature, i.e., a minimal medium. With the dilution rate set at 0.70 h$^{-1}$, the results in Figure 4 demonstrate how metabolic regulation responds to biotic phase kinetics and determines the degree of interaction between $S_1$ and $S_2$. Figure 4 indicates that interactive behavior, noninteractive behavior, or behavior intermediate between these two extremes is observable as the maximum monomer synthesis rates $\mu_{m1}^{max}$, $\mu_{m2}^{max}$, and $\mu_{m3}^{max}$ vary from 10:1:10 to 1:10:1 h$^{-1}$. It is reasonable to presume that monomer synthesis rates will be a function of the identity of the limiting substrates as well as the microorganism. When $S_1$ and $S_2$ are noninteractive, i.e., $\mu_{m1}^{max}$, $\mu_{m2}^{max}$, and $\mu_{m3}^{max}$ = 10:1:10 h$^{-1}$, the contours of constant specific growth rates are approximately perpendicular to one another; therefore, only one substrate controls the specific growth rate for virtually all substrate concentration combinations, even when both substrates are present at subsaturating concentrations. For example, when $s_1/K_{m1,s1} = 0.55$, no jump to a new contour of constant $\mu$ would occur until $s_1/K_{m1,s1}$ is decreased below 1.

**Figure 4.** Steady-state level of interaction between $S_1$ and $S_2$ as determined by the response of metabolic regulation to biotic phase kinetics. Profiles are given for $\mu_{m1}^{max}$, $\mu_{m2}^{max}$, and $\mu_{m3}^{max}$ = 1:10:1 h$^{-1} (- - -)$, 4:6:4 h$^{-1} (- - -)$, 8:2:8 h$^{-1} (- - -)$, and 10:1:10 h$^{-1} (- - -)$ ($D = 0.70$ h$^{-1}$).

**Figure 5.** Steady-state transitions between metabolic pathways for a noninteractive system ($\mu_{m1}^{max}$, $\mu_{m2}^{max}$, $\mu_{m3}^{max}$ = 1:10:1 h$^{-1}$). Transitions are indicated by (a) $v^*_m$ (--), $v^*_m$ (- - -), and $v^*_m$ (- - -); and (b) $\mu_{m1}$ (--), $\mu_{m2}$ (- - -), and $\mu_{m3}$ (- - -) ($D = 0.70$ h$^{-1}$).

In contrast, when $S_1$ and $S_2$ are interactive, i.e., $\mu_{m1}^{max}$, $\mu_{m2}^{max}$, and $\mu_{m3}^{max}$ = 10:1:10 h$^{-1}$, any variation in the concentration of either $S_1$ or $S_2$ will change the specific growth rate when $S_1$ and $S_2$ are present in subsaturating concentrations. For example, when $s_1/K_{m1,s1}$ and $s_2/K_{m2,s2}$ are approximately 8.5 and 8.0, respectively, changing either $s_1$ or $s_2$ would move the specific growth rate to a new contour of constant $\mu$. The results in Figure 4 reflect the actions of the cybernetic variable $v^*_m$, which directs the distribution of $S_1$ and $S_2$ between the available pathways in response to the degree of limitation imposed...
minimum growth rate, which is equivalent to the imposed dilution rate \( D \) as well as the observed specific growth rate \( \mu \) at steady state. The remaining pathways, if unregulated, could potentially support specific growth rates greater than \( D \). In Figures 5–7, the specific growth rate potentially supported by \( M_i \) is calculated from the material balance given by eq 18. After \( m_{ij} \) is set to zero and we assume steady-state conditions, the expression for \( \mu_{mj} \) is given by

\[
\mu_{mj} = \frac{r_{mj} - \sum_{k=1}^{2} \frac{Y_{m,k}}{p_k} r_{pk}}{m_{j}} \quad i = 1, 2, 3 \tag{21}
\]

Furthermore, since the potential specific growth rate supported by each monomer is of interest, no regulation is incorporated in eq 21, i.e., \( \mu_{mj} = 1 \) for all \( i \). If \( \mu_{mj} \) is included in eq 21, then \( \mu_{mj} = D \) for all \( i \). The values of \( \mu_{mj} \), \( \mu_{m2} \), and \( \mu_{m3} \) as calculated from eq 21 are not required for the model. They are calculated here only to demonstrate that the cybernetic variables derived for complementary processes preferentially promote the pathway that supports the minimum specific growth rate. This result is in agreement with the standard rule for determining the specific growth rate when two complementary substrates are defined as noninteractive:

\[
\mu = \min(\mu_{j}) \quad j = 1, 2 \tag{22}
\]

where \( \mu_{mj} \) is the specific growth rate supported by \( S_j \) when it is limiting at a concentration \( s_j \). Both Bader (1978) and Baltzis and Fredrickson (1988) have shown that two substrates may be simultaneously limiting over a range of substrate concentration values, even when the specific growth rate is defined as in eq 22. However, Baltzis and Fredrickson (1988) first assumed variations in cell yield on \( S_1 \) and \( S_2 \) as the status of \( S_1 \) or \( S_2 \) was altered between limiting and nonlimiting in order to predict such a multiply-limited region. Regions in which only \( S_1 \) or \( S_2 \) is controlling, as well as regions in which both substrates control the specific growth rate, are also predicted by the model proposed by eqs 16–19, as indicated from the results in Figures 5–7.

For the noninteractive system in Figure 5, the preferred pathway switches from the synthesis of \( M_2 \) to the synthesis of \( M_1 \) as the ratio \( s_1 K_{m2} s_2 K_{m1} s_2 > 1 \); therefore, only \( S_2 \) controls \( \mu \) when \( s_1 K_{m2} s_2 K_{m1} s_2 < 1 \). Therefore, two regions of singly-limiting conditions are present in Figure 5. At the other extreme are the results presented in Figure 6: no transition between pathways occurs. The synthesis of \( M_2 \) remains the preferred pathway for the entire range of substrate concentrations; therefore, both \( S_1 \) and \( S_2 \) control the specific growth rate at subsaturating substrate concentrations and remain interactive under the conditions of Figure 6. The mixed-interaction results in Figure 7 indicate regions that are under single or double substrate limitations. From Figure 7, if the ratio \( s_1 K_{m2} s_2 K_{m1} s_2 > 5 \) or \( < 0.3 \), then \( \mu_{m2} = 1 \) or \( \mu_{m1} = 1 \), respectively, while \( \mu_{m1} = 1 \) when \( 0.3 < s_1 K_{m2} s_2 K_{m1} s_2 < 5.0 \). Therefore, single limitations in which \( S_1 \) or \( S_2 \) controls \( \mu \) occur when \( s_1 K_{m2} s_2 K_{m1} s_2 > 5 \) or \( < 0.3 \), respectively, while a double limitation of \( S_1 \) and \( S_2 \) occurs when \( 0.3 < s_1 K_{m2} s_2 K_{m1} s_2 < 5.0 \). In summary, by incorporating metabolic regulation within the model equations, regions in which one or more substrates control the specific growth rate occur as a natural consequence of the decision-making processes imple-
before they are incorporated into structural material, Le., their rate of assimilation into common intermediates is generally undergo few, if any, chemical transformations. Prior to these results the concentration of the intermediate common to both noninteractive behavior would be expected to increase it might be expected that if the level of coupling between higher degree of limitation; therefore, the level of interaction must increase.

As the number of chemical conversions an intermediate undergoes while crossing from one pathway to another decreases, its rate of assimilation into common intermediates increases and the level of interaction decreases. Therefore, when limiting complementary substrates are, for example, glucose and riboflavin, the resulting behavior would be expected to be more noninteractive since substrates such as vitamins, cofactors, trace metals, etc., generally undergo few, if any, chemical transformations before they are incorporated into structural material, i.e., their rate of assimilation into common intermediates is relatively high. The opposite would thus be true, i.e., more interactive behavior, when limiting complementary substrates undergo significant degradation as they are incorporated into biomass components. Therefore, in the presence of complementary products, this analysis implies that the degree of regulatory coupling between complementary substrates increases as the synthesis rate, i.e., kinetic coupling, of a common intermediate decreases.

Another aspect of microbial growth subject to limitations involving complementary nutrients is the variation in the measured cell yield coefficient. It has generally been observed experimentally that the cell yield coefficient, with respect to a given substrate, decreases as the status of that substrate changes from limiting to nonlimiting. An area of significant experimental interest therefore is the determination of the variation in cell yield as the limiting substrate varies. One issue addressed within this section is whether some degree of the variation in cell yield is predictable by the incorporation of regulatory processes. If this is possible, then perhaps the amount of experimental data required for model verification can be reduced. The cell yield coefficients for the proposed model are presented in Figure 8 as a function of the substrate concentrations in the feed. The dilution rate is set at 0.4 h⁻¹, while the concentration of S₁,F is set at 5 g/L and the concentration of S₂,F is increased in a stepwise manner. The values for the maximum monomer synthesis rates μ₃₉, max, μ₄₉, max are set to 1:10:1 h⁻¹. From these maximum specific rate values, S₁ and S₂ are assumed to exhibit noninteractive behavior. The molar cell yield coefficient Yᵢ is defined as

\[ Yᵢ = \left( \frac{p₁ + p₂}{s₁,F - sᵢ} \right) MWᵢ \quad i = 1, 2 \] (23)

where \( p₁ + p₂ \) is the total biomass concentration and MWᵢ is the molecular weight of Sᵢ. For comparison purposes, S₁ and S₂ are assumed to be equivalent to glucose and NH₄⁺, respectively. Note that the molar cell yield Yᵢ is not defined for conditions when only Sᵢ is limiting, in contrast to the analysis by Baltzis and Fredrickson (1988).

The results in Figure 8 indicate that a significant reduction in Yᵢ occurs as the status of S₁ is altered from limiting to nonlimiting (i.e., increasing \( s₁,F/s₂,F \)) without...
altering the yield constants and stoichiometric coefficients within the model. Likewise, as the status of $S_2$ changes from nonlimiting to limiting, $Y_1$ increases significantly. The transition for both substrates occurs over the region $1 < a_{12} < 2$ in which $Y_1$ and $Y_2$ change continuously in a smooth manner. The variations in cell yield described in Figure 8 show both qualitative and quantitative agreement with the results previously reported by Rutgers et al. (1990) for microbial growth limited by glucose and ammonium chloride. Neijssel and Tempest (1975) and Cooney et al. (1976) have also reported similar yield variations when microbial growth is limited by complementary substrates. In contrast to reported similar yield variations when microbial growth is limited by glucose and ammonium chloride. Neijssel and Tempest (1975) and Cooney et al. (1976) have also reported similar yield variations when microbial growth is limited by complementary substrates. In contrast to results given in Figure 8, the cell yield coefficients within an unstructured model, which utilizes either interactive or noninteractive criteria to determine $\mu$, are invariant. They remain constant even as the identity of the limiting substrate changes. To describe the results in Figure 8, an unstructured model would require at least four measured yield coefficients. Although the mass balances given by eqs 16–19 require eight yield or stoichiometric coefficients, four of these constants, i.e., $Y_{m_1p_1}, Y_{m_2p_1}, Y_{m_1p_2}$, and $Y_{m_2p_2}$, can most likely be estimated from the established stoichiometry of the pertinent biochemical pathways. However, for the simulation results within this paper, these coefficients were arbitrarily set at 0.5 while the yield coefficients $Y_{m_1m_1}, Y_{m_2m_1}, Y_{m_1m_2}$, and $Y_{m_2m_2}$ were set at values appropriate for previously established cell yields on glucose and NH$_4^+$ (Straight and Ramkrishna, 1994). To further simplify the analysis, the yield coefficients were set such that $Y_{m_1m_1} = Y_{m_2m_1}$ and $Y_{m_2m_2} = Y_{m_1m_2}$. Therefore, specific information on only two of the eight constants above was necessary for the cited results. A standard unstructured model, in this situation, therefore offers no significant advantages over the model proposed here.

Metabolic regulation responds to the composition of the abiotic environment just as it responds to the composition of the biotic environment. The repression of many biosynthetic enzymes in the presence of a complex medium is just one example. Therefore, the type of behavior, i.e., interactive or noninteractive, may also be a function of the type of medium employed. In a complex medium where one or more intermediates, e.g., amino acids, may be available, two substrates previously exhibiting interactive behavior may become noninteractive in nature. To explore this phenomenon, the intermediate $M_2$ is assumed to be available within the medium; therefore, $m_{2P}$ in eq 18 is nonzero. When $M_2$ is present in the feed, the system in Figure 3b is decoupled into two primary pathways, as shown in Figure 9. The decoupled system, similar to the one proposed by Bader (1978), resembles the system in Figure 3a. Figure 10 compares steady-state results when $S_1$, $S_2$, and $S_3$ are present in the medium to the previous result when $M_2$ is not supplied externally. The system is originally interactive in nature since $\mu_{m_1} \mu_{m_2} \mu_{m_3} = 10:1:10$ $h^{-1}$. As the results in Figure 10 show, the presence of $M_2$ in the medium transforms the previously interactive system to a noninteractive system. The substrates $S_1$ and $S_2$ no longer exhibit interactive behavior since their interaction is no longer necessary, i.e., $v_{m_1}$ acts to inhibit the synthesis of their common intermediate $M_2$, which is now available in the feed. While the addition of $M_2$ to the feed acts to transform an interactive system to a noninteractive one, Figure 11 shows that the presence of $M_1$ and $M_2$ in the feed produces little or no interactive behavior in an already noninteractive system with $\mu_{m_1} \mu_{m_2} \mu_{m_3} = 1:10:1$ $h^{-1}$. The significance of the results described by Figures 10 and 11 is that specific pathways may or may not be promoted by modifications within the medium. The incorporation of regulation within the model, however, allows one to identify possible sites that will lead to significant modifications in substrate utilization patterns and to discard those sites that produce insignificant changes in substrate utilization patterns.

Conclusions. The quantitative results within this paper are, of course, a function of the chosen parameter values; however, qualitatively, the simple system in Figure 3b is capable of exhibiting the full spectrum of behavior between and including the extreme cases of interactive and noninteractive behavior due to the incorporation of metabolic regulation. Therefore, even though a structured model may utilize some form of interactive kinetics, the results presented here indicate that the final output of the model need not result in interactive behavior. An alternative structured model could be formulated that may produce results similar to eqs 16–19 by incorporating feedback regulation derived from a kinetic mechanism. However, such a formulation would necessitate the identification of additional interactions and model parameters associated with biotic phase processes, two problems that the proposed cybernetic formulation avoids. Furthermore, simulation results have suggested that a purely kinetic feedback formulation would only restrict rather than promote the synthe-
sis of biosynthetic intermediates, which are needed to minimize the effects due to resource limitations during transient periods (Straight and Ramkrishna, 1994).

It would be useful to have sets of contour plots, as in Figure 4, for different pairs of limiting complementary substrates; however, the difficulties present in obtaining meaningful data are numerous. Bader et al. (1975) have identified many of these problems and emphasized the care needed in order to obtain valid data. The problems identified by Bader et al. (1975), however, are experimental in nature. While the cybernetic framework cannot resolve such problems, it can offer solutions to the problems associated with predicting and understanding microbial growth limited by complementary as well as substitutable substrates by augmenting kinetic models with simple representations of established regulatory processes.

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