Multiplicity and stability of steady states in continuous bioreactors: dissection of cybernetic models

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Abstract

Microbes in industrial bioreactors generally encounter nutrient media that contain a spectrum of components satisfying various nutritional requirements with individual substrates playing roles that may be viewed as substitutable, complementary or combinations of the two to varying extents. Consequently, the pattern of uptake of different nutrients (Pavlou and Fredrickson, Biotechnol. Bioengng 34(7) (1989) 971) by the organism is linked through metabolic regulation of its biochemical pathway to its environment as determined by the composition of the surrounding nutrient medium. Such a situation is indicative of an abundance of steady state multiplicity in continuous bioreactors where different steady states may feature cells with notably different physiological states and consequently vastly different metabolic activities. A striking example of such steady state multiplicity is that observed by Hu et al. (J. Microbiol. Biotechnol. 8(1) 8), and Follstad et al. (Biotechnol. Bioengng 63(6) (1999) 675) with hybridoma cell cultures for the production of antibodies.

A mathematical investigation of the foregoing steady state multiplicity must obviously involve models that incorporate metabolic regulation. In this regard, the cybernetic models developed by Ramkrishna and coworkers (Kompala et al., Biotechnol. Bioengng 28(1986) 1044; Baloo and Ramkrishna, Biotechnol. Bioengng 38(11) (1991a,b) 1337, 1353; Straight and Ramkrishna, Biotechnol. Prog. 10(6) (1994) 574; Ramakrishna et al., Biotechnol. Bioengng 52(1) (1996) 141) represent an ideal framework of which to initiate such an investigation. These models account for regulation on suitably simplified pathways by viewing it as the competition for cellular resources between different enzyme systems. Such competition may produce outright victors denoted by zero enzyme levels of those vanquished or simultaneous expression and activation of all the competing enzyme systems. The cybernetic models feature the foregoing competition with cybernetic variables $u_i$ for the regulation of synthesis of the $i$th enzyme, and $v_j$ for its activation. The latter cybernetic variables are not differentiable at points where reversal of competition occurs so that the application of nonlinear bifurcation analysis based on calculation of the Jacobian must take due account of this technical difficulty.

The strategy proposed here for the bifurcation analysis of cybernetic models is based on a combinatoric approach enumerating all possible consequences of competition between different enzyme systems. Since the system for each combinatoric is differentiable, a bifurcation analysis is therefore enabled. Furthermore, a notable feature is that the procedure analytically reduces the system to one of a single dimension by purely analytical means. The bifurcation analysis is performed on the model of Kompala et al. (Kompala et al., 1986), as well as that of Ramakrishna et al. (Ramakrishna et al., 1996). The latter is a refinement of the former, which describes sequential as well as simultaneous utilization of multiple substrates.

The results of our analysis on both models demonstrate the existence of multiple, stable, steady states for certain ranges of our bifurcation parameters, $D$ the dilution rate and $\gamma$ the fraction of the more preferred substrate in the feed. These multiple steady states refer to different levels of utilization of the more preferred substrate. This multiplicity occurs for the model of Kompala et al. (Kompala et al., 1986) at $D$ near the maximum dilution rate and $\gamma$ suitably small. However, multiplicity exists for the model of Ramakrishna et al. (Ramakrishna et al., 1996) even when the preferred substrate is in excess. The reasons are discussed. The methodology has potential for considerably more detailed cybernetic models and hence should be of practical significance to biotechnology. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Cybernetic modeling; Enzyme systems; Metabolic regulation; Bifurcation; Stability; Numerical simulation

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1. Introduction

The investigation of nonlinear behavior of chemical reactors has been a popular area of chemical engineering research since the pioneering papers of Amundson and coworkers (Bilous & Amundson, 1955; Aris & Amundson, 1958). There have been other landmark papers that have lent fresh impetus to this research area such as those of Uppal, Ray, and Poore (1974) that ushered in the systematic use of bifurcation theory in chemical reaction engineering, and Balakotaiah and Luss (1982) that led to the use of singularity theory. There, of course, have been numerous other papers that deserve mention in regard to developments in this field but further citation is desisted since the sole objective of this paper is to address biological reactors.

The main inspiration for nonlinear analysis of chemical reactors has been their behavior within a non-isothermal setting because of the nonlinearity associated with the temperature dependence of reaction rate constants. Biological reactors, on the other hand, have been relatively constrained to operate under isothermal conditions so that any nonlinearity in their behavior generally arises from the kinetics of change. Thus, investigation of steady state multiplicity of continuous biochemical reactors has depended almost exclusively on such behavior arising from substrate inhibition kinetics (Agarwal, Lee, Lim, & Ramkrishna, 1982). However, this paper is predicated on the premise that the real source of multiplicity in bioreactors lies in the phenomenon of metabolic regulation, an omnipresent feature of biochemical pathways that endows cells to make choice decisions at metabolic crossroads. A striking example is the observation of multiple steady state behavior (Hu, Zhou, & Europa, 1998; Follstad, Balcarel, Stephanopoulou, & Wang, 1999) of hybridoma cells. The steady states involve cells of widely varying metabolic activities. In some cases, the different steady states can be perceived only if measurements are available of distinct cellular constituents that are involved in their contrasting pathways as gross measurements such as those of cell mass or external nutrient concentrations may not reveal such multiplicities. Nevertheless, the distinction may be vital as product formation may be linked to a specific steady state whose preferential stability may be the chief target of the mathematical investigation.

Clearly, the multiplicity behavior just cited can be explored only with mathematical models that address the regulatory processes of microorganisms. Thus, the numerous kinetic models that have been employed for biochemical processes, which are notably oblivious to the presence of metabolic regulation, cannot be commissioned for this task. In this connection, it is now known that the cybernetic models of Ramkrishna and coworkers (Kompala, Ramkrishna, Jansen, & Tsao, 1986; Baloo & Ramkrishna, 1991a,b), have successfully described several well-known situations of metabolic regulation. Consequently, they represent potentially the most effective models to investigate multiplicity behavior in bioreactors arising from metabolic switching. The objective of this paper is to initiate the treatment of steady state multiplicity and stability of continuous biochemical reactors that are fed with two substitutable nutrients with the cybernetic models of Kompala et al. (1986) and those of Ramakrishna, Ramkrishna, and Konopka (1996).

The nonlinear analysis of cybernetic models is complicated by the fact that the cybernetic variables compound the kinetic nonlinearity. Further, the cybernetic variables \( (v_i) \) which are concerned with control of enzyme activity are not differentiable with respect to the substrate or enzyme levels at points where metabolic switching occurs. An immediate consequence of this feature is the loss of the facility of the implicit function theorem, which depends on the existence of a non-vanishing Jacobian at the solution. Furthermore, the methods of nonlinear analysis, which depend upon the differentiability of the tangent vector field at the bifurcation point, cannot be used if metabolic switching occurs there. Thus, the methods of singularity theory cannot be used indiscriminately.

The investigation of steady state multiplicity proceeds by reducing the dimensionality of the problem. In some cases, the nonlinearity is permissive enough to allow an analytical reduction to a one-dimensional problem. More often, however, the dimensionality may be amenable to reduction by numerical procedures. In this paper, we present a methodology that performs a numerical reduction of dimensionality by being computationally sensitive to metabolic switching. This sensitivity is accomplished by a systematic combinatorial treatment of potential metabolic switches while assessing the regulated rates of various metabolic processes. Thus one may expect that the methodology will identify situations where bifurcation points coincide with points of metabolic switching thereby providing the required alert against the use of techniques that call for differentiation at bifurcation.

The basic goal of this paper is to develop a quantitative approach suitable for the nonlinear analysis of complex cybernetic models. This consists in the evolution of a convenient algebraic strategy to process the nonlinearity characteristic of cybernetic models, that, in conjunction with a numerical procedure, will accomplish the maximum reduction in dimensionality. For the development of such a quantitative approach we select the setting of relatively simple cybernetic models. Of course, the underlying notion is that the methodology that emerges from such an effort will serve to guide the nonlinear analysis of more complex cybernetic models that are essential to address relevant applications to biotechnology. The reason for this contention arises from the similarity between the regulatory features in the models chosen here versus those in the more complex models. The complex models have more detailed pathways, however.
Our choice of the models of Kompala et al. (1986) and of Ramakrishna et al. (1996) is based on their possessing a pathway structure that is a reasonable compromise between one too simple to accommodate a detailed regulatory structure and one that is too complicated for initiating nonlinear analysis. This compromise is reflected in our including multiple substrate feeds for which metabolic regulation may lead to multiple uptake patterns, but neglecting maintenance effects that become important at lower dilution rates. Thus the results of the analysis are more relevant to interpret the reactor behavior at higher dilution rates.

2. Analysis of the model of Kompala et al. (1986)

2.1. Brief introduction

Kompala et al. (1986) proposed a “goal seeking” or cybernetic model to explain microbial growth on multiple substrates. This model was successful in explaining the diauxie phenomenon in which bacteria such as Klebsiella oxytoca consume substitutable substrates such as glucose and arabinose sequentially in batch cultures as a result of catabolite inhibition. The model utilized data obtained from growth on single substrates and did not require an a priori specification of the order in which the substrates are consumed. This cybernetic model could also account for the simultaneous utilization of substitutable substrates in continuous cultures at low dilution rates. Although this model does not account for maintenance phenomena occurring at low dilution rates, it simulates continuous bioreactors at dilution rates close to the maximum growth rate, where maintenance effects are not pronounced.

2.2. Description of model equations

We now present the reaction scheme and the model equations which are presented here in a slightly different form from Kompala et al.’s original work, to suit our analysis. The assimilation of the ith substrate $S_i$ by biomass $B$ is assumed to be catalyzed by the ith key enzyme $E_i$, which represents the entire cellular machinery contributing to the utilization of $S_i$, as shown:

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

The key enzyme $E_i$ is induced in the presence of $S_i$ according to

$$ B \xrightarrow{S_i} E_i + B_i', $$

where $B_i'$ is $B$ excluding $E_i$. The kinetic rate expressions for the above two reactions are written as

$$ r_i c, \quad \text{where} \quad r_i = \frac{Y_i s_i e_i}{K_i + s_i}, $$

$$ r_1 c, \quad \text{where} \quad r_1 = \frac{2 s_i}{K_1 + s_i}, $$

and $c$ represents biomass concentration. The above equations are modified by cybernetic variables $v_i$ and $u_i$, respectively to give the actual rates of substrate utilization and enzyme synthesis given by $r_i v_i$ and $r_i u_i$, where

$$ u_i = \frac{r_i}{\sum_j r_j} \quad \text{and} \quad v_i = \frac{r_i}{\max_j(r_j)}. $$

The complete model equations for a continuous reactor are as follows:

$$ \frac{d s_i}{d t} = -Y_i^{-1} r_i v_i c + D(s_i' - s_i), \quad (1) $$

$$ \frac{d e_i}{d t} = r_i u_i - \left( \sum_j r_j v_j + \beta_i \right) e_i, \quad (2) $$

$$ \frac{dc}{dt} = \left( \sum_j r_j v_j - D \right) c. \quad (3) $$

2.3. A strategy for analysis of multiplicity

Consider two substrates for the sake of simplicity and the situation when washout does not occur. The pertinent equations are the steady state forms of Eqs. (1)–(3). Eq. (2) should not be used to simulate the enzyme concentrations in washout as it is derived for enzyme levels per unit biomass and biomass is zero in washout. The equations for analysis then are

$$ D(s_1' - s_1) = Y_1^{-1} r_1 v_1 c, \quad (4) $$

$$ D(s_2' - s_2) = Y_2^{-1} r_2 v_2 c, \quad (5) $$

$$ r_1 u_1 = (D + \beta_1) e_1, \quad (6) $$

$$ r_2 u_2 = (D + \beta_2) e_2, \quad (7) $$

$$ r_1 v_1 + r_2 v_2 = D, \quad (8) $$

where Eq. (8) has been used in writing simplified equations (6) and (7). Eqs. (4), (5) and (8) have cybernetic ‘$v$’ variables which cannot be differentiated with respect to the substrate concentrations and enzyme levels. Hence singularity theory should not be applied indiscriminately to such a system. The strategy outlined herein is an approach to unearth the multiplicity features of the bioreactor equations (4)–(8) by anticipating sharp corners which disallow differentiation. While the strategy is successful in its endeavor, it unfortunately compromises on seeking the most singular equilibrium point (steady state). This difficulty with the proportional law could of course be overcome by differentiable approximations but whether or not any higher order singularity (and the consequent multiplicity) that might result from them retains its capacity to model regulation is an issue in doubt.

Our mission is to seek steady states which feature the different consequences of the competition between the two different enzyme systems. For two competing enzyme
systems, these consequences include two situations: (i) one enzyme system prevailing over the other producing an exclusive victor, and (ii) a simultaneous manifestation of both enzyme systems with varying degrees of success, however, of one over the other. Note in particular that exclusive victors arise only in the absence of constitutive levels of enzyme synthesis. Thus the strategy, which is developed in this paper, is based on anticipation of the consequences of such competition. Such an approach is rational because whether or not a steady state with an envisaged consequence does actually arise in the system, is an issue of stability and hence a matter of concern only subsequent to the identification of the different steady states.

A bifurcation plot tracing the variation of any of the system variables with a chosen parameter must include steady states of either category (i) or (ii) for every competition between enzyme systems. While bifurcation may be perceived to occur as transitions through types (i) and (ii), it is apparent that a branch of steady states entirely of type (ii) could feature a catch-up point at which one enzyme system lagging behind the other overtakes the other. This catch-up point is clearly a sharp corner caused by a discontinuity in the derivative of the variable with respect to the parameter. If a turning point bifurcation exists on this branch of type (ii), its coincidence with a catch-up point (sharp corner) is a distinct possibility so that the turning point is characterized by not the vanishing of a derivative but a jump in the derivative occurring with a concurrent change in sign.

Our strategy is based on recognition of branches of types (i) and (ii) as arising from looking first at the enzyme balances (6) and (7). We assume zero constitutive levels for the present. Eqs. (6) and (7) must be considered for three different possibilities. These include: (A) \( e_2 = 0 \), which deems the \( e_1 \) system an outright victor, (B) \( e_1 = 0 \), with the \( e_2 \) system as the outright victor, and (C) neither enzyme levels vanishing. Clearly, situations (A) and (B) of Fig. 1 belong to case (i) while (C) belongs to case (ii). The main reason for considering these situations separately is due to each case leading to a distinct set of simplified algebraic equations that may be investigated for multiple solutions. Thus we shall deal with three different branches of solutions with two of them representing, as outright victors, either of the two enzymes, and the third where simultaneous expression of both enzymes occurs.

These different metabolic states of the cell help simplify the model equations. For instance, case A \( (e_2 = 0) \) precludes the uptake of \( S_2 \) \( (e_2 = 0 \Rightarrow v_2 = 0 \Rightarrow s_2 = s_2^c) \) as per Eq. (5)) whereas cases B and C allow its utilization. Also, \( u_2 = 0 = v_2 \) and \( u_1 = 1 = v_1 \) if \( e_2 = 0 \), and a substantial simplification in the model equations is thereby possible. On the contrary we cannot make any definite statement about the values of the cybernetic \( u \) and \( v \) variables when \( e_1 \) and \( e_2 \) are both non-zero in case C. These cases illustrate beyond doubt that different metabolic states of the cell lead to different levels of simplification of the original equations, a fact that will be further asserted when we employ our solution methodology. Also, a methodology is now surfacing which clearly points out that the first step towards tracking down the numerical solutions is the consideration of different metabolic states of the cell, which are characterized by different enzyme systems going to zero levels, and different competing reactions in a metabolic pathway dominating one another. The methodology is outlined as follows.

1. Examine the reaction network of the model as well as its mathematical framework, in particular investigate the definitions of the cybernetic variables to identify all the sets of competing reactions.
2. Identify all the enzymes that catalyze competing reactions and can reach zero levels in steady states not leading to washout.
3. Prepare a table of combinatorial cases arising out of different enzymes taking zero and non-zero levels in steady state. All the competing enzymes in a pathway cannot simultaneously be zero in steady state, as this would lead to zero rates of competing reactions and the cybernetic variables in that case would not be defined as they would be of the type \( (0/0) \).
4. In situations where all the enzymes in a competition take non-zero values identify combinatorial cases based on the relative rates of the competing reactions at steady state.
5. For each combinatorial case, complete the table by allocating the cybernetic \( v \) variable involved, a value of 0, 1 or \( \leq 1 \) depending on the corresponding enzyme reaching zero level, all the competing enzymes reaching zero levels, and all enzymes reaching non-zero levels respectively.
6. Investigate the simplified steady state system for each combinatorial case by first looking at the enzyme balances. Make use of the definitions of cybernetic \( v \) variables and their properties to achieve further reduction of the system, before proceeding to the substrate and biomass balances.
7. Use the differentiable model equations in each combinatorial case to evaluate analytically the Jacobian matrix for linear stability analysis.

We now apply the above steps to Kompala et al.’s model. On applying steps (1) and (2) we identify

<table>
<thead>
<tr>
<th>Case</th>
<th>( e_1 )</th>
<th>( e_2 )</th>
<th>( v_1 )</th>
<th>( v_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>( \neq 0 )</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>( \neq 0 )</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C-1</td>
<td>( \neq 0 )</td>
<td>( \neq 0 )</td>
<td>1</td>
<td>( \leq 1 )</td>
</tr>
<tr>
<td>C-2</td>
<td>( \leq 1 )</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 1. Different combinatorial cases in the model of Kompala et al. (1986).
enzymes \( e_1 \) and \( e_2 \) as candidates for zero solutions. Application of step (3) of this strategy to the above set of equations leads to combinatorial cases A–C shown in Fig. 1 and helps us complete the first 3 columns of the table. Application of step (4) further differentiates case C to give sub-cases C-1 and C-2. Step (5) helps complete the table by adding columns (4) and (5). Step (6) is carried out to yield solution algorithms for the different cases as shown in Appendix A followed by step (7), shown next, to examine the stability of different states.

### 2.3.1. Linear stability analysis

Once the steady states are determined, the stability of the different states can be determined using linear stability analysis. To accomplish this, we evaluate the Jacobian matrix of the system around a state and examine its eigenvalues. One difficulty in evaluating the Jacobian is the non-differentiability of the cybernetic \( v \) variable. However, as mentioned before our methodology surmounts this by using the Jacobian of the differentiable system obtained by replacing the max function in the cybernetic \( v \) variable by its appropriate argument. Once the steady state is known, the maximum of \( r_1 \) and \( r_2 \) can be determined a priori and the appropriate Jacobian matrix expression can be used. At this stage, it is appropriate to recall that the Jacobian is defined only for a specific combinatoric.

### 2.4. Presentation and discussion of results

We present the bifurcation plots as a function of the two parameters \( \gamma \) (fraction of the preferred substrate in the feed) and \( D \) (the dilution rate). The perspective of the steady state picture varies substantially with the choice of the planes on which the behavior is projected because of the multidimensional situation at hand. Thus in some planes, spurious intersections of different branches are perceived while in others they are clearly separated. Of course no real intersection can exist between branches in the multidimensional space if there is any plane in which it does not occur. In this regard, although there are key planes in which the regulatory picture will emerge most lucidly, it will of course be useful to corroborate this perspective by viewing them in different planes.

Since the chief instruments of regulation are the cybernetic variables, a plot of either \( u_1 \) (or \( u_2 \)) or \( v_1 \) (or \( v_2 \)) versus the bifurcation parameter \( \gamma \) as shown in Fig. 5 at a dilution rate of \( D = 0.85 \) should provide the most graphic view of the varying regulatory scenario. Recall that the parameter \( \gamma \) represents the fraction of the preferred substrate \( (S_1) \) in the feed for a fixed total concentration of the two substitutable substrates. Furthermore, continuous lines are used to distinguish stable portions of a branch from dotted unstable portions. When the fraction of preferred substrate is sufficiently small in the feed, the only stable steady state is the one involving exclusive use of the less preferred substrate as in plots of \( u_1 \) and \( u_2 \) in Fig. 5 (which reflects the lack of constitutive enzyme synthesis for the more preferred substrate). As \( \gamma \) is increased, uptake of the preferred substrate is initiated at some stage (well before 0.5 as an indication of its preference) along with the less preferred substrate. This uptake of the preferred substrate progressively increases over that of the less preferred substrate until at the turning point, there is a jump in the extent to which the preferred substrate is consumed. There is some question as to whether the turning point coincides with the catch-up point of the preferred substrate because they appear to almost coincide. In fact the catch-up point \( (T) \) actually occurs at a slightly smaller value of \( \gamma \). Further increase in \( \gamma \) beyond the turning point progressively leads to exclusive utilization of the preferred substrate at a transcritical bifurcation point. This trend is corroborated by \( v_1 \) and \( v_2 \) plots in Fig. 5 although less impressively because the cybernetic variable \( v \) does not have the capacity to distinguish between preference and exclusive preference.

Plots of \( e_1 \) and \( e_2 \) in Fig. 4 are almost exact replicas of plots of \( u_1 \) and \( u_2 \) in Fig. 5 and hence contain the same information, although we have taken the opportunity of designating the \( e_1 = 0 \) (or \( e_2 = 0 \)) line as \( s_1 = s_1^f \) (or \( s_2 = s_2^f \)) since \( S_1 \) (or \( S_2 \)) flows unutilized through the reactor. Fig. 4 also shows the substrate concentrations as a function of \( \gamma \). Note here that the figure for \( s_1 \) is a zoomed in version.

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### Table 1: Bifurcation parameter for glucose–arabinose

<table>
<thead>
<tr>
<th>Bifurcation parameter</th>
<th>No. of States</th>
<th>Stability of different states</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \gamma \leq 0.033 )</td>
<td>1 SU</td>
<td>S</td>
</tr>
<tr>
<td>( 0.033 &lt; \gamma &lt; 0.2185 )</td>
<td>3 U U</td>
<td>S</td>
</tr>
<tr>
<td>( \gamma = 0.2185 )</td>
<td>4 U U</td>
<td>S</td>
</tr>
<tr>
<td>( 0.2185 &lt; \gamma \leq 0.3667 )</td>
<td>5 U US</td>
<td>S</td>
</tr>
<tr>
<td>( 0.3667 &lt; \gamma &lt; 0.375 )</td>
<td>5 U U 2S 1U</td>
<td></td>
</tr>
<tr>
<td>( \gamma = 0.375 )</td>
<td>4 U U 1S 1N</td>
<td></td>
</tr>
<tr>
<td>( 0.375 &lt; \gamma &lt; 0.708 )</td>
<td>3 U U S</td>
<td></td>
</tr>
<tr>
<td>( 0.708 \leq \gamma \leq 1 )</td>
<td>1 S</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 2.** Steady states and their stability at different values of the bifurcation parameter for glucose–arabinose in the model of Kompala et al. (1986), S—stable, U—unstable, N—undetermined.

**Fig. 3.** Bifurcation points and bifurcation phenomena in the model of Kompala et al. (1986).
to reveal different bifurcation phenomena and the steady state $s_1 = s_1^f$ really extends upwards beyond the segment shown, up to $\gamma = 0.708$. The corroboration, which Fig. 4 shows of the multiplicity trends in Fig. 5, suffers from having to display the spurious intersection between $e_1 = 0$ and $e_2 = 0$ lines. Such an intersection also appears in Fig. 9 which shows the biomass concentration $c$ as a function of $\gamma$. Fig. 2 shows the different states that exist in different regions of the bifurcation diagram along with their stability. Fig. 3 shows the different bifurcation points along with the characterization of the bifurcation that occurs.
The most interesting result of this analysis is that for $0.2185 < \gamma < 0.375$, there are two stable states, with similar concentrations of $s_1$, but remarkably different levels of $s_2$ as well as biomass as shown in Fig. 6. This leads to two states, one with a high biomass concentration, due to consumption of $S_1$ as well as $S_2$ and the other with a relatively low biomass concentration as a result of growth on $S_1$ but almost no utilization of $S_2$. Thus this model simulates two steady states in this range of $\gamma$, one of which denotes simultaneous utilization, and the other almost preferential utilization (as there is only slight consumption of $S_2$) as is evident from Fig. 4 where $s_2$ in state C-1 is very close to that in state B which represents a metabolic state of $s_2 = s_2'$. Thus it would be interesting to carry out experiments, and approach this region from either ends of the bifurcation diagram, by starting on the one hand with a culture with a pure arabinose ($S_2$) feed ($\gamma = 0$, $s_2' = 0$, $s_1' = 1 \text{ g/l}$) and slowly increasing the fraction of $S_1$ in the feed to arrive at the region of interest, and on the other hand starting with a pure glucose ($S_1$) feed ($\gamma = 1$, $s_2' = 0$, $s_1' = 1 \text{ g/l}$) and slowly increasing the concentration of arabinose in the feed. It would be interesting to experimentally verify this simultaneous existence of multiple stable steady states as this would have implications in fermentation and bioremediation.

Shown in Fig. 9 is the bifurcation diagram depicting biomass concentration $c$ versus $D$ the dilution rate at $\gamma = 0.3$. This shows that there are essentially three different types of steady states, at low dilution rates, one depicting simultaneous utilization of $S_1$ and $S_2$ (though not obvious from the diagram of biomass, this is state C-2) showing high biomass concentration and the other two depicting preferential utilization of $S_1$ ($e_2 = 0$, $s_2 = s_2'$, state B) and $S_2$ ($e_1 = 0$, $s_1 = s_1'$, state A) respectively. The diagram shows that at low dilution rates, both substrates are consumed and state C-2 is stable, and as dilution rate is increased, it undergoes a catchup point (sharp corner) which coincides with a turning point. The unstable state then undergoes a smooth turning point with gain of stability, and a further increase in the dilution rate shows a transcritical bifurcation to state B, which at higher dilution rate shows washout, indicated by $c = 0$. In fact state A remains unstable all along. A word of caution, however, is due here as the biomass concentration predicted by this model at low dilution rates is higher than experimentally observed as this does not account for maintenance effects; however, the results hold near the dilution rate close to the maximum growth rate where the interesting bifurcation phenomena are observed.

Fig. 7 shows the bifurcation diagram of $u_1$ which is chosen to be an indicator of the metabolic state of the cell; recall that states A, B, C-1 and C-2 are denoted by values of $u_1$ of 0, 1, $\geq 0.5$, $\leq 0.5$ respectively. As one can see from the figure, the system shows a cusp catastrophe (Golubitsky & Schaeffer, 1985) indicated by two turning
points at high dilution rates, and slowly merging into a hysteresis point at low dilution rates. At still lower dilution rates, the hysteresis point vanishes. The magenta lines denote steady states of type B, whereas black lines indicate states of type A. Blue and red lines indicate families of steady state of types C-1 and C-2 respectively. Projected on the $\gamma-D$ plane, as shown in Fig. 8 for clarity are the two magenta lines, denoting a change in the number of solutions from 1 to 3 (going from region I to II and also V to VI), due to a transcritical bifurcation as well as a boundary crossing. The two blue lines indicate the two turning points, with their intersection showing the hysteresis bifurcation, one would encounter by moving along $\gamma$ at a constant $D$. The red line shows the catch-up point, which is a sharp corner indicated by a change in color from red to blue in the solution trajectories shown in three dimensional space. The legend on the figure shows the different steady states and the types of stable (and unstable) branches in the different regions in $\gamma-D$ space.

3. Analysis of the model of Ramakrishna et al. (1996)

3.1. Brief introduction

The model of Kompala et al. (1986) was successful in explaining diauxic behavior of microbes in the presence of substitutable substrates. It could explain sequential utilization in batch cultures, simultaneous utilization in continuous cultures at low dilution rates and preferential utilization at high dilution rates. It was however unsuccessful in predicting the simultaneous utilization of certain carbon-energy sources such as mixtures of sugars and organic acids observed in batch cultures. This is attributed to the rigidity of the kinetic structure of the model which arises due to the limited number of metabolic pathways accounted for due to the lumping of biomass formation from substrates into a single step.

Ramakrishna et al. (1996) have developed a more expanded kinetic structure that uses the same cybernetic principles of the model of Kompala et al., but acknowledges the role of biosynthetic precursors. Their model describes diauxic growth as well as simultaneous substrate uptake in batch cultures as also situations where the pattern of substrate uptake switches from sequential to simultaneous utilization and vice versa. In what follows, we describe the model of Ramakrishna et al., point out some model deficiencies we came across, suggest modifications to the model equations and apply our solution methodology to get the complete steady state picture for the revised model.

3.2. Description of model equations

The expanded kinetic scheme of Ramakrishna et al. (1996) comprises of two substrates $S_1$ and $S_2$, two intermediates $M_1$ and $M_2$ and four key enzymes $E_1-E_4$ as shown in Fig. 10. Thus, substrate $S_1$ forms a lumped pool of precursors denoted by $M_1$ in a process catalyzed by the key enzyme $E_1$. The precursors pool $M_1$ can then form the other lumped precursor pool $M_2$ required for the formation of biomass $B$ using key enzyme $E_3$. $M_2$ on the other hand can be formed from $S_2$ using key enzyme $E_4$ and then synthesize $M_1$ using $E_5$. Thus there are two pathways for the formation of $M_1$ as well as $M_2$ when the microbe is growing on two substrates and these alternatives create a framework that allows preferential or simultaneous utilization as demonstrated in what follows. If $S_1 \rightarrow M_1$ and $M_1 \rightarrow M_2$ are the preferred alternatives for the synthesis of the precursors, then the scenario is preferential utilization of $S_1$ over $S_2$ in continuous cultures or diauxic growth in batch cultures. However, if the preferred alternative for the synthesis of $M_2$ is $S_2 \rightarrow M_2$ with $M_1$ being synthesized from $S_1$, simultaneous utilization in batch or continuous cultures is observed.

3.2.1. Rate expressions for the expanded model

The rate of synthesis of $M_1$ from $S_1$ in a slightly modified form from the original work of Ramakrishna et al.
(1996) is considered as

\[ r_1 = \left( \frac{\mu_1 s_1 e_1}{K_1 + s_1} \right) \]

and the rate of synthesis of enzyme \( E_1 \) is

\[ r_{e_1} = \left( \frac{\alpha_1 s_1}{K_{e_1} + s_1} \right). \]

This rate expression only includes the inducible rate of enzyme synthesis, and the overall rate will include a constitutive rate of enzyme synthesis given by \( r_{e_i}^* \). The rate expressions for processes (2)–(4) are similarly formulated. The growth process \( M_1 + M_2 \rightarrow B \) is assumed to follow a multiple saturation form of growth and is given by

\[ r_g = r_g^{\text{max}} \left( \frac{m_1}{K_{g_1} + m_1} \right) \left( \frac{m_2}{K_{g_2} + m_2} \right). \]

3.2.2. Regulation and model equations

The cybernetic variables that describe the substitutable processes for the synthesis of \( M_1 \) and \( M_2 \) are formulated as derived by Straight and Ramkrishna (1994) and are given below:

\[ u_1 = \frac{Y_1 r_1}{Y_1 r_1 + r_2}, \quad u_2 = \frac{r_2}{Y_1 r_1 + r_2}, \quad u_3 = \frac{r_3}{r_3 + Y_4 r_4}, \]

\[ u_4 = \frac{Y_4 r_4}{r_3 + Y_4 r_4}. \]

\[ v_1 = \frac{Y_1 r_1}{\max(Y_1 r_1, r_2)}, \quad v_2 = \frac{r_2}{\max(Y_1 r_1, r_2)}, \]

\[ v_3 = \frac{r_3}{\max(r_3, Y_4 r_4)}, \quad v_4 = \frac{Y_4 r_4}{\max(r_3, Y_4 r_4)}. \]

The complete set of model equations for a continuous reactor at a dilution rate \( D \) is as follows:

\[ \frac{ds_1}{dt} = -r_{e_1} v_1 c + D(s_1^f - s_1), \quad \] (9)

\[ \frac{ds_2}{dt} = -r_{e_2} v_2 c + D(s_2^f - s_2), \quad \] (10)

\[ \frac{dm_1}{dt} = Y_1 r_1 v_1 + r_2 v_2 - \frac{r_g}{Y_{m_1}} Y_{m_1} - r_3 v_3 - r_g m_1, \]

\[ \frac{dm_2}{dt} = Y_4 r_4 v_4 + r_3 v_3 - \frac{r_g}{Y_{m_2}} Y_{m_2} - r_2 v_2 - r_g m_2, \]

\[ \frac{dc}{dt} = (r_g - D)c, \]

\[ \frac{de_1}{dt} = r_{e_1}^* + r_{e_1} u_1 - (r_g + \beta_1) e_1, \]

\[ \frac{de_2}{dt} = r_{e_2}^* + r_{e_2} u_2 - (r_g + \beta_2) e_2, \]

\[ \frac{de_3}{dt} = r_{e_3}^* + r_{e_3} u_3 - (r_g + \beta_3) e_3, \]

\[ \frac{de_4}{dt} = r_{e_4}^* + r_{e_4} u_4 - (r_g + \beta_4) e_4, \]

where the terms \( (r_g/Y_{m_1}) \) and \( (r_g/Y_{m_2}) \) denote the depletion in \( M_1 \) and \( M_2 \) levels due to biomass formation. Terms of the type \( r_g x \) where \( x \) is either of \( m_1, m_2 \) and the four enzymes, denote dilution of intracellular intermediate pools due to growth. Terms of the type \( \beta_i e_i, \forall i = 1–4 \) denote the degradation of enzymes by a first order process called turnover.

3.3. Modifications to the model of Ramakrishna et al. (1996)

The model of Ramakrishna et al. (1996) explains the observed phenomena of simultaneous utilization in batch cultures as well as diauxie, when observed, and so also simultaneous as well as preferential utilization in continuous cultures. However, it has a technical flaw which we seek to eliminate. The intermediate levels \( (m_1, m_2) \) are defined in terms of mass per unit cell mass, so that the sum of the intermediates should at most add up to unity. The species balance written by Ramakrishna et al. (1996) do not account for this constraint so that it is possible to have values of \( m_1 \) and \( m_2 \) higher than unity. (This problem does not exist with enzyme levels as the enzymes have a turnover constant \( \beta_i \) that limits the maximum enzyme level even under low values of the growth rate \( r_g \).) In order to complete the mass balance for the intermediates we propose the following modifications to the original formulation that ensure that the intracellular species levels sum to unity. (Since the enzyme levels are generally very small, their contribution to this conservation constraint is not significant.) Fig. 11 shows a schematic of the modified formulation where biomass \( B \) is composed of \( M_1, M_2 \) and \( C' \). As shown in Fig. 11 the intermediates \( M_1 \) and \( M_2 \) form the rest of biomass (other than \( M_1 \) and
denoted by $C'$ as per the reaction $M_1 + M_2 \rightarrow C'$ in a double saturation dependence given by

$$\dot{r}_g = \dot{r}_{g}^{max} \frac{m_1}{m_1 + K_{g1}} \frac{m_2}{m_2 + K_{g2}}.$$  

Note that this expression is identical to the growth rate expression in the original work of Ramakrishna et al. (1996). This modification leads to changes in the mass balances of $M_1$ and $M_2$, introduces an additional balance on $C'$ and changes the growth rate expression $\dot{r}_g$. The equations for $s_1, s_2, e_i, \forall i = 1–4$ and $c$ remain unchanged in the modified model formulation. The modified equations for $M_1$ and $M_2$ as well as the balance on $C'$ are shown below:

$$\frac{d m_1}{dt} = Y_{1} r_1 v_1 + r_2 v_2 - \frac{\dot{r}_g}{Y_{m1c}} r_3 v_3 - r_g m_1, \quad (18)$$

$$\frac{d m_2}{dt} = Y_{4} r_4 v_4 + r_3 v_3 - \frac{\dot{r}_g}{Y_{m2c}} r_2 v_2 - r_g m_2, \quad (19)$$

$$\frac{dc'}{dt} = \dot{r}_g - r_g c'. \quad (20)$$

In Eqs. (18) and (19) the terms ($r_g/Y_{m1c}$) and ($r_g/Y_{m2c}$) have changed to ($\dot{r}_g/Y_{m1c}$) and ($\dot{r}_g/Y_{m2c}$) respectively due to contribution of $M_1$ and $M_2$ to $c'$ instead of $B$ in the original formulation. The expression for growth can be obtained by making use of the equality

$$m_1 + m_2 + c' = 1$$

which implies

$$\frac{d(m_1 + m_2 + c')}{dt} = 0.$$  

Substituting Eqs. (18)–(20) in the above and simplifying for $\dot{r}_g$ leads to

$$\dot{r}_g = Y_{1} r_1 v_1 + Y_{4} r_4 v_4 + \dot{r}_g \left(1 - \frac{1}{Y_{m1c}} - \frac{1}{Y_{m2c}}\right).$$

Growth rate is thus determined from a balance on the pool of intermediates and is the net rate of change of intracellular species, instead of being an explicit expression as before. Eqs. (9), (10), (18)–(20) and (13)–(17) were simulated for continuous as well as batch reactors (with $D = 0$) and found to match the transients predicted by Ramakrishna et al. (1996) with modification of some model parameters. These modified model parameters are shown in Fig. 13. The variable $\mu_i$ in our analysis is the ratio of $r_i^{max}$ and $e_i^{max}$ shown in Fig. 13. We now proceed with the multiplicity analysis for the modified model formulation, the steady state equations for which are Eqs. (21)–(27). We neglect constitutive rates of enzyme synthesis in this model analysis as it makes solution extremely difficult. This is because constitutive rates of enzyme synthesis destroy zero enzyme level solutions, and make the steady state equations intractable to reduction of dimension. Thus the results obtained hold for extremely low values of constitutive rates of enzyme synthesis. The $r_i^{max}$ terms are left out of Eq. (27). Also Eq. (26) has been used to replace $r_g$ by $D$ wherever it appears:

$$\frac{r_1 v_1 c}{Y_{m1c}} = D(s_1^f - s_1), \quad (21)$$

$$\frac{r_4 v_4 c}{Y_{m2c}} = D(s_2^f - s_2), \quad (22)$$

$$Y_{1} r_1 v_1 + r_2 v_2 = \frac{\dot{r}_g}{Y_{m1c}} r_3 v_3 + D m_1, \quad (23)$$

$$Y_{4} r_4 v_4 + r_3 v_3 = \frac{\dot{r}_g}{Y_{m2c}} r_2 v_2 + D m_2, \quad (24)$$

$$\dot{r}_g = D c', \quad (25)$$

$$r_g = D, \quad (26)$$

$$r_{ei} u_i = (D + \beta_i) e_i \quad \forall i = 1 – 4. \quad (27)$$

### 3.4 Discussion of strategy

The different steps employed in our methodology as applied to the model of Ramakrishna et al. (1996) are shown below:

1. The model of Ramakrishna et al. (1996) contains two sets of substitutable competitions. Reaction (1) competes with reaction (2) for the production of $M_1$. Also reaction (3) competes with reaction (4) for making $M_2$. This information can be obtained by looking at the schematic of the model framework and examining the cybernetic variables involved.

2. Enzymes $e_1–e_4$ are the different enzymes involved in the competing sets of reactions and these may take zero values in the absence of constitutive levels of enzyme synthesis.
3. The first 5 columns of Fig. 12 show the results of this step where the different enzyme zero combinatorics are considered. The figure also illustrates that cases of the type \( e_1 = 0 = e_2 \) are not considered.

4. The combinatorics in Fig. 12 split further in sub-cases denoted by numbers depending on the relative values of \( Y_1 r_1 \) and \( r_2 \) and also \( r_3 \) and \( Y_4 r_4 \).

5. Columns 5 and 6 are assigned to complete Fig. 12.

6. Steps (6) and (7) are employed along lines similar to that for Kompala’s model, the steps for which are shown in Appendix A.

3.5. Presentation and discussion of results

We choose the glucose (\( S_1 \))–fumarate (\( S_2 \)) system with dilution rate \( D \) as the bifurcation parameter. Figs. 14 and 15 show the result of our analysis at a total substrate concentration of 1 g/l distributed equally between \( S_1 \) and \( S_2 \) in the feed. Also shown in Fig. 14 are the schematics of model pathways that are active in different regions of \( u_1 – u_4 \) plane. Thus at, say, a high value \( u_1 \) and low value of \( u_4 \), \( S_1 \) is a better source of \( M_1 \) than \( M_2 \) and \( M_1 \) is a better source of \( M_2 \) than \( S_2 \) as indicated by thick versus dotted pathways in the reaction schematic. The axes denote states with cybernetic variables at unity (or zero) and indicate that the corresponding enzyme system (or its competing system) was an exclusive victor. Any point away from the axes would have all enzyme systems activated, albeit to different extents. The dotted lines with \( u_1 = 0.5 \) and \( u_4 = 0.5 \) denote possible locations of catchup points, as the corresponding cybernetic \( v \) variables would touch unity at these points. The arrows marked with \( D \) show increasing values of the dilution rate, and the trajectory that would be followed if one were to step up the dilution rate slowly in a continuous culture.

Due to the large number of combinatorics involved, the bifurcation plots for this system comprise a large number of steady states. In this discussion, we focus on the stable steady states, and do not go into the details of the bifurcation phenomena associated with unstable steady states because of the large number of states involved. In terms of substrate utilization, we can broadly classify the states into three categories, one where only \( S_1 \) is consumed, another where only \( S_2 \) is consumed and the third where both \( S_1 \) and \( S_2 \) were consumed.

**Fig. 13.** Modified model parameters in the model of Ramakrishna et al. (1996).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Original Value</th>
<th>Modified Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_1^{new} )</td>
<td>2</td>
<td>0.92</td>
</tr>
<tr>
<td>( r_2^{new} )</td>
<td>0.784</td>
<td>0.392</td>
</tr>
<tr>
<td>( r_3^{new} )</td>
<td>1.165</td>
<td>0.5826</td>
</tr>
<tr>
<td>( r_4^{new} )</td>
<td>0.58</td>
<td>0.27</td>
</tr>
<tr>
<td>( Y_{r, c} )</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>( Y_{r, e} )</td>
<td>0.31</td>
<td>0.155</td>
</tr>
<tr>
<td>( Y_{m, r} )</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>( Y_{m, e} )</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

**Fig. 14.** \( u_4 \) versus \( u_1 \) at \( \gamma = 0.5 \) for the model of Ramakrishna et al. (1996). Thick and dotted lines denote stable and unstable steady states respectively.
are consumed. Thus, Fig. 15 shows three different levels of biomass, the intermediate, low and high levels respectively, in the three types of steady states. At low values of dilution rate, the stable state is a state of high biomass concentration which stands for simultaneous utilization of \( S_1 \) as well as \( S_2 \), while the other states of exclusive \( S_1 \) or \( S_2 \) utilization are unstable, in fact the state of preferential utilization of \( S_2 \) (the less preferred substrate) is seen to be unstable all along, a fact that was also observed for Kompala’s model. The stable state at low dilution rates shows up away from the axes in the \( u_1-u_4 \) plot which means that all enzyme systems are activated, and we do not have exclusive victors. Also, in Fig. 14 \( u_1, u_4 < 0.5 \), which means that \( v_1, v_4 < 1 \) and these are the states of type F-4 of Fig. 13. As dilution rate is increased however, there is a catch-up point, wherein \( u_1 \) becomes equal to 0.5 and hence equal to \( u_2 \) and the enzyme systems 1 and 2 are equal competitors for the production of \( M_1 \). This is a state of type F-2. A further sharp corner is shown by a crossing of the \( u_4 = 0.5 \) dotted line and this denotes a transcritical bifurcation to state F-1.

At higher dilution rates, a transcritical bifurcation occurs as our stable steady state trajectory loses stability to a branch of the type \( u_1 = 1 \) leading to a slight decrease in the biomass concentration, at a dilution rate of around 0.5. This leads to a state of the type G-2, which undergoes turning point bifurcation that coincides with a catch-up point (sharp corner), as we cross the \( u_4 = 1 \) line, along with loss of stability leading to state G-1. As we negotiate a turning point, if we follow the unstable branch, \( D \) decreases, indicated by a dotted line with decreasing values of biomass. A smooth turning point, with exchange of stability leads to a stable steady state branch, with a decreased biomass concentration, which then undergoes a transcritical bifurcation, leading to state of type \( D \), which shows preferential utilization of \( S_1 \). Such a state continues to be represented on the \( u_1 = 1 \) axis, until the concentration of biomass decreases to zero, after which only washout type of states are stable at higher dilution rates.

Thus our analysis shows that a state of simultaneous utilization of \( S_1 \) as well as \( S_2 \) is stable at low dilution rates, whereas states where only \( S_1 \) or \( S_2 \) is utilized are unstable, as seen in Fig. 15. However, as the dilution rate is increased, the system slowly changes to one in which only \( S_1 \) is utilized, wherein a slight hysteresis type behavior is observed, with multiple stable steady states. At higher dilution rates, the state with preferential utilization of \( S_1 \) washes out of the reactor. This agrees with the general observation on substitutable substrates by Pavlou and Fredrickson (1989) who have studied substrates with widely varying consumption characteristics, from substitutable to complementary along with different substrate inhibitions, etc. This is also the observation of Narang (1998) who has investigated microbial growth on substitutable substrates using a kinetic model. However, a significant difference from the cited work lies in the prediction of stable multiple steady states for certain parameter ranges not found with Narang’s kinetic model (Narang, 1998).

To be able to compare the models of Ramakrishna et al. (1996) and Kompala et al. (1986), steady state simulations with Kompala et al.’s model were carried out with substrates displaying a maximum growth rate of 0.72 and 0.38 which are equivalent to glucose and fumarate simulations in the model of Ramakrishna et al. (1996). The results are shown in Fig. 16. At \( \gamma = 0.5 \), the model of Kompala et al. (1986) for these dummy substrates displays, at low dilution rates, a state of simultaneous utilization which gradually shifts to one of preferential utilization on \( S_1 \) indicated by intermediate levels of biomass, before washout occurs at growth rates higher than the maximum growth rate on \( S_1 \). Although this is in tune with simulations of the model of Ramakrishna.
et al. (1996) at the same value of \( \gamma \) shown in Fig. 15, stable multiple steady states are not observed. This indicates that the detailed framework in the model of Ramakrishna et al. (1996) extends multiplicity, a result one would have expected, considering the comparatively expanded framework in the model of Ramakrishna et al. (1996).

4. Conclusions

A strategy for bifurcation analysis of cybernetic models which involve non-differentiable cybernetic variables has been developed in this work. This strategy is based on combinatoric enumeration of the various consequences of competition between different key enzyme systems. The advantage of the procedure is two-fold. Firstly, alternatives associated with outright victors (in which one of the enzyme levels is zero) lead to algebraically convenient equations that can be processed for reduction in dimensionality. Secondly, for simultaneous activation each alternative is free of the non-differentiability of the max function in the v-cybernetic variables. This not only leads to algebraic simplification but also enables the calculation of the Jacobian for linear stability analysis. The bifurcation analysis reveals sharp “catch-up” points (i.e., points at which reversal of competition occurs) that may be transcritical bifurcation points, turning points or, in some cases, neither.

As one would expect, the stability analysis reveals for both models that washout occurs at dilution rates exceeding the growth rate on the more preferred substrate at its feed concentration. Further, multiple stable steady states involving simultaneous utilization of both substrates to different extents are found from both models at higher dilution rates below the maximum growth rate. However, the model of Ramakrishna et al. (1996) allows simultaneous utilization of mixed substrates and multiplicity over a wider range of substrate concentrations than the model of Kompala et al. (1986). Thus although steady state multiplicity is predicted by the model of Kompala et al. (1986) it is suppressed when the more preferred substrate is present in slight excess. On the other hand, the model of Ramakrishna et al. (1996) predicts multiplicity even in such circumstances. We have paid no attention to start-up conditions in the bioreactor which will ensure the attainment of a specific steady state. However, it should be clear that the initial enzyme levels will play an important role in determining the steady state which is reached eventually. Thus a fed batch mode of operation, as was done by Hu et al. (1998) with suitable feeding strategies might well provide the proper preparation for initiating continuous operation so that a particular steady state may be attained. The role of cybernetic models in rationalizing such a procedure should be evident.

We envisage the treatment presented here to provide the direction for the analysis of more detailed cybernetic models that have potential for high multiplicity in bioreactors with significant changes in physiological state and hence metabolic activity that may be associated with the formation of specific biotechnology products.

### Notation

- **\( B \)**: biomass
- **\( B'_i \)**: biomass other than the \( i \)th enzyme
- **\( c \)**: biomass concentration
- **\( C' \)**: biomass other than intermediates
- **\( c' \)**: concentration of \( C' \)
- **\( D \)**: dilution rate
- **\( E_i \)**: \( i \)th key enzyme system
- **\( e_i \)**: specific level of the \( i \)th key enzyme system
- **\( K_{gi} \)**: saturation constant for the \( i \)th reaction
- **\( K_{ei} \)**: saturation constant for the expression of the \( i \)th key enzyme system
- **\( K_{si} \)**: saturation constant for growth or formation of \( C' \) from the \( i \)th intermediate
- **\( M_i \)**: \( i \)th intermediate
- **\( m_i \)**: specific level of the \( i \)th intermediate
- **\( r_i \)**: rate of the \( i \)th reaction
- **\( r_{i\text{max}} \)**: maximum rate of the \( i \)th reaction
- **\( e_{i\text{max}} \)**: maximum level of the \( i \)th enzyme
- **\( r_e \)**: enzyme expression rate of the \( i \)th key enzyme system
- **\( r^e_c \)**: constitutive rate of the expression of the \( i \)th key enzyme system
- **\( r_g \)**: growth rate
- **\( r_{g\text{max}} \)**: maximum growth rate
- **\( r_{g'\text{max}} \)**: rate of formation of \( C' \)
- **\( r_{g'\text{max}} \)**: maximum rate of formation of \( C' \)
- **\( S_i \)**: \( i \)th substrate
- **\( s_i \)**: concentration of the \( i \)th substrate
- **\( S_f \)**: feed concentration of the \( i \)th substrate
- **\( u_i \)**: cybernetic variable governing the enzyme expression rate of the \( i \)th key enzyme system
- **\( v_i \)**: cybernetic variable governing the activity of the \( i \)th key enzyme system
- **\( Y_i \)**: yield coefficient for the \( i \)th reaction
- **\( Y_{m,c} \)**: yield coefficient
- **\( Y_{s,c} \)**: yield coefficient

### Greek letters

- **\( \alpha_i \)**: maximum enzyme expression rate of the \( i \)th key enzyme system
- **\( \beta_i \)**: turnover rate of the \( i \)th key enzyme system
- **\( \gamma \)**: fraction of \( S_1 \) in a mixed feed of \( S_1 \) and \( S_2 \)
- **\( \mu_i \)**: \( r_{i\text{max}}/e_{i\text{max}} \)
Acknowledgements

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Appendix A.

A.1. Model of Kompala et al.

In the following we demonstrate how the solution algorithms for the different combinatorial cases in Kompala et al.'s (1986) model are arrived at.

A.1.1. Case A

We are looking for solutions of the type \( e_1 = 0 \). Eqs. (4) and (6) lead to \( e_1 = 0 \) and \( s_1 = s_1' \) respectively. This implies \( e_1 = 0 = u_1 \) and \( v_2 = 1 = u_2 \). Eqs. (5), (7) and (8) then lead to

\[
\begin{align*}
    c &= (s_1' - s_2)Y_1, \\
    e_2 &= \frac{1}{(D + \beta_1)} \left( \frac{x_1s_1}{K_{e_1} + s_1} \right), \\
    r_2 &= \frac{\mu_2s_2}{s_2 + K_2} = D.
\end{align*}
\]

Substitution of Eq. (A.2) in Eq. (A.3) leads to a quadratic equation in \( s_2 \) given by

\[
\left( 1 - \frac{\mu_2s_2}{D + \beta_2} \right) s_2^2 + (K_2 + K_{e_2})s_2 + K_2K_{e_2} = 0
\]

which has at least one negative root. The possible positive root is given by

\[
s_2 = \frac{-(K_2 + K_{e_2}) - \sqrt{(K_2 + K_{e_2})^2 - 4(1 - \frac{\mu_2s_2}{D + \beta_2})K_2K_{e_2}}}{2(1 - \frac{\mu_2s_2}{D + \beta_2})}.
\]

This root will be real as well as positive if \( \mu_2s_2 > D + \beta_2 \). The implications of this inequality are profound as it gives us a criterion for the survival of biomass on substrate \( S_2 \). For biomass to survive on the \( i \)th substrate alone, the product of the maximum growth rate on that substrate and the maximum enzyme induction rate should be greater than the product of the dilution rate (the rate at which biomass dilutes out of the reactor) and \( (D + \beta_i) \) which is the rate at which the \( i \)th key enzyme dilutes out of biomass! This criterion is necessary, though not sufficient, as the calculation of \( s_2 \) is not guaranteed to be less than \( s_1' \) which is required for the biomass to be positive as per Eq. (A.1). One should bear in mind that this criterion does not guarantee the stability of this steady state, but only comments about its existence. After calculation of \( s_2 \), one can use Eqs. (A.3) and (A.2) to calculate \( c \) and \( e_2 \) respectively.

A.1.2. Case B

Case B is very similar to Case A, due to the symmetry of the topology of the model.

A.1.3. Cases C-1 and C-2

These cases are the most general cases, and analytical solution is not possible; however, we can reduce the number of equations by substituting several variables in terms of a few others in order to facilitate numerical solution. We therefore use Eqs. (4)–(8) to obtain numerical solutions. Let us divide Eq. (6) by Eq. (7) to obtain

\[
\frac{r_{e_1}}{r_{e_2}} = \frac{(D + \beta_1)e_1}{(D + \beta_2)e_2} \left( \frac{u_2}{u_1} \right) = \left( \frac{D + \beta_1}{D + \beta_2} \right) \frac{r_{e_1}}{r_{e_2}}
\]

after substitution of the cybernetic \( u \) variables. Substituting the kinetics of enzyme synthesis and the growth rates leads to

\[
\left( \frac{x_1s_1}{K_{e_1} + s_1} \right) \left( \frac{K_{e_1} + s_2}{\alpha_2s_2} \right) = \left( \frac{D + \beta_1}{D + \beta_2} \right) \left( \frac{\mu_2s_2}{K_2 + s_2} \right) \left( \frac{K_1 + s_1}{\mu_1s_1} \right).
\]

The above is a quadratic equation in \( s_1 \) as well as \( s_2 \) and we can use it to obtain \( s_2 \) after guessing \( s_1 \). Letting

\[
K = \left( \frac{D + \beta_2}{D + \beta_1} \right) \left( \frac{\mu_1s_1}{K_1 + s_1} \right) \left( \frac{x_1s_1}{K_{e_1} + s_1} \right)
\]

the foregoing equation becomes

\[
K = \left( \frac{\mu_2s_2}{K_2 + s_2} \right) \left( \frac{\alpha_2s_2}{K_{e_1} + s_2} \right)
\]

which then becomes a quadratic equation in \( s_2 \) given by

\[
s_2 = \frac{(K_2 + K_{e_2})}{(1 - \mu_2s_2/K)} s_2 + \frac{K_2K_{e_2}}{(1 - \mu_2s_2/K)} = 0.
\]

Thus there can be at most two solutions for \( s_2 \) given the guess value of \( s_1 \). One can examine the coefficients of the equation above to conclude that there can be at most one positive root so that \( s_2 \) is uniquely related to \( s_1 \). This root which can be positive depending on the guess value of \( s_1 \) as well as the model parameters is given by

\[
s_2 = \frac{-(K_2 + K_{e_2}) - \sqrt{(K_2 + K_{e_2})^2 - 4(1 - \frac{\mu_2s_2}{K})K_2K_{e_2}}}{2(1 - \frac{\mu_2s_2}{K})}.
\]

Dividing Eq. (4) by Eq. (5) gives

\[
\frac{Y_1D(s_1' - s_1)}{Y_2D(s_2' - s_2)} = \left( \frac{r_{e_1}v_1}{r_{e_2}v_2} \right) = \left( \frac{r_1}{r_2} \right)^2
\]
which on substitution of the substrate utilization kinetics leads to

\[
\frac{e_1}{e_2} = \left(\frac{K_1 + s_1}{\mu_1 s_1}\right) \left(\frac{\mu_2 s_2}{K_2 + s_2}\right) \sqrt{\frac{Y_1(s_1^f - s_1)}{Y_2(s_2^f - s_2)}}
\]  

(A.5)

Using Eqs. (6) and (7) along with the fact that \( u_1 + u_2 = 1 \) leads to

\[
\frac{(D + \beta_1) e_1}{r_{e_1}} + \frac{(D + \beta_2) e_2}{r_{e_2}} = 1
\]

so that we may write

\[
e_2 = \frac{(D + \beta_1) e_1}{r_{e_1}} \left(\frac{(D + \beta_2) e_2}{r_{e_2}}\right)^{-1}.
\]

(A.6)

Adding Eqs. (4) and (5) and using Eq. (8)

\[
c = Y_1(s_1^f - s_1) + Y_2(s_2^f - s_2).
\]

(A.7)

Using the equations above, we can calculate \( s_2, e_1, e_2 \) and \( c \) uniquely using a guess value of \( s_1 \). Since the value of \( s_1 \) is only a guess value, we need to verify our guess numerically as the value of \( s_1 \) for which Eq. (8) is satisfied. Thus, our solution algorithm for this case can be written as follows.

**Algorithm.**

1. Discretize the physically meaningful interval \((0-s_1^f)\) of \( s_1 \) into a predecided number of steps. The step size should be small to increase the accuracy of the results.
2. For each of the discretized values \( s_1 \) we take, use Eq. (A.4) to calculate \( s_2 \). Discard the solution if \( s_2 \) is complex, \( s_2 < 0 \) or \( s_2 > s_1^f \).
3. Calculate \( e_1 \) and \( e_2 \) using Eqs. (A.5) and (A.6). Calculate \( c \) using Eq. (A.7).
4. Calculate the reaction rates and cybernetic variables \( u_1, u_2, v_1 \) and \( v_2 \).
5. Find out, if there exists, the value of \( s_1 \) around which Eq. (8) is satisfied within numerical error.

**A.2. Model of Ramkrishna et al.**

The algebraic strategy for Ramprasad’s model follows from the same principles that were used for Kompala’s model. The details are not included here for succinctness but can be obtained by writing to the authors.

**References**


