



## **Comparative Characterization of the Fermentation Pathway of *Saccharomyces cerevisiae* Using Biochemical Systems Theory and Metabolic Control Analysis: Model Definition and Nomenclature**

RAUL CURTO

*Departament de Bioquímica i Fisiologia, Facultat de Químiques, Universitat de Barcelona, 08028 Barcelona, Spain*

ALBERT SORRIBAS

*Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida, 25006, Lleida, Spain*

AND

MARTA CASCANTE

*Departament de Bioquímica i Fisiologia, Facultat de Químiques, Universitat de Barcelona, 08028 Barcelona, Spain*

*Received 11 May 1994; revised 16 September 1994*

---

### ABSTRACT

Mathematical tools that involve the determination of systemic responses to small changes in metabolites or enzymes have demonstrated their utility for analyzing metabolic pathways. The different methodologies based on these ideas allow for modeling and analyzing biochemical pathways focusing on the coordinate behavior of the whole system. However, one must become familiar with the difference in nomenclature and methodology to relate the models and results obtained by applying these techniques and to appreciate their potential for answering fundamental questions about biochemical systems. In the following three papers we show how this can be facilitated by comparing the nomenclature, methodology, and results of the two leading techniques in this area, metabolic control analysis and biochemical systems theory, using a model of the fermentation pathway in *Saccharomyces cerevisiae* as a reference system. In the present paper we review the nomenclature, technical concepts, and related experimental measurements while creating a practical dictionary for the reference system that makes the relatedness of the two approaches more apparent. In the second paper, subtitled Steady-State Analysis, we show that both approaches give the same picture for many systemic responses of the reference system. In the third paper of this series, subtitled Model Validation and Dynamic Behavior, we show that the quality of the model can be assessed by studying the sensitivity to changes in the system parameters. We hope to illustrate the usefulness of these tools in providing an interpretation of the experimental measurements in a specific metabolic pathway.

## INTRODUCTION

The complexity of biochemical pathways makes it necessary to use tools that are able to deal with a great number of components and interactions. Because of these requirements, mathematical models and computer simulations based on these models are the elective tools for analyzing a great amount of data and for drawing conclusions on the structure and properties of the reference system. In building such models, the first step is to consider a scheme showing the flow of material and the regulatory signals within the system. Then appropriate mathematical expressions for each of the processes involved in the system are selected and the model is defined. The third step involves selecting an appropriate set of parameters so that the model can reproduce a given set of experimental data.

It is common to consider the available *in vitro* information,  $K_m$ , rate laws, etc., as a basis for building a model, especially in selecting the mathematical representation and in defining its parameters. However, there is increasing evidence that following this strategy in complex models may lead to ill-conditioned models, even if accurate *in vitro* data for the individual reactions are used [1–5]. As a consequence, simulations and results based on these models will produce wrong conclusions about the system's properties. This suggests that more general modeling strategies should be examined and that parameter values determined under conditions *in vivo* rather than *in vitro* should be used if the goal is to study the intact system.

Of the several possibilities for systemic modeling, methods that involve the determination of systemic responses to small changes in metabolites or enzymes are most attractive because of their experimental feasibility (for reviews see [6–14] and references therein). Within this context, two major strategies have been defined to relate systemic and molecular properties: (1) Derivation of a theoretical framework using explicit kinetic representations and (2) derivation of specific theorems using implicit kinetic representations. The first strategy has resulted in a specific framework known as biochemical systems theory (BST) [10, 11, 14–20]. The second strategy led to metabolic control analysis (MCA) [also known as metabolic control theory (MCT)] [6, 8, 9, 21–24]. The relatedness of these approaches has been addressed in a number of studies [10, 11, 17, 18, 25–27], and discussion of the mathematical basis of each approach and analysis of theoretical examples indicates to what extent BST and MCA are related and what kinds of results can be obtained by applying either of these approaches [10, 12, 13, 17–19, 25–28]. However, there is no example in which, using experimental data,

the same metabolic pathway has been completely characterized by both approaches.

The potential of these kinds of mathematical tools for analyzing and characterizing biochemical pathways justifies the effort of providing examples of their capabilities. In addition to the theoretical comparisons indicated above, the analysis of common examples can help in this direction. In this series of papers we address, among others, several basic matters: (a) the interpretation of different nomenclatures, (b) the characterization of a system's steady state, (c) the requirements for building the mathematical description, and (d) how to validate the resulting model.

We have chosen as an experimental reference system the anaerobic fermentation pathway of the yeast *Saccharomyces cerevisiae*. This pathway was chosen because detailed knowledge of the rates of glucose uptake and of glycerol and ethanol formation *in vivo* are available. Further, the intracellular concentrations of substrate and effectors for most key enzymes at different steady-state conditions have been measured, and a detailed model of the pathway kinetics has been published [29, 30]. In addition, this system has been characterized by the corresponding flux control coefficients calculated from a steady-state model based on kinetic expressions obtained experimentally. The complexity of this metabolic pathway, according to the description given by Galazzo and Bailey [29, 30] (Figure 1), will help us show how to deal with many of the difficulties that an investigator may encounter in applying a systems theory such as BST or MCA. With that, we hope that the analyses of the specific pathway in these papers illustrate the practical utility of these approaches.

Because of the extent of the material to be included, we have organized it in three parts. First, in this paper, we discuss the model definition and the nomenclature used by MCA and BST. We build the corresponding models of the reference system and give the complete set of equivalences in both nomenclatures (BST or MCA). The conversion tables we include in this paper constitute a practical dictionary that will help readers pass easily from one theory to the other. The principal points to be addressed are those concerning the treatment of the enzymes and the interpretation of the basic concepts underlying the different nomenclatures used by the two approaches. We pay particular attention to the meaning of the aggregation in the S-system variant within BST so that those familiar only with MCA can understand how to use this variant of BST.

In the second paper [31] we turn to the characterization of the pathway properties. This goal involves steady-state characterization

through the computation of logarithmic gains (control coefficients). In the third paper [32] we use sensitivity analysis to establish the validity of the steady-state characterization. We also consider the dynamics of the system and the prediction of changes after a large perturbation of the steady state.

#### ABBREVIATIONS

The following abbreviations appear frequently throughout this series of papers.

##### *Enzymes*

In	Glucose uptake
HK	Hexokinase (E.C. 2.7.1.1) ATP:D-hexose 6-phospho-transferase
PFK	Phosphofructokinase (E.C. 2.7.1.11) ATP:D-fructose-6-phosphate 1-phosphotransferase
GAPD	Glyceraldehyde 3-phosphate dehydrogenase (E.C. 1.2.1.12) D-Glyceraldehyde-3-phosphate:NAD <sup>+</sup> oxidoreductase
PK	Pyruvate kinase (E.C. 2.7.1.40) ATP:pyruvate O <sub>2</sub> -phosphotransferase
POL	Polysaccharide production (glycogen + trehalose)
GOL	Glycerol production
$V_j$	Maximal velocity of the enzyme at step $j$ .

##### *Metabolites*

$G_{in}$	Glucose inside
G6P	Glucose-6-phosphate
F6P	Fructose-6-phosphate
FDP	Fructose-1,6-diphosphate
3PG	3-Phosphoglycerate
G3P	Glyceraldehyde-3-phosphate
PEP	Phosphoenolpyruvate

##### *Theoretical Approaches*

MCA	Metabolic control analysis
BST	Biochemical systems theory
GMA	Generalized mass action representation

## METHODS

### *THE REFERENCE EXPERIMENTAL SYSTEM*

The anaerobic fermentation pathway from glucose to ethanol, glycerol, and polysaccharide in the yeast *Saccharomyces cerevisiae*, as char-

acterized by Galazzo and Bailey [29, 30], will be used in the present study as a reference system. The structure and components of this pathway are shown in Figure 1. According to these authors, the enzymes of the intermediate steps not shown in this scheme catalyze very fast reactions, so that equilibrium conditions can be assumed. Incomplete information is available concerning some of the reactions of the model. Both the MCA and BST models will follow the same assumptions for these reactions that were defined in the original model of Galazzo and Bailey [29, 30]. These reactions are indicated in the Results section.

Steady-state intracellular concentrations of substrates and effectors and the rates of glucose uptake and of glycerol and ethanol formation, as determined by Galazzo and Bailey, are shown in Tables 1 and 2 [29, 30; Galazzo and Bailey, personal communication]. They correspond to two basic cell environments: suspended and alginate-entrapped cells. In each situation, two different pH values are tested: 4.5 and 5.5. This produces four different experimental conditions. The values obtained experimentally are shown in Tables 1 and 2. In these data, the experimental values corresponding to the internal metabolites were readjusted for each set of conditions by numerical computation from the kinetic model in order to make the fluxes calculated from the rate laws agree with the experimental flux values (Bailey, personal communication). The final values are close to the experimental values estimated from  $^{31}\text{P}$  nuclear magnetic resonance measurements in vivo [29, 30].

#### *KINETIC DATA*

The rate equations for each step in the pathway and the parameter values of each rate expression used in the present study are the same as those used in the model of Galazzo and Bailey [29, 30] and are not reproduced here. These equations are used, when needed, to compute the corresponding parameters for both the MCA and BST approaches. It should be stressed that we are using this model as a reference system and that no attempt has been made to improve the data used by the original authors.

## RESULTS

### *IDENTIFICATION AND NOMENCLATURE OF THE COMPONENTS OF THE SYSTEM*

The first step in the description of the system is to identify and label each individual component. In a metabolic pathway, this involves several factors, such as metabolites and enzymes, velocities, and mass balances. Hence, we began by examining how MCA and BST represent

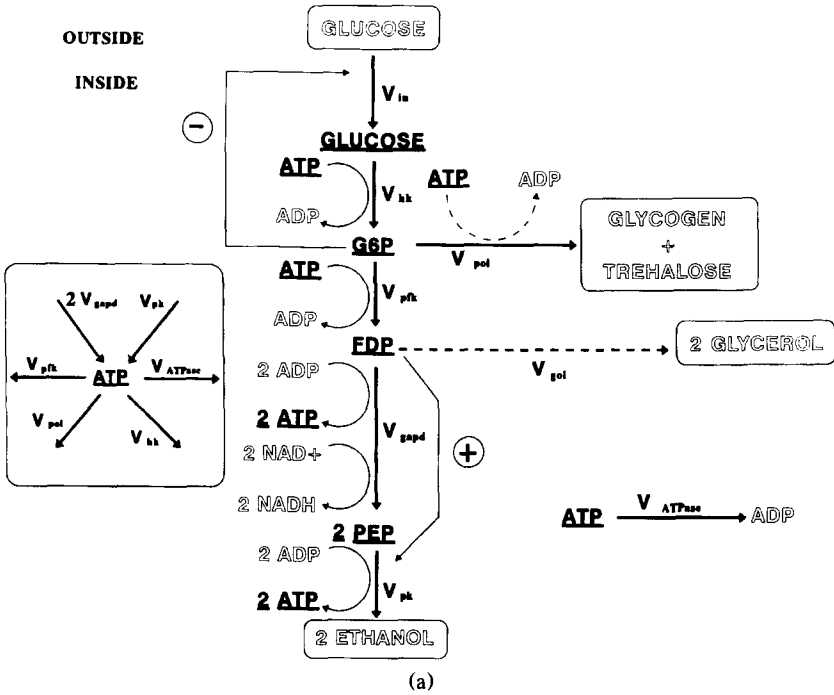


FIG. 1. Anaerobic fermentation pathway of yeast *Saccharomyces cerevisiae* from glucose to ethanol, glycerol, and polysaccharides. (a) Biochemical description; (b) BST nomenclature; (c) MCA nomenclature.

the system. To relate these approaches, we consider both the *general mass action* (GMA) and *S-system* representations within BST (see [17, 18] for definitions; see also Equations (5) and (6) below).

#### Dependent Variables

In both the MCA and BST methods (in either the GMA or S-system variants within BST), the metabolites, which are synthesized and degraded in the pathway, are considered dependent variables and designated as  $X_i$  (these variables are also called  $S_i$  in some applications within MCA). The subscript  $i$  is a correlative number from 1 to  $n$ , where  $n$  is the total number of dependent variables.

In the metabolic pathway of Figure 1, we recognize the following set

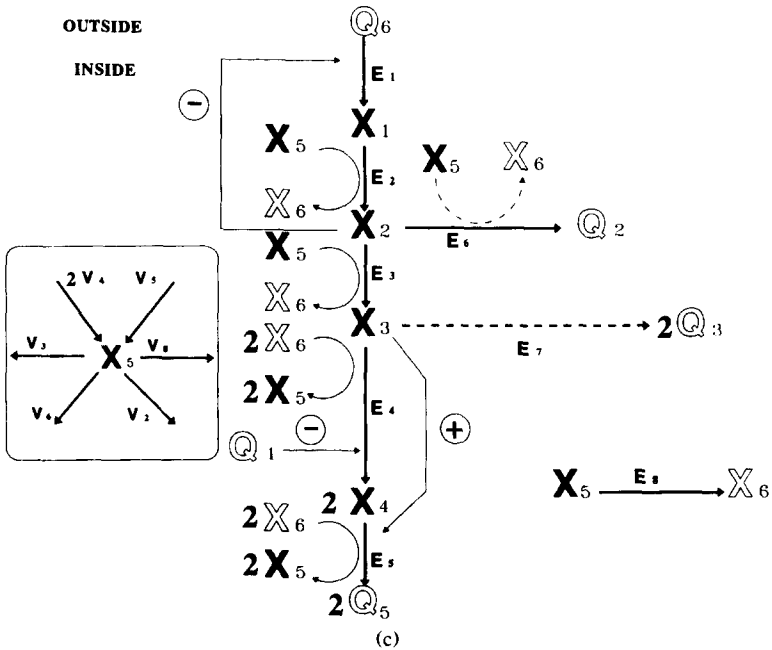
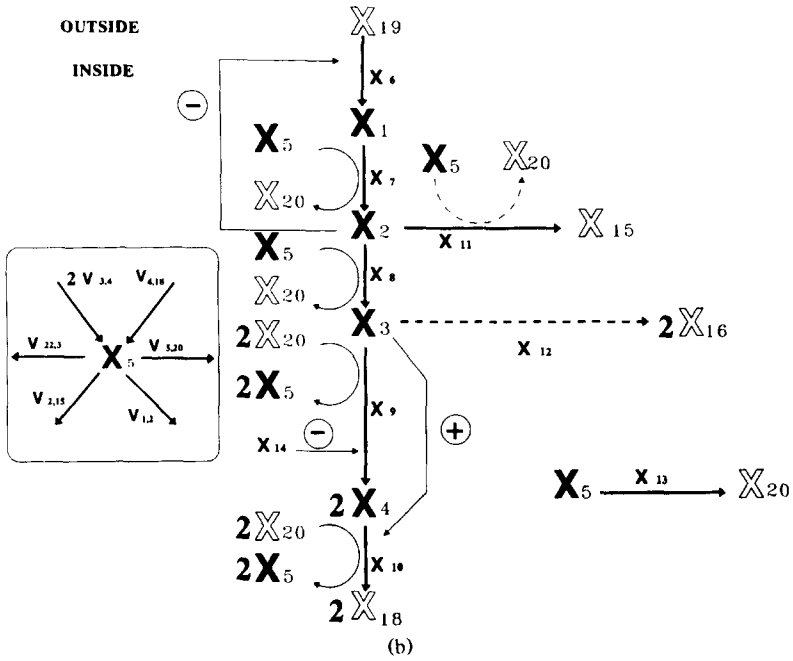


FIG. 1. Continued.

TABLE 1

Steady-State Concentrations and Maximum Velocities for the Metabolites and Fluxes of the Fermentation Pathway Considered<sup>a</sup>

System Variables	Suspended cells, pH = 4.5	Suspended cells, pH = 5.5	Immobilized cells, pH = 4.5	Immobilized cells, pH = 5.5
$G_{in}$	0.0345	0.0276	0.1304	0.1169
G6P	1.011	1.6647	2.7178	3.047
FDP	9.144	5.8152	4.7862	5.3797
PEP	0.0095	0.0079	0.0597	0.4989
ATP	1.1278	1.722	1.2504	1.9214
$V_{in}^M$	19.7	19.7	45.6	45.6
$V_{HK}^M$	68.5	68.5	68.5	68.5
$V_{PFK}^M$	31.7	31.7	31.7	31.7
$V_{GAPD}^M$	49.9	49.9	49.9	49.9
$V_{PK}^M$	3440	3440	3440	3440
$V_{POL}^M$	14.31	14.31	14.31	14.31
$V_{GOL}^M$	203	231.9	237.7	259.7
$V_{ATPase}^M$	25.1	12.1	25	14.3
NADH/NAD+	0.042	0.042	0.007	0.011

<sup>a</sup>Conditions and technical commentaries to these values can be found in [29, 30]. Concentrations are millimolar (mM), and for  $V_j$  are millimolar per minute (mM/min).

TABLE 2

Steady-State Fluxes Measured in Different Conditions for the Fermentation Pathway Considered<sup>a</sup>

Fluxes in steady state	Suspended cells, pH = 4.5	Suspended cells, pH = 5.5	Immobilized cells, pH = 4.5	Immobilized cells, pH = 5.5
$V_{in}$	15.96	13.54	35.54	34.33
$V_{HK}$	15.96	13.54	35.54	34.33
$V_{PFK}$	15.94	12.79	27.35	25.85
$V_{GAPD}$	15.06	11.98	25.59	24.03
$V_{PK}$	30.11	23.96	51.17	48.06
$V_{POL}$	0.014	0.754	8.19	8.479
$V_{GOL}$	1.777	1.615	3.54	3.629
$V_{ATPase}$	28.31	20.84	31.26	27.48

<sup>a</sup>Conditions and technical commentaries to these values can be found in Galazzo and Bailey [29, 30]. Units are millimolar per minute (mM/min).



of dependent variables:

$G_{in}$	$X_1$
G6P	$X_2$
FDP	$X_3$
PEP	$X_4$
ATP	$X_5$

### *Independent Variables*

The independent variables are those variables that are unaffected by the dynamics of the system, and they can be considered constant under the studied conditions. In some cases, this includes variables that change due to processes that can be considered constant with respect to the time scale of the target system, as could be the case of enzyme levels. Further, it is convenient to include in this set of independent variables those that are affected by the dynamics of the system but participate in other pathways that contribute to maintain them constant. In our example, this is the case also of the NADH/NAD<sup>+</sup> ratio. For simplicity, this ratio will be considered constant in each experimental condition since fluorescence measurements performed by Galazzo and Bailey [29] indicate small variations of this ratio. Independent variables include effectors, source and sink metabolites, enzyme concentrations (provided they can be varied independently of the system dynamics),  $K_m$  and  $V_m$ , temperature, and others. The nomenclature for the independent variables is different in MCA and BST, partly because MCA considers different categories of independent variables whereas BST includes any kind of independent variable in the same class.

In MCA, in general, the interest is focused on control by the enzyme concentrations or on their maximum velocity. Accordingly, this is the basic set of independent variables in this approach. MCA designates each *enzyme concentration* (or each  $V_j$  for each individual step reaction) as  $E_j$ , where the subscript  $j$  takes values from 1 to the total number of enzymes. Metabolite concentrations that are independent variables, that is, source and sink metabolites and inhibitors, are named *external effectors* in the MCA terminology. They are considered a separate set of independent variables and are designed as  $Q_j$ , where the subscript  $j$  extends from 1 to the number of external effectors considered.

In BST all independent variables of interest in the system are formally included under the same set, be they enzyme concentrations (or  $V_j$ ), source metabolites, inhibitors, or any other kind of variable. Independent variables are designated  $X_j$ , with the subscript  $j$  ranging

TABLE 3

Independent variable	MCA nomenclature	BST nomenclature
$V_{in}$	$E_1$	$X_6$
$V_{HK}$	$E_2$	$X_7$
$V_{PFK}$	$E_3$	$X_8$
$V_{GAPD}$	$E_4$	$X_9$
$V_{PK}$	$E_5$	$X_{10}$
$V_{POL}$	$E_6$	$X_{11}$
$V_{GOL}$	$E_7$	$X_{12}$
$V_{ATPase}$	$E_8$	$X_{13}$
NADH/NAD <sup>+</sup> ratio	$Q_1$	$X_{14}$
Polysaccharide pool	$Q_2$	$X_{15}$
Glycerol pool	$Q_3$	$X_{16}$
UDPG	$Q_4$	$X_{17}$
Ethanol	$Q_5$	$X_{18}$
Glucose <sub>OUT</sub>	$Q_6$	$X_{19}$

from  $n + 1$  to  $n + m$ , where  $n$  is the number of dependent variables and  $m$  is the number of independent variables considered.

In the illustrative pathway shown in Figure 1a–c, we recognize the set of independent variables listed in Table 3, which are named differently in MCA and BST according to the above described rules.

#### *Constrained Variables*

Some variables related to mass conservation constraints such as [ADP] and [AMP] have not been included. Their concentrations are a function of [ATP] ( $X_5$ ), the  $K_{eq}$  of the adenylate kinase reaction, and the sum of the adenine nucleotides, [AMP] + [ADP] + [ATP] = 3 mM, measured by Galazzo and Bailey [29]. Then, [ADP] and [AMP] can be calculated from the [ATP] values and from these relationships and need not to be considered in the set of dependent variables. We use these constraints to express both [AMP] and [ADP] as a function of [ATP], the  $K_{eq}$  of the adenylate kinase equation, and the adenylate pool, with [ATP] the only variable to be considered. Following these results, [AMP] and [ADP] are substituted in each rate law. This way of including the constrained variables in a model yields exactly the same results as the general method proposed by Savageau [33] within BST (see also [25]) or the method proposed by Fell and Sauro [34] within MCA. However, the former method of solving for the constrained variables and then substituting the solution directly into the rate laws cannot always be used because in more complex constraints there is no analytical solution. Nevertheless, such complex constraints can be handled by the more general methods mentioned above.

[F6P], [G3P], and [3PG] are also related to the set of dependent variables by the following equilibrium ratios:

$$[\text{F6P}]/[\text{G6P}] = 0.3, \quad [\text{G3P}]/[\text{FDP}] = 0.01, \quad [\text{PEP}]/[\text{3PG}] = 0.1.$$

These constraints have been measured by Galazzo and Bailey [30]. Hence, we can drop [F6P], [G3P], and [3PG] from the set of dependent variables.

Usually, MCA assigns correlative numbers following the last dependent variable for these constrained variables. BST uses correlative numbers following the last independent variable. Hence, [AMP], [ADP], [F6P], [G3P], and [3PG] are designated in the following manner:

Nonexplicit dependent variables	MCA nomenclature	BST nomenclature
ADP	$X_6$	$X_{20}$
AMP	$X_7$	$X_{21}$
F6P	$X_8$	$X_{22}$
G3P	$X_9$	$X_{23}$
3PG	$X_{10}$	$X_{24}$

#### IDENTIFICATION AND NOMENCLATURE OF THE DYNAMIC PROCESSES

##### *Velocities of Individual Steps*

In MCA, first we assign an ordinal number  $j$  to each step. Each velocity of each step is then named  $v_j$ . The subscript  $j$  extends from 1 to the total number of steps considered in the pathway. In general, these numbers follow the scheme used for the enzymes.

In BST several numbering schemes are used depending upon the focus. For individual reactions we define  $v_{ij}$  to indicate the rate of transformation of  $X_i$  into  $X_j$  [17, 18, 25]. This is a correlative notation in which the subscripts  $i, j$  identify the reaction with its substrate and product. In the GMA representation, within BST we define  $V_{ir}^+$  as the rate of synthesis of  $X_i$  via the  $r$ th parallel reaction. Similarly, we define  $V_{ir}^-$  as the rate of degradation of  $X_i$  via the  $r$ th parallel reaction. The subscript  $r$  is dropped when there are no parallel reactions. A third numbering scheme is defined for the S-system representation, as will be discussed below.

According to these rules, the velocities of the individual steps considered in the metabolic pathway of Figure 1 are designated in the

following manner:

Step velocity	MCA	BST	
		Correlative	GMA
$v_{in}$	$v_1$	$v_{19,1}$	$V_1^+$
$v_{HK}$	$v_2$	$v_{1,2}$	$V_2^+$
$v_{PFK}$	$v_3$	$v_{22,3}$	$V_3^+$
$v_{GAPD}$ (FDP degradation)	$v_4$	$v_{3,4}$	$V_4^+/2$
$v_{PK}$	$v_5$	$v_{4,18}$	$V_4^-$
$v_{POL}$	$v_6$	$v_{2,15}$	$V_{2,2}^-$
$v_{GOL}$ (glycerol production)	$v_7$	$v_{3,16}$	$2V_{3,2}^-$
$v_{ATPase}$	$v_8$	$v_{5,20}$	$V_{5,4}^-$

### Aggregated Velocities

As a foundation for deriving the S-system representation within BST [17, 28], we define a net rate of synthesis ( $V_i^+$ ) and a net rate of degradation ( $V_i^-$ ) for each dependent metabolite ( $X_i$  with  $i = 1$  to  $n$ ). The net rate of synthesis is obtained after aggregating the rates of the different individual steps that account for the synthesis of the considered metabolite. The net rate of degradation is obtained after aggregating the different processes. Hence, in the S-system representation, the mass balance of a given internal metabolite is always the difference between  $V_i^+$  and  $V_i^-$ .

According to these rules, we can write the following net rates of synthesis and degradation for the metabolic pathway of Figure 1:

$$\begin{aligned}
 V_1^+ &= v_{19,1}, & V_1^- &= v_{1,2}, \\
 V_2^+ &= v_{1,2}, & V_2^- &= v_{22,3} + v_{2,15}, \\
 V_3^+ &= v_{22,3}, & V_3^- &= v_{3,16}/2 + v_{3,4}, \\
 V_4^+ &= 2v_{3,4}, & V_4^- &= v_{4,18}, \\
 V_5^+ &= 2v_{3,4} + v_{4,18}, & V_5^- &= v_{1,2} + v_{22,3} + v_{2,15} + v_{5,20}.
 \end{aligned}$$

### BASIC ELEMENTS OF MCA

In MCA, rate laws are not explicitly described. MCA uses the normalized partial derivatives of velocities with regard to an independent or dependent variable as a basic element for describing the system. These derivatives are designated as *elasticities*, and they are named in different ways depending on the variable considered. Basically,

*Elasticity.*  $X_i$  is a dependent variable,

$$\left( \frac{\partial v_j}{\partial X_i} \right)_0 \left( \frac{X_{i_0}}{v_{j_0}} \right) = \epsilon_{X_i}^{v_j} = \epsilon_i^j,$$

*Special Elasticity.*  $Q_i$  is an external effector,

$$\left( \frac{\partial v_j}{\partial Q_i} \right)_0 \left( \frac{Q_{i_0}}{v_{j_0}} \right) = \kappa_{Q_i}^{v_j} = \kappa_i^j$$

$\pi$ -Elasticity.  $E_i$  is an enzyme,

$$\left( \frac{\partial v_j}{\partial E_i} \right)_0 \left( \frac{E_{i_0}}{y_{j_0}} \right) = \pi_{E_i}^{v_j} = \pi_i^j$$

The values of these parameters for each of the processes of the reference system are shown in Table 4.

#### BASIC ELEMENTS OF BST

In BST, rate laws are explicitly described. The form of the rate law depends upon the representation of the mass balance equations being used.

#### Mass Balance Equations

The mass balances for the pathway of Figure 1 are

Node equations	GMA	S-System
$\dot{X}_1 = v_{19,1} - v_{1,2}$	$V_1^+ - V_1^-$	$V_1^+ - V_1^-$
$\dot{X}_2 = v_{1,2} - v_{22,3} - v_{2,15}$	$V_2^+ - V_{2,1}^- - V_{2,2}^-$	$V_2^+ - V_2^-$
$\dot{X}_3 = v_{22,3} - v_{3,4} - \frac{v_{3,16}}{2}$	$V_3^+ - V_{3,1}^- - V_{3,2}^-$	$V_3^+ - V_3^-$
$\dot{X}_4 = 2v_{3,4} - v_{4,18}$	$V_4^+ - V_4^-$	$V_4^+ - V_4^-$
$\dot{X}_5 = 2v_{3,4} + v_{4,18} - v_{1,2} - v_{22,3} - v_{2,15} - v_{5,20}$	$V_{5,1}^+ + V_{5,2}^+ - V_{5,1}^- - V_{5,2}^- - V_{5,3}^- - V_{5,4}^-$	$V_5^+ - V_5^-$

#### Representation of Rate Laws in the GMA Variant Within BST

As the first step, we consider the mathematical representation of the experimental system by following the GMA variant within BST. This provides a clear side-by-side translation of the results to those obtained in MCA. To build up this representation, each rate law is written as an

TABLE 4  
Elasticities and GMA Kinetic Orders Computed for the  
Fermentation Pathway of *Saccharomyces cerevisiae*<sup>a</sup>

	Suspended cells, pH = 4.5	Suspended cells, pH = 5.5	Immobilized cells, pH = 4.5	Immobilized cells, pH = 5.5
$g_{12} = \epsilon_{X_2}^{V_1}$	-0.2344	-0.4549	-0.2829	-0.3284
$g_{16} = \pi_{E_1}^{V_1}$	1	1	1	1
$h_{11} = \epsilon_{X_1}^{V_2}$	0.7464	0.7908	0.4396	0.4728
$h_{15} = \epsilon_{X_5}^{V_2}$	0.0243	0.014	0.0435	0.0275
$h_{17} = \pi_{E_2}^{V_2}$	1	1	1	1
$g_{32} = \epsilon_{X_2}^{V_3}$	0.7318	1.019	0.1524	0.263
$g_{35} = \epsilon_{X_5}^{V_3}$	-0.3941	-1.777	-0.0255	-0.4303
$g_{38} = \pi_{E_3}^{V_3}$	1	1	1	1
$h_{22,2} = \epsilon_{X_2}^{V_6}$	8.6107	7.0542	0.7349	0.3661
$h_{211,2} = \pi_{E_6}^{V_6}$	1	1	1	1
$g_{43} = \epsilon_{X_3}^{V_4}$	0.6159	0.7004	0.3546	0.4061
$g_{45} = \epsilon_{X_5}^{V_4}$	0.1308	0.2038	0.0954	0.1473
$g_{49} = \pi_{E_4}^{V_4}$	1	1	1	1
$g_{414} = \kappa_{Q_1}^{V_4}$	-0.6088	-0.69	-0.3219	-0.03798
$h_{33,2} = \epsilon_{X_3}^{V_7^b}$	0.05	0.179	0.007	0.0001
$h_{34,2} = \epsilon_{X_4}^{V_7^b}$	0.533	0.603	0.188	0.023
$h_{35,2} = \epsilon_{X_5}^{V_7^b}$	-0.0822	-0.6907	-0.1823	-1.1451
$h_{312,2} = \pi_{E_7}^{V_7}$	1	1	1	1
$h_{43} = \epsilon_{X_3}^{V_8}$	0.05	0.179	0.007	0.0001
$h_{44} = \epsilon_{X_4}^{V_8}$	0.533	0.603	0.188	0.023
$h_{45} = \epsilon_{X_5}^{V_8}$	-0.0822	-0.6907	-0.1823	-1.1451
$h_{410} = \pi_{E_5}^{V_8}$	1	1	1	1
$h_{55,4} = \epsilon_{X_5}^{V_8}$	1	1	1	1
$h_{513,4} = \pi_{E_8}^{V_8}$	1	1	1	1

<sup>a</sup>Experimental data of Gallazzo and Bailey [29, 30]. In each case, these parameters are computed from the original rate laws derived experimentally and published by these authors [see Equation (2) and the corresponding definition for the elasticity parameters].

<sup>b</sup>It should be noted that the three kinetic orders corresponding to the effect of FDP, PEP, and ATP on  $v_{\text{GOL}}$  have the same values as the kinetic orders with respect to  $v_{\text{PK}}$ . This is because  $v_{\text{GOL}}$  was considered to be proportional to  $v_{\text{PK}}$  [29, 30].

appropriate product of power law functions. For a system consisting of  $n$  dependent and  $m$  independent variables, these rate laws are written

$$V_{ir}^+ = \alpha_{ir} \prod_{j=1}^{n+m} X_j^{g_{ij,r}}, \quad V_{ir}^- = \beta_{ir} \prod_{j=1}^{n+m} X_j^{h_{ij,r}}. \quad (1)$$

The power law representation can be derived from first principles for arbitrary rate laws [33,35–37] and is obtained by specifying a rate constant (the multiplicative term) and writing one power term for each variable that directly influences the rate law in question (metabolites, effectors, enzymes, etc.). See [17] for details. The exponent parameters in each power term are referred to as *kinetic orders*. The kinetic orders are defined as

$$\left( \frac{\partial V_{ir}^+}{\partial X_j} \right)_0 \left( \frac{X_{j_0}}{V_{ir_0}^+} \right) = g_{ij,r}, \quad \left( \frac{\partial V_{ir}^-}{\partial X_j} \right)_0 \left( \frac{X_{j_0}}{V_{ir_0}^-} \right) = h_{ij,r}, \quad (2)$$

where the subscript 0 indicates evaluation at a given steady state.

In each case, the first subscript,  $i$ , is associated with the corresponding  $V_i$  and the second subscript,  $j$ , is associated with the variable  $X_j$  that modulates the process. The third subscript,  $r$ , is an ordinal that is added to distinguish each individual process when there is more than a single process accounting for the synthesis (or degradation) of  $X_i$ . The  $r$  subscript is omitted when there is only a single reaction contributing to the flux for synthesis (or degradation).

The rate constants are defined as

$$\alpha_{ir} = V_{ir_0}^+ \prod_{j=1}^{n+m} X_{j_0}^{-g_{ij,r}}, \quad \beta_{ir} = V_{ir_0}^- \prod_{j=1}^{n+m} X_{j_0}^{-h_{ij,r}}. \quad (3)$$

For the model pathway described in Figure 1, the variables that directly influence a rate law are identified by inspection of the metabolic scheme. To make this explicit, and to facilitate the construction in the power law terms, we will indicate these influences by first making them explicit in the mass balance equations:

$$\begin{aligned} \dot{x}_1 &= V_1^+(X_2, X_6) - V_1^-(X_1, X_5, X_7), \\ \dot{x}_2 &= V_2^+(X_1, X_5, X_7) - V_{2,1}^-(X_2, X_5, X_8) - V_{2,2}^-(X_2, X_{11}), \\ \dot{x}_3 &= V_3^+(X_2, X_5, X_8) - V_{3,1}^-(X_3, X_5, X_9, X_{14}) - V_{3,2}^-(X_3, X_4, X_5, X_{12}), \\ \dot{x}_4 &= V_4^+(X_3, X_5, X_9, X_{14}) - V_4^-(X_3, X_4, X_5, X_{10}), \\ \dot{x}_5 &= V_{5,1}^+(X_3, X_5, X_9, X_{14}) + V_{5,2}^+(X_3, X_4, X_5, X_{10}) - V_{5,1}^-(X_1, X_5, X_7) \\ &\quad - V_{5,2}^-(X_2, X_{11}) - V_{5,3}^-(X_2, X_5, X_8) - V_{5,4}^-(X_5, X_{13}). \end{aligned} \quad (4)$$

The extracellular concentration of glucose does not appear as a variable because we consider this metabolite constant [29]. Since [ATP] does not appear explicitly in the rate expression of  $v_{\text{POL}}$  [29], we have

not included this variable explicitly in  $V_{2,2}^-$  and  $V_{5,2}^-$ . Further, since Galazzo and Bailey considered  $v_{\text{GOL}}$  to be proportional to  $v_{\text{PK}}$ , the kinetic orders  $h_{33,2}$ ,  $h_{34,2}$ , and  $h_{35,2}$  have exactly the same values as  $h_{43}$ ,  $h_{44}$ , and  $h_{45}$ . [ATP] and [PEP] also are not involved in the  $v_{\text{GOL}}$  process (see Table 4).

From Equation (4) the GMA equations can be written as

$$\begin{aligned}
 \dot{X}_1 &= \alpha_1 X_2^{g_{12}} X_6^{g_{16}} - \beta_1 X_1^{h_{11}} X_5^{h_{15}} X_7^{h_{17}}, \\
 \dot{X}_2 &= \alpha_2 X_1^{g_{21}} X_3^{g_{25}} X_7^{g_{27}} - \beta_{2,1} X_2^{h_{22,1}} X_5^{h_{25,1}} X_8^{h_{28,1}} - \beta_{2,2} X_2^{h_{22,2}} X_{11}^{h_{211,2}}, \\
 \dot{X}_3 &= \alpha_3 X_2^{g_{32}} X_5^{g_{35}} X_8^{g_{38}} - \beta_{3,1} X_3^{h_{33,1}} X_5^{h_{35,1}} X_9^{h_{39,1}} X_{14}^{h_{314,1}} \\
 &\quad - \beta_{3,2} X_3^{h_{33,2}} X_4^{h_{34,2}} X_5^{h_{35,2}} X_{12}^{h_{312,2}}, \\
 \dot{X}_4 &= \alpha_4 X_3^{g_{43}} X_5^{g_{45}} X_9^{g_{49}} X_{14}^{g_{414}} - \beta_4 X_3^{h_{43}} X_4^{h_{44}} X_5^{h_{45}} X_{10}^{h_{410}}, \\
 \dot{X}_5 &= \alpha_{5,1} X_3^{g_{53,1}} X_5^{g_{55,1}} X_9^{g_{59,1}} X_{14}^{g_{514,1}} + \alpha_{5,2} X_3^{g_{53,2}} X_4^{g_{54,2}} X_5^{g_{55,2}} X_{10}^{g_{510,2}} \\
 &\quad - \beta_{5,1} X_1^{h_{51,1}} X_5^{h_{55,1}} X_7^{h_{57,1}} - \beta_{5,2} X_2^{h_{52,2}} X_{11}^{h_{511,2}} \\
 &\quad - \beta_{5,3} X_2^{h_{52,3}} X_5^{h_{55,3}} X_8^{h_{58,3}} - \beta_{5,4} X_5^{h_{55,4}} X_{13}^{h_{513,4}}. \tag{5}
 \end{aligned}$$

In Equations (5) we have included all the enzyme concentrations (maximum velocities) as independent variables to make the interpretation of the results more transparent and facilitate the comparison of MCA and BST.

Precursor-product relationships determine the conservation of flow between some components. This means that the corresponding parameters have the same value. For example, in our case, the degradation of  $X_1$  is the same as the synthesis of  $X_2$ , and so the corresponding power laws are the same. Accordingly it should be noted that the following equivalences appear between the kinetic orders and rate constants in the above GMA representation:

$$\begin{array}{lll}
 \alpha_2 = \beta_1 = \beta_{5,1}, & \alpha_3 = \beta_{2,1} = \beta_{5,3}, & \alpha_4 = 2\beta_{3,1} = \alpha_{5,1}, \\
 \beta_4 = \alpha_{5,2}, & \beta_{5,2} = \beta_{2,2}, & h_{11} = g_{21} = h_{51,1}, \\
 g_{32} = h_{22,1} = h_{52,3}, & g_{43} = h_{33,1} = g_{53,1}, & h_{43} = g_{53,2}, \\
 h_{52,2} = h_{22,2}, & h_{15} = g_{25} = h_{55,1}, & g_{35} = h_{25,1} = h_{55,3}, \\
 g_{45} = h_{35,1} = g_{55,1}, & h_{44} = g_{54,2}, & h_{511,2} = h_{211,2}, \\
 h_{17} = g_{27} = h_{57,1}, & g_{38} = h_{28,1} = h_{58,3}, & g_{49} = h_{39,1} = g_{59,1}, \\
 h_{45} = g_{55,2}, & g_{414} = g_{314,1} = g_{514,1}, & h_{410} = g_{510,2}.
 \end{array}$$



These equivalences allow one to identify the final set of parameters involved in the GMA representation of the reference system (see Table 4).

The values of the kinetic order set in the GMA representation can easily be computed from the rate laws and the operational steady-state values of system variables described in the original papers of Galazzo and Bailey [29, 30]. Despite the different names and nomenclature, it should be noted that the different elasticity coefficients have the same interpretation as the kinetic orders in GMA, as can be appreciated by inspection of their definition once the correspondence between the nomenclature used to indicate variables and fluxes is realized. Hence, there is a *one-to-one* correspondence between the elasticity coefficients and the kinetic orders in the GMA representation [18]. The list of elasticity coefficients that can be defined in the example, their values at each experimental condition considered, and the corresponding equivalences with the GMA kinetic orders appear in Table 4.

#### *Representation of Rate Laws in the S-System Variant Within BST*

The experience accumulated using BST indicates that it is often better to transform Equation (5) to the S-system representation [17, 18, 25, 38, 39]. The advantages include a greater efficiency in characterizing the system, the possibility of obtaining an explicit solution for the steady-state equations, greater accuracy in predicting the dynamic response of the system, and the possibility of investigating the stability of the steady state [10, 16–18]. The corresponding S-system equations are obtained by defining a power law representation for each of the aggregated fluxes  $V_i^+$  and  $V_i^-$  (see the mass balance equations).

$$\begin{aligned}
 \dot{X}_1 &= \alpha_1 X_2^{g_{12}} X_6^{g_{16}} - \beta_1 X_1^{h_{11}} X_5^{h_{15}} X_7^{h_{17}}, \\
 \dot{X}_2 &= \alpha_2 X_1^{g_{21}} X_5^{g_{25}} X_7^{g_{27}} - \beta_2 X_2^{h_{22}} X_5^{h_{25}} X_8^{h_{28}} X_{11}^{h_{211}}, \\
 \dot{X}_3 &= \alpha_3 X_2^{g_{32}} X_5^{g_{35}} X_8^{g_{38}} - \beta_3 X_3^{h_{33}} X_4^{h_{34}} X_5^{h_{35}} X_9^{h_{39}} X_{12}^{h_{312}} X_{14}^{h_{314}}, \\
 \dot{X}_4 &= \alpha_4 X_3^{g_{43}} X_5^{g_{45}} X_9^{g_{49}} X_{14}^{g_{414}} - \beta_4 X_3^{h_{43}} X_4^{h_{44}} X_5^{h_{45}} X_{10}^{h_{410}}, \\
 \dot{X}_5 &= \alpha_5 X_3^{g_{53}} X_4^{g_{54}} X_5^{g_{55}} X_9^{g_{59}} X_{10}^{g_{510}} X_{14}^{g_{514}} \\
 &\quad - \beta_5 X_1^{h_{51}} X_2^{h_{52}} X_5^{h_{55}} X_7^{h_{57}} X_8^{h_{58}} X_{11}^{h_{511}} X_{13}^{h_{513}}.
 \end{aligned} \tag{6}$$

Equations (5) (the generalized mass action representation) and (6) (the S-system representation) differ in those metabolites that have more than one process either in their synthesis ( $X_5$  in our case) and/or their degradation ( $X_2$ ,  $X_3$ , and  $X_5$  in our case) [17, 25, 38]. For these cases,

the correspondence between the GMA and S-system representations is

$$\begin{aligned} V_5^+ &= V_{5,1}^+ + V_{5,2}^+, & V_2^- &= V_{2,1}^- + V_{2,2}^-, & V_3^- &= V_{3,1}^- + V_{3,2}^-, \\ V_5^- &= V_{5,1}^- + V_{5,2}^- + V_{5,3}^- + V_{5,4}^-. \end{aligned} \quad (7)$$

### *Solving the Steady-State Equations*

The aggregation strategy is a particular characteristic of the S-system variant of BST and allows us to solve the steady-state equations explicitly. If we look at the S-system representation of our system, the steady-state equations can be written

$$\begin{aligned} 0 &= \alpha_1 X_2^{g_{12}} X_6^{g_{16}} - \beta_1 X_1^{h_{11}} X_5^{h_{15}} X_7^{h_{17}}, \\ 0 &= \alpha_2 X_1^{g_{21}} X_3^{g_{25}} X_7^{g_{27}} - \beta_2 X_2^{h_{22}} X_5^{h_{25}} X_8^{h_{28}} X_{11}^{h_{211}}, \\ 0 &= \alpha_3 X_2^{g_{32}} X_3^{g_{35}} X_8^{g_{38}} - \beta_3 X_3^{h_{33}} X_4^{h_{34}} X_5^{h_{35}} X_9^{h_{39}} X_{12}^{h_{312}} X_{14}^{h_{314}}, \\ 0 &= \alpha_4 X_3^{g_{43}} X_5^{g_{45}} X_9^{g_{49}} X_{14}^{g_{414}} - \beta_4 X_3^{h_{43}} X_4^{h_{44}} X_5^{h_{45}} X_{10}^{h_{410}}, \\ 0 &= \alpha_5 X_3^{g_{53}} X_4^{g_{54}} X_5^{g_{55}} X_9^{g_{59}} X_{10}^{g_{510}} X_{14}^{g_{514}} \\ &\quad - \beta_5 X_1^{h_{51}} X_2^{h_{52}} X_5^{h_{55}} X_7^{h_{57}} X_8^{h_{58}} X_{11}^{h_{511}} X_{13}^{h_{513}}. \end{aligned} \quad (8)$$

If none of the  $\alpha_i$  or  $\beta_i$  terms is equal to zero, a logarithmic transformation of the system variables reduces the equations to a set of linear algebraic equations. Upon identifying  $y_i = \log X_i$  and  $b_i = \log(\beta_i / \alpha_i)$ , Equations (8) can be written as

$$\begin{aligned} &\left( \begin{bmatrix} 0 & g_{12} & 0 & 0 & 0 \\ g_{21} & 0 & 0 & 0 & g_{25} \\ 0 & g_{32} & 0 & 0 & g_{35} \\ 0 & 0 & g_{43} & 0 & g_{45} \\ 0 & 0 & g_{53} & g_{54} & g_{55} \end{bmatrix} - \begin{bmatrix} h_{11} & 0 & 0 & 0 & h_{15} \\ 0 & h_{22} & 0 & 0 & h_{25} \\ 0 & 0 & h_{33} & h_{34} & h_{35} \\ 0 & 0 & h_{43} & h_{44} & h_{45} \\ h_{51} & h_{52} & 0 & 0 & h_{55} \end{bmatrix} \right) \cdot \begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \end{pmatrix} \\ &+ \begin{pmatrix} g_{16} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & g_{27} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & g_{38} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & g_{49} & 0 & 0 & 0 & 0 & g_{414} \\ 0 & 0 & 0 & g_{59} & g_{510} & 0 & 0 & 0 & g_{514} \end{pmatrix} \end{aligned}$$

$$\begin{aligned}
 & - \begin{bmatrix} 0 & h_{17} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & h_{28} & 0 & 0 & h_{211} & 0 & 0 & 0 \\ 0 & 0 & 0 & h_{39} & 0 & 0 & h_{312} & 0 & h_{314} \\ 0 & 0 & 0 & 0 & h_{410} & 0 & 0 & 0 & 0 \\ 0 & h_{57} & h_{58} & 0 & 0 & h_{511} & 0 & h_{513} & 0 \end{bmatrix} \cdot \begin{pmatrix} y_6 \\ y_7 \\ y_8 \\ y_9 \\ y_{10} \\ y_{11} \\ y_{12} \\ y_{13} \\ y_{14} \end{pmatrix} \\
 & = \begin{pmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \\ b_5 \end{pmatrix}. \tag{9}
 \end{aligned}$$

Equation (9) can be written as

$$([G]_D - [H]_D) \cdot y]_D + ([G]_I - [H]_I) \cdot y]_I = b], \tag{10}$$

where  $[G]_D$ ,  $[H]_D$ ,  $[G]_I$ ,  $[H]_I$ ,  $y]_D$ ,  $y]_I$ , and  $b]$  can be written systematically by simple inspection of the S-system equations, that is:

- $[G]_D$ :  $n \times n$  matrix whose elements are the kinetic orders  $g_{ij}$ , where  $X_j$  is a dependent variable ( $g_{ij} = 0$  if  $X_j$  does not affect  $V_i^+$ ).
- $[G]_I$ :  $n \times m$  matrix whose elements are the kinetic orders  $g_{ij}$ , where  $X_j$  is an independent variable ( $g_{ij} = 0$  if  $X_j$  does not affect  $V_i^+$ ).
- $[H]_D$ :  $n \times n$  matrix whose elements are the kinetic orders  $h_{ij}$ , where  $X_j$  is a dependent variable ( $h_{ij} = 0$  if  $X_j$  does not affect  $V_i^-$ ).
- $[H]_I$ :  $n \times m$  matrix whose elements are the kinetic orders  $h_{ij}$ , where  $X_j$  is an independent variable ( $h_{ij} = 0$  if  $X_j$  does not affect  $V_i^-$ ).
- $y]_D$ :  $n$ -dimensional vector of dependent variables (logarithmic scale).
- $y]_I$ :  $m$ -dimensional vector of the independent variables (logarithmic scale).
- $b]$ :  $n$ -dimensional vector whose elements are the parameters  $b_i$ .

If, additionally, we define

$$[A]_D = [G]_D - [H]_D \quad \text{and} \quad [A]_I = [G]_I - [H]_I,$$

then Equation (10) can be written as

$$[A]_{\text{D}} \cdot y]_{\text{D}} + [A]_{\text{I}} \cdot y]_{\text{I}} = b], \quad (11)$$

which yields an explicit solution for  $y]_{\text{D}}$ , provided that  $[A]_{\text{D}}$  has an inverse:

$$y]_{\text{D}} = -[A]_{\text{D}}^{-1} \cdot [A]_{\text{I}} \cdot y]_{\text{I}} + [A]_{\text{D}}^{-1} \cdot b]. \quad (12)$$

This equation has important implications. It allows us to characterize the response of the system after a change in an independent variable or a parameter of the representation. In the next two papers [31, 32] we use this property to characterize the experimental pathway.

Aggregation of fluxes through pools to define an S-system has been claimed to be a artificial procedure that destroys the original structure of the system. However, it should be noted that elasticities and GMA kinetic orders are also parameters of a given aggregation strategy through individual processes. This is specially clear for reversible reactions. This issue is reviewed in [38] and developed further in [25]. Differentiation and normalization of Equations (7) yields the following relationships:

$$\begin{aligned} h_{22} &= \frac{h_{22,1}V_{2,1}^- + h_{22,2}V_{2,2}^-}{V_2^-}, & h_{25} &= \frac{h_{25,1}V_{2,1}^-}{V_2^-}, & h_{28} &= \frac{h_{28,1}V_{2,1}^-}{V_2^-}, \\ h_{211} &= \frac{h_{211,2}V_{2,2}^-}{V_2^-}, & h_{33} &= \frac{h_{33,1}V_{3,1}^- + h_{33,2}V_{3,2}^-}{V_3^-}, & h_{34} &= \frac{h_{34,2}V_{3,2}^-}{V_3^-}, \\ h_{35} &= \frac{h_{35,1}V_{3,1}^- + h_{35,2}V_{3,2}^-}{V_3^-}, & h_{39} &= \frac{h_{39,1}V_{3,1}^-}{V_3^-}, & h_{312} &= \frac{h_{312,2}V_{3,2}^-}{V_3^-}, \\ h_{314} &= \frac{h_{314,1}V_{3,1}^-}{V_3^-}, & h_{51} &= \frac{h_{51,1}V_{5,1}^-}{V_5^-}, \\ h_{52} &= \frac{h_{52,2}V_{5,2}^- + h_{52,3}V_{5,3}^-}{V_5^-}, & h_{55} &= \frac{h_{55,1}V_{5,1}^- + h_{55,3}V_{5,3}^- + h_{55,4}V_{5,4}^-}{V_5^-}, \\ h_{57} &= \frac{h_{57,1}V_{5,1}^-}{V_5^-}, & h_{58} &= \frac{h_{58,3}V_{5,3}^-}{V_5^-}, & h_{511} &= \frac{h_{511,2}V_{5,2}^-}{V_5^-}, \\ h_{513} &= \frac{h_{513,4}V_{5,4}^-}{V_5^-}, & g_{53} &= \frac{g_{53,1}V_{5,1}^+ + g_{53,2}V_{5,2}^+}{V_5^+}, & g_{54} &= \frac{g_{54,2}V_{5,2}^+}{V_5^+}, \\ g_{55} &= \frac{g_{55,1}V_{5,1}^+ + g_{55,2}V_{5,2}^+}{V_5^+}, & g_{514} &= \frac{g_{514,1}V_{5,1}^+}{V_5^+}, & g_{59} &= \frac{g_{59,1}V_{5,1}^+}{V_5^+}, \\ g_{510} &= \frac{g_{510,2}V_{5,2}^+}{V_5^+}. \end{aligned}$$

TABLE 5

Kinetic Orders for the S-System Representation of the Experimental System<sup>a</sup>

Calculated kinetic orders	Suspended cells, pH = 4.5	Suspended cells, pH = 5.5	Immobilized cells, pH = 4.5	Immobilized cells, pH = 5.5
$h_{22}$	0.739	1.355	0.286	0.288
$h_{25}$	-0.394	-1.678	-0.019	-0.324
$h_{28}$	0.999	0.944	0.77	0.753
$h_{211}$	0.001	0.056	0.23	0.247
$h_{33}$	0.584	0.667	0.332	0.377
$h_{34}$	0.03	0.038	0.012	0.002
$h_{35}$	0.119	0.147	0.077	0.056
$h_{39}$	0.944	0.937	0.935	0.93
$h_{312}$	0.056	0.063	0.065	0.07
$h_{314}$	-0.575	-0.6464	-0.301	-0.353
$h_{51}$	0.198	0.223	0.153	0.169
$h_{52}$	0.196	0.383	0.099	0.103
$h_{55}$	0.372	-0.035	0.314	0.18
$h_{57}$	0.265	0.283	0.347	0.357
$h_{58}$	0.265	0.267	0.267	0.269
$h_{511}$	0.0002	0.016	0.08	0.088
$h_{513}$	0.47	0.435	0.305	0.286
$g_{53}$	0.333	0.439	0.181	0.203
$g_{54}$	0.266	0.301	0.094	0.011
$g_{55}$	0.024	-0.243	-0.043	-0.497
$g_{514}$	-0.304	-0.345	-0.161	-0.19
$g_{59}$	0.5	0.5	0.5	0.5
$g_{510}$	0.5	0.5	0.5	0.5

<sup>a</sup>These parameters are computed using Equation (2) for the aggregated fluxes. Correspondence of these parameters with those in Table 4 is indicated in the text.

The kinetic orders corresponding to the aggregated rate laws appearing in the S-system representation can be calculated according to these equations. As has been pointed out in [18], this fact also clarifies the relationships between the elasticities of MCA and kinetic orders of BST. The values of these kinetic orders are given in Table 5 for the four experimental conditions. The numerical values of  $\alpha_i$  and  $\beta_i$  are calculated from these kinetic order values and steady-state concentration values of metabolites, and these appear in Table 6. Since we have been using aggregated rate laws, some of the parameters in the S-system representation are not independent. This is clear from the meaning of the aggregated fluxes.

TABLE 6  
Rate Constant Parameters for the S-System Representation<sup>a</sup>

Rate constant	Suspended cells, pH = 4.5	Suspended cells, pH = 5.5	Immobilized cells, pH = 4.5	Immobilized cells, pH = 5.5
$\alpha_1$	0.8122	0.8667	1.0344	1.0848
$\alpha_2$	2.8632	3.3579	1.2593	1.3588
$\alpha_3$	0.5232	0.6304	0.7454	0.8055
$\alpha_4$	0.022	0.014	0.1166	0.0797
$\alpha_5$	0.0913	0.08784	0.1102	0.0976
$\beta_1$	2.8632	3.3579	1.2593	1.3588
$\beta_2$	0.5239	0.5572	1.0158	1.1807
$\beta_3$	0.0148	0.010276	0.0672	0.048
$\beta_4$	0.0945	0.1372	0.026	0.03
$\beta_5$	3.2097	3.5023	3.2621	3.5332

<sup>a</sup>These parameters are computed using Equation (3) for the aggregated fluxes. In each experimental condition, the steady-state values indicated in Tables 1 and 2 were used.

## DISCUSSION

Mathematical methods play an important role in providing new insights in metabolic studies. Among the several alternatives, the MCA and BST approaches have achieved a leading position as tools for analyzing biochemical pathways. Application of both approaches to the same experimental system permit one to understand the basis of both methodologies and their relatedness and differences. The results presented in this paper show the following.

(1) MCA and BST differ in the consideration of independent variables. MCA considers source metabolite concentrations, external effector concentrations, enzyme concentrations, etc., as separate sets of independent variables. Further, MCA has focused mainly on enzyme concentrations. In contrast, BST makes no distinctions among the kinds of independent variables the experimenter may be interested in. In particular, enzyme levels can be considered independent variables [17, 18]. In any case, it is trivial to identify each component in either of the considered sets; relating the corresponding MCA and BST sets of variables is not difficult, and a dictionary list identifying the meaning of metabolites and fluxes can be written down following the rationale shown in this paper. It is a matter of taste to prefer MCA or BST nomenclature at this level. There is no fundamental reason for not using a single set of independent variables. In BST, for simplicity, because it may be preferable for computation, and because a uniform

notation allows one to see important symmetries and other relationships in the explicit solutions, it is considered to be more convenient to keep track of a single set rather than to maintain several different sets (especially in developing software to compute all the desired steps in analyzing the pathway). It is important to realize that both MCA and BST refer to the same concepts and that the differences at this level are reduced to nomenclature preferences and are thus subjective.

(2) To construct a BST model, either in the basic GMA variant or in the preferred S-system form, all that is needed is a scheme of the pathway, a list of dependent and independent variables, and a list of fluxes. At this level, this is the same information needed to build up the MCA description. However, MCA and BST differ beyond this point. The BST approach derives an explicit representation for each of the processes involved, whereas MCA does not. This has important implications for the development of tools to predict the system behavior. We will show these implications in the following papers [31, 32].

(3) MCA elasticities and BST kinetic orders are related to the same concepts. We have illustrated this relatedness by using the fermentation pathway of *Saccharomyces cerevisiae*. First, the kinetic orders of the GMA representation within BST have a one-to-one translation with the elasticities of MCA. Second, the kinetic orders of the S-system representation are easily related to those of the GMA representation. Hence, we have clearly shown in our example that the *same experimental measurements* (those reported by Galazzo and Bailey [29, 30]) give the basic parameters for both BST and MCA. The fact that the S-system variant within BST uses aggregated fluxes should not be seen as an obstacle to interpreting the corresponding kinetic orders. Again, this is a matter of being acquainted with the nomenclature.

(4) Elasticities and kinetic orders share the same definition: a partial logarithmic derivative evaluated at a given operating point. Hence, their values will change depending on the operating point considered (see Tables 4 and 5). This is true in MCA and in BST. In some cases this variation may be great, in others slight. This has important consequences, which will be seen when we discuss the implications of the description of the system at a given steady state [31].

In conclusion, we have shown that the differences between MCA and BST at the level of fundamental variables and parameters can be overcome by a simple glossary of terms. As Tables 4 and 5 show, the basic parameters are interchangeable and reflect the same concept: how an infinitesimal change in a variable affects a given process. Then, at this level, the information required to build up the representation of the target system is the same.

The differences arise when we consider the system description. MCA does not consider an explicit representation for each rate law. BST develops an explicit representation using the power law concept, which involves important features for further analyzing the target pathway. The implications of this choice will be clear when we consider the kinds of questions that can be answered following each approach. This is the subject of [31, 32].

*Our work is funded by a grant from the Comissió Interdepartamental de Recerca i Innovació Tecnològica of the Generalitat de Catalunya (DGYCIT-CIRIT, (1991) QFN91-4203), and a grant from DGYCIT (PB 92-0852). R. Curto is a Ph.D. student funded by CIRIT BQF92. A. Sorribas is partially funded by an Ajuda de Recerca de l'Ajuntament de Lleida. We thank Dr. J. E. Bailey of ETH (Switzerland) and Dr. P. M. Schlosser of CIIT (United States) for kindly providing us with all experimental data used in this paper and for fruitful discussions.*

#### REFERENCES

- 1 F. Shiraishi and M. A. Savageau, The tricarboxylic acid cycle in *Dictyostelium discoideum*. I. Formulation of alternative kinetic representations, *J. Biol. Chem.* 267:22912–22918 (1992).
- 2 F. Shiraishi and M. A. Savageau, The tricarboxylic acid cycle in *Dictyostelium discoideum*. II. Evaluation of model consistency and robustness, *J. Biol. Chem.* 267:22919–22925 (1992).
- 3 F. Shiraishi and M. A. Savageau, The tricarboxylic acid cycle in *Dictyostelium discoideum*. III. Analysis of steady state and dynamic behavior, *J. Biol. Chem.* 267:22926–22933 (1992).
- 4 F. Shiraishi and M. A. Savageau, The tricarboxylic acid cycle in *Dictyostelium discoideum*. IV. Resolution of discrepancies between alternative methods of analysis, *J. Biol. Chem.* 267:22934–22943 (1992).
- 5 F. Shiraishi and M. A. Savageau, The tricarboxylic acid cycle in *Dictyostelium discoideum*. V. Systemic effects of including protein turnover in the current model, *J. Biol. Chem.* 268:16917–16928 (1993).
- 6 H. Kacser and J. W. Porteous, Control of metabolism: what do we have to measure?, *Trends Biochem. Sci.* 12:5–14 (1987).
- 7 B. Crabtree and E. A. Newsholme, A systematic approach to describing and analyzing metabolic control systems, *Trends Biochem. Sci.* 12:4–12 (1987).
- 8 D. A. Fell, Metabolic control analysis—a survey of its theoretical and experimental development, *Biochem. J.* 286:313–330 (1992).
- 9 R. Heinrich, S. Schuster, and H. G. Holzhutter, Mathematical analysis of enzymatic reaction systems using optimization principles, *Eur. J. Biochem.* 201:1–21 (1991).
- 10 M. A. Savageau, Metabolite channeling—implications for regulation of metabolism and for quantitative description of reactions in vivo, *J. Theor. Biol.* 152:85–92 (1991).



- 11 M. A. Savageau, Dominance according to metabolic control analysis—major achievement or house of cards?, *J. Theor. Biol.* 154:131–136 (1992).
- 12 M. A. Savageau, E. O. Voit, and D. H. Irvine, Biochemical systems theory and metabolic control theory. 1. Fundamental similarities and differences, *Math. Biosci.* 86:127–145 (1987).
- 13 M. A. Savageau, E. O. Voit, and D. H. Irvine, Biochemical systems theory and metabolic control theory. 2. The role of summation and connectivity relationships, *Math. Biosci.* 86:147–169 (1987).
- 14 E. O. Voit, *Canonical Nonlinear Modeling: S-System Approach to Understanding Complexity*, Van Nostrand Reinhold, New York, 1991.
- 15 M. A. Savageau, The behavior of intact biochemical control systems, *Curr. Topics Cell Reg.* 6:63–130 (1972).
- 16 M. A. Savageau, *Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology*, Addison-Wesley, Reading, MA, 1976.
- 17 A. Sorribas and M. A. Savageau, A comparison of variant theories of intact biochemical systems. 1. Enzyme–enzyme interactions and biochemical systems theory, *Math. Biosci.* 94:161–193 (1989).
- 18 A. Sorribas and M. A. Savageau, A comparison of variant theories of intact biochemical systems. 2. Flux-oriented and metabolic control theories, *Math. Biosci.* 94:195–238 (1989).
- 19 M. A. Savageau and A. Sorribas, Constraints among molecular and systemic properties—implications for physiological genetics, *J. Theor. Biol.* 141:93–115 (1989).
- 20 A. Sorribas, S. Samitier, E. I. Canela, and M. Cascante, Metabolic pathway characterizations from transient response data obtained in situ: parameter estimation in S-system models, *J. Theor. Biol.* 162:81–102 (1993).
- 21 H. Kacser and J. A. Burns, Control of enzyme flux, *Symp. Soc. Exp. Biol.* 27:65–104 (1973).
- 22 R. Heinrich and T. A. Rapoport, A linear steady-state treatment of enzymatic chains, *Eur. J. Biochem.* 42:89–95 (1974).
- 23 H. V. Westerhoff and Y. D. Chen, How do enzyme activities control metabolite concentrations? An additional theorem in the theory of metabolic control, *Eur. J. Biochem.* 142:425–430 (1984).
- 24 A. Cornish-Bowden and M. L. Cárdenas, *Control of Metabolic Processes*, NATO ASI Ser., Ser. A: Life Sciences, Plenum, New York, 1990.
- 25 A. Sorribas and M. A. Savageau, Strategies for representing metabolic pathways within biochemical systems theory—reversible pathways, *Math. Biosci.* 94:239–269 (1989).
- 26 M. Cascante, R. Franco and E. I. Canela, Use of implicit methods from general sensitivity theory to develop a systematic approach to metabolic control. I. Unbranched pathways, *Math. Biosci.* 94:271–288 (1989).
- 27 M. Cascante, R. Franco, and E. I. Canela, Use of implicit methods from general sensitivity theory to develop a systematic approach to metabolic control. II. Complex systems, *Math. Biosci.* 94:289–309 (1989).
- 28 M. A. Savageau, Biochemical systems theory: operational differences among variant representations and their significance, *J. Theor. Biol.* 151:509–530 (1991).

- 29 J. L. Galazzo and J. E. Bailey, Fermentation pathway kinetics and metabolic flux control in suspended and immobilized *Saccharomyces cerevisiae*, *Enzyme Microb. Technol.* 12:162–172 (1990).
- 30 J. Galazzo and J. Bailey, Fermentation pathway kinetics and metabolic flux control in suspended and immobilized *Saccharomyces cerevisiae*, Errata, *Enzyme Microb. Technol.* 13:363 (1991).
- 31 M. Cascante, R. Curto, and A. Sorribas, Comparative characterization of the fermentation pathway of *Saccharomyces cerevisiae* using biochemical systems theory and metabolic control analysis: steady-state analysis, *Math. Biosci.*, this issue.
- 32 A. Sorribas, R. Curto, and M. Cascante, Comparative characterization of the fermentation pathway of *Saccharomyces cerevisiae* using biochemical systems theory and metabolic control analysis: model validation and dynamic behavior, *Math. Biosci.*, this issue.
- 33 M. A. Savageau, Allometric morphogenesis of complex systems: derivation of the basic equations from first principles, *Proc. Natl. Acad. Sci. USA* 76:6023–6025 (1979).
- 34 D. A. Fell and H. M. Sauro, Metabolic control and its analysis. Additional relationships between elasticities and control coefficients, *Eur. J. Biochem.* 148:555–561 (1985).
- 35 M. A. Savageau, Biochemical systems analysis. I. Some mathematical properties of the rate law for the component enzymatic reactions, *J. Theor. Biol.* 25:365–369 (1969).
- 36 M. A. Savageau, Biochemical systems analysis. II. Steady state solutions for an  $n$ -pool system using a power-law approximation, *J. Theor. Biol.* 25:370–379 (1969).
- 37 M. A. Savageau, Critique of the enzymologist's test tube, in *Foundations of Medical Cell Biology*, Vol. 3A, E. Bittar, Ed., JAI Press, Greenwich, CT, 1992, pp. 45–108.
- 38 E. O. Voit and M. A. Savageau, Accuracy of alternative representations for integrated biochemical systems, *Biochemistry* 26:6869–6880 (1987).
- 39 M. Cascante, A. Sorribas, R. Franco, and E. I. Canela, Biochemical systems theory: increasing predictive power by using second-order derivatives measurements, *J. Theor. Biol.* 149:521–535 (1991).