Extension of Metabolic Control Analysis to Chemostat Bioreactors

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A method of determining control coefficients and pseudo-first-order kinetic parameters is presented for cell growth, substrate consumption, and product formation in a chemostat bioreactor. From the equations that relate the control coefficients to process variables, such as feed concentrations of the limiting substrate and the product of interest, a perturbation method is developed to determine control coefficients from steady-state measurements. This method combined with the transient response analysis provides a practical way for determining control coefficients and estimating kinetic properties in chemostat bioreactor systems. To determine the kinetic parameters, one measures cell mass, concentrations of the limiting substrate and the product of interest following a step change in the feed concentration until the system reaches a new steady state. The time courses of these variables are processed to obtain control coefficients, which yield the pseudo-first-order kinetic constants for cell growth, substrate consumption, and product formation. Only steady-state responses are needed in calculation, if a step change in cell concentration in the feed stream can also be introduced without significantly perturbing cell physiology. This method is useful in characterizing the kinetics of whole cell bioreactions: results from chemostat experiments can be used to design operating strategies for batch or fed-batch bioreactions. It is generally applicable to continuous-stir-tank reactors with interacting parallel reactions.

Introduction

In the past two decades, quantitative approaches, such as metabolic control analysis (MCA) (Kacser and Burns, 1973; Heinrich and Rapoport, 1974) and biochemical systems theory (Savageau, 1976), have evolved for analyzing the control of metabolic systems. In the context of MCA, emphasis has been on the use of control coefficients as a measure of control strength. These control coefficients are specialized sensitivity coefficients, defined as the (normalized) partial derivative of a system response with respect to the activity (or rate) of a reaction step. They are internally related by some constraints that depend only on the stoichiometry, which have been termed the structural properties of the control coefficients (Reder, 1988). The control coefficients are typically used to pinpoint the controlling steps in intracellular metabolic systems. Although there have been some efforts to apply MCA in bioreactor systems (Westerhoff et al., 1991; Small, 1994), the potential of MCA applied to bioreactor systems has not been fully exploited. In this study, we extend the analysis to continuous bioreactor systems that have a steady state. Instead of determining the rate-controlling steps, our objective is to develop an experimental method to extract macroscopic kinetic properties via control coefficients.

Kinetics of bacterial or cell cultures play an important role in the design and optimization of bioreaction processes. With this information, one can better design medium formulation, feeding rates, and operating strategies. However, obtaining detailed kinetic parameters requires extensive experimentation and the functional form of the kinetic rate expressions. For example, one can measure the initial rates in batch cultures under various conditions, or measure the steady-state rates of continuous cultures at different steady states. This information can then be used to estimate kinetic parameters with a postulated kinetic expression. Alternatively, one can estimate kinetic parameters directly from transient experiments in either batch or continuous cultures with given kinetic functions. However, such estimation is sensitive to ex-

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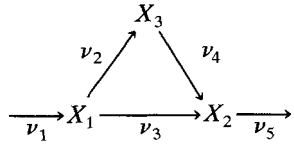
perimental errors, and results may be easily corrupted as the number of parameters increases.

Instead of determining the kinetic parameters directly, we propose to determine the control coefficients from which the pseudo-first-order kinetic constants (elasticity coefficients) can be calculated. This approach avoids problems when some of the kinetic parameters cannot be determined accurately. Control coefficients help to identify steps that are unimportant to the system response of interest. Reaction steps that have relatively small control coefficients can be ignored or lumped, since they have relatively small impact on overall response (Delgado and Liao, 1995). Our group has developed a dynamic approach to determine the control coefficients from transient data (Delgado and Liao, 1992a,b). This approach employs a linear approximation of the kinetics around the steady state, and thus results may be misleading for systems with highly nonlinear kinetics. In this study, we derive equations which relate the control coefficients to process variables. From these equations, we develop a perturbation method for determining the control coefficients from steady-state measurements, and the results are less sensitive to nonlinearity. The combination of these two approaches provides a practical way for determining control coefficients and estimating kinetic properties in chemostat bioreactor systems.

Following a brief introduction of terminology, the control and elasticity coefficients, and their properties used in this study, the relationships are derived between the control coefficients and process variables with the objective to determine control coefficients from simple experiments. Derivations of equations are discussed in the Appendices for clarity and continuity. The utility of these relationships is discussed in the examples that follow the equations.

Metabolic Control Analysis

MCA was conceived for studying metabolic networks with arbitrary pathway structures such as



Scheme 1

in which X_i denotes the ith metabolite and ν_j is the flux through the jth reaction step. MCA aims to characterize the sensitivity of steady-state metabolic responses with respect to changes in enzyme activities or parameters without use of full mathematical models (for reviews, see Kell and Westerhoff, 1986; Fell, 1992; Liao and Delgado, 1993). The analysis is based on a set of sensitivity coefficients known as control and elasticity coefficients; these determine how changes in a system parameter or variable affect a steady-state response.

Control coefficients

The control coefficients and other similar terms were defined by Savageau (1971), Kacser and Burns (1973), and Heinrich and Rapoport (1974). If the system has a unique and stable steady state, one can define the (normalized) flux control coefficient as

$$C_{\nu_j}^{J_i} = \frac{\nu_j}{J_i}\left(\frac{\partial J_i}{\partial \theta_j}\right)\left(\frac{\partial \nu_j}{\partial \theta_j}\right)_{ss}^{-1} = \left(\frac{\partial \ln J_i}{\partial \ln \nu_j}\right)_{ss} \quad i, j = 1, \ldots, r \quad (1a)$$

or the simple (nonnormalized) flux control coefficient as

$$Z_j^i = \left(\frac{\partial J_i}{\partial \theta_j}\right)\left(\frac{\partial \nu_j}{\partial \theta_j}\right)_{ss}^{-1} = \left(\frac{\partial J_i}{\partial \nu_j}\right)_{ss} \quad i, j = 1, \ldots, r \quad (1b)$$

where ν_j is the rate function of step j, J_i is the steady-state rate of the ith reaction, θ_j is a parameter affecting ν_j, and r is the number of reaction steps in the system. Note that for simplicity it was assumed that θ_j affects only ν_j and no other reaction rate. Although such parameters may not exist in the actual system, one can always define them to aid the derivations. Most commonly, the flux control coefficients are used to define the rate-controlling steps by quantifying the change of steady-state flux per unit change of the rate function of the specific step. The usual form of the control coefficients is normalized by the steady-state values (Eq. 1a). We chose, however, to use the non-normalized form (Eq. 1b) because of its convenience in our analysis. The above control coefficients are the ν-type control coefficient defined in Liao and Delgado (1993) because the partial derivative is taken with respect to the rate of a reaction step, rather than the enzyme concentration. If the partial derivative is taken with respect to an adjustable parameter, it is generally called the *response coefficient*. The distinction between the ν-type control and other coefficients is important, because the ν-type control coefficients are related by a set of structural properties which are useful in both theoretical derivation and experimental determination of these values. The definition of control coefficients has been discussed by many researchers (Fell, 1992; Schuster and Heinrich, 1992; Liao and Delgado, 1993).

Similar to Eq. 1, the (normalized) metabolite concentration control coefficients are defined as

$$C_{\nu_j}^{\sigma_i} = \frac{\nu_j}{\sigma_i}\left(\frac{\partial \sigma_i}{\partial \theta_j}\right)\left(\frac{\partial \nu_j}{\partial \theta_j}\right)_{ss}^{-1} = \left(\frac{\partial \ln \sigma_i}{\partial \ln \nu_j}\right)_{ss} \quad i = 1, \ldots, m; \quad j = 1, \ldots, r \quad (2a)$$

in which σ_i is the steady-state metabolite concentration of metabolite X_i, ν_j is the reaction rate of step j, and m is the number of internal metabolites in the reaction network, that is, without counting external substrates and products. Similarly, the non-normalized form of these control coefficients is

$$\Gamma_j^i = \left(\frac{\partial \sigma_i}{\partial \theta_j}\right)\left(\frac{\partial \nu_j}{\partial \theta_j}\right)_{ss}^{-1} = \left(\frac{\partial \sigma_i}{\partial \nu_j}\right)_{ss} \quad i = 1, \ldots, m; \quad j = 1, \ldots, r \quad (2b)$$

We have again assumed that θ_j affects ν_j only.

Elasticity coefficients

The elasticity coefficients express how the rate of a reaction in isolation responds to a perturbation in an effector

metabolite. The normalized and nonnormalized forms of elasticity coefficients have been defined (Kacser and Burns, 1973; Burns et al., 1985; Reder, 1988) as

$$\epsilon_{x_i}^{\nu_j} = \frac{x_i}{\nu_j}\left(\frac{\partial \nu_j}{\partial x_i}\right)_{ss} = \left(\frac{\partial \ln \nu_j}{\partial \ln x_i}\right)_{ss} \qquad i=1,\ldots, m; \quad j=1,\ldots, r \quad (3a)$$

and

$$E_i^j = \left(\frac{\partial \nu_j}{\partial x_i}\right)_{ss} \qquad i=1,\ldots, m; \quad j=1,\ldots, r \quad (3b)$$

respectively, in which x_i is an effector metabolite that changes the reaction rate ν_j.

Structural properties of control coefficients

The structural approach to MCA by Reder (1988) provides a general framework to derive relationships among the control coefficients and elasticity coefficients. The method leads to a comprehensive characterization of the control coefficients and to generalized relationships between them, commonly known as the summation and connectivity theorems. The structural properties depend only on the stoichiometry, not on the functional form of the reaction kinetics. To simplify the mathematical treatment, all the control and elasticity coefficients in the derivations are nonnormalized: they are defined in terms of simple partial derivatives.

If we consider a biochemical system formed by m metabolites linked by r reactions, the metabolite concentrations, reaction rates, and stoichiometry are related by

$$\frac{d\boldsymbol{x}}{dt} = \boldsymbol{N}\boldsymbol{v} \quad (4)$$

in which $\boldsymbol{x}$ is a $(m\times 1)$ concentration vector, $\boldsymbol{v}$ is a $(r\times 1)$ reaction rate vector, t is time, and $\boldsymbol{N}$ $(m\times r)$ is the stoichiometric matrix in which the element of the ith row and jth column is the stoichiometric coefficient of metabolite i in reaction j. If the system has a steady or quasi-steady state, the flux and metabolite control coefficients have the following properties

$$\boldsymbol{Z}\boldsymbol{K} = \boldsymbol{K} \quad (5)$$

$$\boldsymbol{\Gamma}\boldsymbol{K} = \boldsymbol{0} \quad (6)$$

in which the columns of $\boldsymbol{K}$ $(r\times m)$ contains a basis for the kernel of the stoichiometric matrix $\boldsymbol{N}$, such that $\boldsymbol{N}\boldsymbol{K} = \boldsymbol{0}$, and $\boldsymbol{Z}$ $(r\times r)$ and $\boldsymbol{\Gamma}$ $(m\times r)$ are matrices whose elements are the flux and the metabolite concentration control coefficients defined in Eqs. 1b and 2b, respectively. Note that these equations are valid for any reaction system that can be described by Eq. 4 and has a stable, steady or quasi-steady state. The matrix $\boldsymbol{K}$ can be easily determined analytically by solving a system of linear equations or numerically with computer assisted algebra software (such as Mathematica, Maple, or Matlab). Other structural properties exist, but are not used in the following derivations. It is straightforward to express the above relationships in terms of normalized control coefficients using the non-normalized coefficients (Reder, 1988).

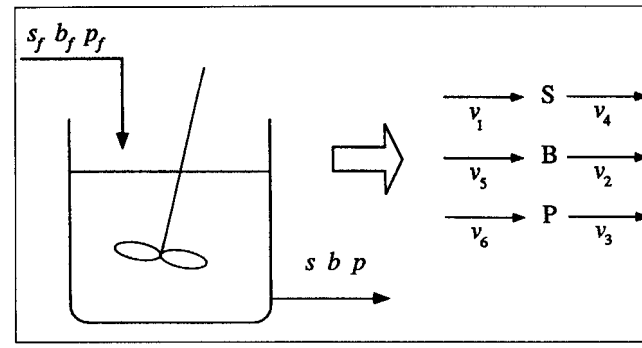


Figure 1. Continuous bioreactor system and equivalent reaction scheme.

s_f, b_f, and p_f are the feed concentrations, and s, b, p are the outlet concentrations. The reaction rates ν_i $(i=1, \ldots, 6)$ and their relationship to the concentrations of S, B, and P are defined in Eqs. 8a to 8f.

Application of MCA to Chemostat Bioreactors

The simplest chemostat bioreactor can be analyzed in terms of three components, substrate (S), biomass (B), and product (P), and we denote their concentrations by s, b, and p, respectively (see Figure 1). We assume that the system is perfectly mixed such that the system compositions are identical to that of the outlet stream; s_f, b_f, and p_f are the concentrations of substrate, biomass, and product in the feed, respectively, and D is the dilution rate, which is defined as the ratio of the feed volumetric flow rate to the system volume. The system is modeled by the set of differential equations

$$\frac{ds}{dt} = (s_f - s)D - Q_s b \quad (7a)$$

$$\frac{db}{dt} = -(b - b_f)D + \mu b \quad (7b)$$

$$\frac{dp}{dt} = -(p - p_f)D + Q_p b \quad (7c)$$

Equations 7a to 7c account for the material balances of substrate, biomass, and the product, respectively. Also, μ is the specific growth rate, and Q_s and Q_p are the specific rates of substrate consumption and product formation, respectively. To apply MCA, we rewrite the system into a pathway form with three parallel reactions, in which the flow, generation and consumption rates are defined as (see Figure 1)

$$\nu_1 = (s_f - s)D \quad (8a)$$

$$\nu_2 = (b - b_f)D \quad (8b)$$

$$\nu_3 = (p - p_f)D \quad (8c)$$

$$\nu_4 = Q_s b \quad (8d)$$

$$\nu_5 = \mu b \quad (8e)$$

$$\nu_6 = Q_p b \quad (8f)$$

To parallel the terminology in MCA, S, B, and P will be referred to as "metabolites," v_i will be called "fluxes," and the steady state of v_i will be denoted J_i. Although each flux is determined by intracellular enzyme kinetics, it is common, as a first approximation, to express the apparent kinetics in terms of extracellular variables s, b, and p. Therefore, we can treat the chemostat system as a set of parallel reactions as shown in Figure 1, without concern of the intracellular variables. In general, these reactions interact with each other because their rates are influenced commonly by s, b, p, and operating parameters.

Determination of Control Coefficients

According to the equivalent reaction scheme we postulate in Figure 1, N and K matrices in Eqs. 4–6 are given by

$$N=\begin{bmatrix}1 & 0 & 0 & -1 & 0 & 0\\ 0 & -1 & 0 & 0 & 1 & 0\\ 0 & 0 & -1 & 0 & 0 & 1\end{bmatrix} \tag{9}$$

and

$$K=\begin{bmatrix}1 & 0 & 0\\ 0 & 1 & 0\\ 0 & 0 & 1\\ 1 & 0 & 0\\ 0 & 1 & 0\\ 0 & 0 & 1\end{bmatrix} \tag{10}$$

where the rows of N correspond to S, B, P, and the ith column corresponds to v_i. The matrices of control coefficients Z and Γ have dimensions of (6×6) and (3×6), respectively, and their elements are related by Eqs. 5 and 6 as

$$\begin{aligned} &Z_1^1+Z_4^1=1 \quad Z_1^2+Z_4^2=0 \quad Z_1^3+Z_4^3=0\\ &Z_2^1+Z_5^1=0 \quad Z_2^2+Z_5^2=1 \quad Z_2^3+Z_5^3=0\\ &Z_3^1+Z_6^1=0 \quad Z_3^2+Z_6^2=0 \quad Z_3^3+Z_6^3=1 \end{aligned} \tag{11}$$

and

$$\begin{aligned} &\Gamma_1^S+\Gamma_4^S=0 \quad \Gamma_1^B+\Gamma_4^B=0 \quad \Gamma_1^P+\Gamma_4^P=0\\ &\Gamma_2^S+\Gamma_5^S=0 \quad \Gamma_2^B+\Gamma_5^B=0 \quad \Gamma_2^P+\Gamma_5^P=0\\ &\Gamma_3^S+\Gamma_6^S=0 \quad \Gamma_3^B+\Gamma_6^B=0 \quad \Gamma_3^P+\Gamma_6^P=0 \end{aligned} \tag{12}$$

Note that J_4, J_5, and J_6, are equal to J_1, J_2 and J_3 respectively, because of the chemostat material balances. Thus, the control coefficients for J_4, J_5, and J_6, are identical to those of J_1, J_2, and J_3. These constraints reduce the number of control coefficients that need to be determined. Instead of estimating 36 flux control coefficients and 18 metabolite concentration control coefficients in matrices Z and Γ, we now need to determine only nine flux (Z_j^i with $i, j=1, 2$, and 3) and nine metabolite concentration (Γ_i^m with $m=S$, B and P, and $i=1$, 2, and 3) control coefficients.

Dynamic approach to MCA

Conventional approaches for determining the control coefficients require the knowledge of the reaction kinetics. However, these kinetics are not always available or easily measurable. The dynamic approach (Delgado and Liao, 1991, 1992a,b; Liao and Delgado, 1992) circumvents this problem by using linear approximation around the steady state and transient metabolite data to determine the control coefficients. It was shown that the transient metabolite concentrations do not vary freely, but are constrained as the system moves towards the steady state. To derive such a relationship we begin from the following equation (Delgado and Liao, 1992a), which assumes local linearity around the steady state

$$\boldsymbol{Zv}=\boldsymbol{J} \tag{13}$$

where $\boldsymbol{v}$ is the $r\times 1$ transient flux vector and $\boldsymbol{J}$ is the $r\times 1$ steady-state flux vector. Substituting Eq. 11 into the above equation, we obtain

$$\begin{aligned} &Z_1^1(v_1-v_4)+Z_2^1(v_2-v_5)+Z_3^1(v_3-v_6)=(J_1-v_4)\\ &Z_1^2(v_1-v_4)+Z_2^2(v_2-v_5)+Z_3^2(v_3-v_6)=(J_2-v_5)\\ &Z_1^3(v_1-v_4)+Z_2^3(v_2-v_5)+Z_3^3(v_3-v_6)=(J_3-v_6) \end{aligned} \tag{14}$$

The evaluation of v_4, v_5, and v_6 in the transient state involves numerical differentiation of the transient metabolite concentrations, and results will be sensitive to experimental errors. To avoid this problem, we integrate both sides of the above expressions with respect to time and obtain an integral form of Eq. 14

$$\begin{bmatrix}Z_1^1 & Z_2^1 & Z_3^1\\ Z_1^2 & Z_2^2 & Z_3^2\\ Z_1^3 & Z_2^3 & Z_3^3\end{bmatrix}\begin{bmatrix}\int_{t_0}^{t}\frac{ds}{dt}dt\\ -\int_{t_0}^{t}\frac{db}{dt}dt\\ -\int_{t_0}^{t}\frac{dp}{dt}dt\end{bmatrix}=\begin{bmatrix}J_1(t-t_0)-\int_{t_0}^{t}Q_s b\,dt\\ J_2(t-t_0)-\int_{t_0}^{t}\mu b\,dt\\ J_3(t-t_0)-\int_{t_0}^{t}Q_p b\,dt\end{bmatrix} \tag{15}$$

$$\begin{bmatrix}Z_1^1 & Z_2^1 & Z_3^1\\ Z_1^2 & Z_2^2 & Z_3^2\\ Z_1^3 & Z_2^3 & Z_3^3\end{bmatrix}\begin{bmatrix}s-s_0\\ -(b-b_0)\\ -(p-p_0)\end{bmatrix}$$

$$=\begin{bmatrix}D(s_f-s_{ss})(t-t_0)+\int_{t_0}^{t}\frac{ds}{dt}-(s_f-s)D\,dt\\ -D(b_f-b_{ss})(t-t_0)-\int_{t_0}^{t}\frac{db}{dt}+(b-b_f)D\,dt\\ -D(p_f-p_{ss})(t-t_0)-\int_{t_0}^{t}\frac{dp}{dt}+(p-p_f)D\,dt\end{bmatrix} \tag{16}$$

or

$$\begin{bmatrix} Z_1^1 & Z_2^1 & Z_3^1 \\ Z_1^2 & Z_2^2 & Z_3^2 \\ Z_1^3 & Z_2^3 & Z_3^3 \end{bmatrix} \begin{bmatrix} s-s_0 \\ -(b-b_0) \\ -(p-p_0) \end{bmatrix}$$

$$= \begin{bmatrix} D(s_f-s_{ss})(t-t_0)+(s-s_0)+\int_{t_0}^{t}(s-s_f)D\,dt \\ -D(b_f-b_{ss})(t-t_0)-(b-b_0)-\int_{t_0}^{t}(b-b_f)D\,dt \\ -D(p_f-p_{ss})(t-t_0)-(p-p_0)-\int_{t_0}^{t}(p-p_f)D\,dt \end{bmatrix} \quad (17)$$

where s_0, b_0, and p_0 are initial substrate, biomass, and product concentrations, respectively, s_{ss}, b_{ss}, and p_{ss} are the steady-state values of S, B and P, respectively, and t_0 is the initial time. Thus, one can measure the concentrations of S, B and P as a function of time after a perturbation and use linear regression according to Eq. 17 to estimate control coefficients (Z_j^i with $i, j = 1, 2$, and 3). The transient data of s, b and p are used as regressors and the righthand side of Eq. 17 as dependent variables. Although sophisticated numerical integration algorithms are available, the trapezoidal rule is adequate for the integral term in the above equation. Although it may be possible to develop an optimal perturbation to minimize effects of measurement error or nonlinearity, such investigation is beyond the scope of this article.

Similarly, a relationship between the transient metabolites concentrations and the metabolite concentration control coefficients can be derived based on

$$\Gamma \boldsymbol{v} = \boldsymbol{\sigma} - \boldsymbol{x} \quad (18)$$

which is the version of Eq. 13 for the metabolite concentration control coefficients (Delgado and Liao, 1992b). Following a procedure that parallels that used to derive Eq. 17 we obtain

$$\begin{bmatrix} \Gamma_1^S & \Gamma_2^S & \Gamma_3^S \\ \Gamma_1^B & \Gamma_2^B & \Gamma_3^B \\ \Gamma_1^P & \Gamma_2^P & \Gamma_3^P \end{bmatrix} \begin{bmatrix} s-s_0 \\ -(b-b_0) \\ -(p-p_0) \end{bmatrix} = \begin{bmatrix} \int_{t_0}^{t}(s_{ss}-s)\,dt \\ \int_{t_0}^{t}(b_{ss}-b)\,dt \\ \int_{t_0}^{t}(p_{ss}-p)\,dt \end{bmatrix} \quad (19)$$

Therefore, by use of the transient data of s, b, and p, one can estimate Γ_i^m ($m = S$, B and P, and $i = 1$, 2, and 3) via linear regression. In the chemostat system, the metabolite concentration control coefficients can also be calculated directly from the flux control coefficients and the dilution rate. The equation for this calculation will be discussed shortly.

Example 1. Consider a system described by Eqs. 7a to 7c having the following kinetic expressions

$$\mu = \frac{U_{\max} s/K_s}{1 + s/K_s + p/K_P} \quad (20)$$

$$Q_s = \alpha_s \mu + \beta_s \quad (21)$$

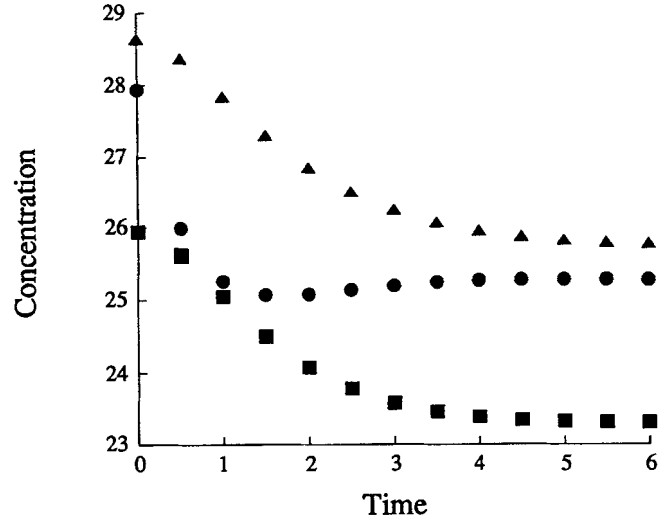


Figure 2. Transient response for the dynamic system used in the examples.

Symbols are: biomass, $b \times 3$ (■); substrate, $s \times 1.5$ (●); and product, p (▲). The system was perturbed from its original steady state, $s_{ss} = 18.65$; $b_{ss} = 8.65$; and $p_{ss} = 28.64$ by changing the input substrate concentration s_f from 50 to 45; b_f and p_f are zero, and the dilution rate D is 0.8. The new steady state is $s_{ss} = 16.84$; $b_{ss} = 7.77$; $p_{ss} = 25.74$.

$$Q_p = \alpha_p \mu + \beta_p \quad (22)$$

where $U_{\max} = 4$, $K_S = 3$, $K_P = 1.2$, $\alpha_s = 3$, $\beta_s = 0.5$, $\alpha_p = 2.5$, and $\beta_p = 0.65$. Initially, the system is allowed to reach a steady state with the following parameter values: $D = 0.8$, $s_f = 50$, $b_f = 0$, and $p_f = 0$. To determine the control coefficients using the transient data, we perturb the system by decreasing s_f to 45. The concentrations of S, B, and P are then measured periodically; the simulated experimental results are shown in Figure 2. The transient data between $t = 0$ and $t = 6$ are then used in linear regressions according to Eqs. 17 and 19. The control coefficients estimated from these regressions are

$$\begin{bmatrix} Z_1^1 & Z_2^1 & Z_3^1 \\ Z_1^2 & Z_2^2 & Z_3^2 \\ Z_1^3 & Z_2^3 & Z_3^3 \end{bmatrix}_{\text{calculated}} = \begin{bmatrix} 0.61 & -1.48 & 0.36 \\ 0.17 & -0.27 & 0.11 \\ 0.57 & -1.65 & 0.34 \end{bmatrix} \quad (23)$$

and

$$\begin{bmatrix} \Gamma_1^S & \Gamma_2^S & \Gamma_3^S \\ \Gamma_1^B & \Gamma_2^B & \Gamma_3^B \\ \Gamma_1^P & \Gamma_2^P & \Gamma_3^P \end{bmatrix}_{\text{calculated}} = \begin{bmatrix} 0.49 & 1.84 & -0.45 \\ 0.21 & -1.59 & 0.13 \\ 0.71 & -2.07 & -0.83 \end{bmatrix} \quad (24)$$

These control coefficients agree reasonably well with the exact values determined from the numerical solution (Caracotsios and Stewart, 1985) using all the known kinetic parameters

$$\begin{bmatrix} Z_1^1 & Z_2^1 & Z_3^1 \\ Z_1^2 & Z_2^2 & Z_3^2 \\ Z_1^3 & Z_2^3 & Z_3^3 \end{bmatrix}_{\text{exact}} = \begin{bmatrix} 0.64 & -1.63 & 0.40 \\ 0.18 & -0.28 & 0.11 \\ 0.58 & -1.73 & 0.36 \end{bmatrix} \quad (25)$$

and

$$\begin{bmatrix} \Gamma_1^S & \Gamma_2^S & \Gamma_3^S \\ \Gamma_1^B & \Gamma_2^B & \Gamma_3^B \\ \Gamma_1^P & \Gamma_2^P & \Gamma_3^P \end{bmatrix}_{\text{exact}} = \begin{bmatrix} 0.45 & 2.03 & -0.50 \\ 0.22 & -1.60 & 0.14 \\ 0.73 & -2.16 & -0.80 \end{bmatrix} \quad (26)$$

Because this approach relies on the linear approximation of the rate laws around the steady state, it is sensitive to nonlinearity. The fitness of Eqs. 17 and 19 depends on the size of perturbation. Residual plots (plots of the normalized differences between the measured and estimated independent variable used in the regression) can be used to judge the confidence level of the control coefficients estimated.

Steady-State Perturbation Approach. To improve the estimates of the control coefficients, we develop another methodology based on measurements of steady-state perturbations only. This method takes advantage of the operating variables s_f, b_f, and p_f, which act only on ν_1, ν_2, and ν_3, respectively. Thus, we can write the control coefficients as

$$Z_j^i = \left(\frac{\partial J_i}{\partial M_j}\right)\left(\frac{\partial \nu_j}{\partial M_j}\right)^{-1} \quad (27)$$

and

$$\Gamma_j^i = \left(\frac{\partial \sigma_i}{\partial M_j}\right)\left(\frac{\partial \nu_j}{\partial M_j}\right)^{-1} \quad (28)$$

where M_j denotes a manipulated parameter that only affects ν_j. Here, $M_1 = s_f$, $M_2 = b_f$, and $M_3 = p_f$. It is important to note that Eqs. 27 and 28 are equivalent to Eqs. 1 and 2, respectively; the use of M_j instead of θ_j is to stress the fact that the parameter that affects ν_j is a manipulated variable as opposed to θ_j, which is arbitrary.

Following the above definition of the flux control coefficients, one can derive the following equations

$$\begin{bmatrix} Z_1^1 & Z_2^1 & Z_3^1 \\ Z_1^2 & Z_2^2 & Z_3^2 \\ Z_1^3 & Z_2^3 & Z_3^3 \end{bmatrix} = \begin{bmatrix} 1-\frac{\partial s_{ss}}{\partial s_f} & \frac{\partial s_{ss}}{\partial b_f} & \frac{\partial s_{ss}}{\partial p_f} \\ \frac{\partial b_{ss}}{\partial s_f} & 1-\frac{\partial b_{ss}}{\partial b_f} & -\frac{\partial b_{ss}}{\partial p_f} \\ \frac{\partial p_{ss}}{\partial s_f} & -\frac{\partial p_{ss}}{\partial b_f} & 1-\frac{\partial p_{ss}}{\partial p_f} \end{bmatrix}$$

$$\approx \begin{bmatrix} 1-\frac{\Delta s_{ss}}{\Delta s_f} & \frac{\Delta s_{ss}}{\Delta b_f} & \frac{\Delta s_{ss}}{\Delta p_f} \\ \frac{\Delta b_{ss}}{\Delta s_f} & 1-\frac{\Delta b_{ss}}{\Delta b_f} & -\frac{\Delta b_{ss}}{\Delta p_f} \\ \frac{\Delta p_{ss}}{\Delta s_f} & -\frac{\Delta p_{ss}}{\Delta b_f} & 1-\frac{\Delta p_{ss}}{\Delta p_f} \end{bmatrix} \quad (29)$$

and

$$\begin{bmatrix} \Gamma_1^S & \Gamma_2^S & \Gamma_3^S \\ \Gamma_1^B & \Gamma_2^B & \Gamma_3^B \\ \Gamma_1^P & \Gamma_2^P & \Gamma_3^P \end{bmatrix} = \frac{1}{D}\begin{bmatrix} \frac{\partial s_{ss}}{\partial s_f} & -\frac{\partial s_{ss}}{\partial b_f} & -\frac{\partial s_{ss}}{\partial p_f} \\ \frac{\partial b_{ss}}{\partial s_f} & -\frac{\partial b_{ss}}{\partial b_f} & -\frac{\partial b_{ss}}{\partial p_f} \\ \frac{\partial p_{ss}}{\partial s_f} & -\frac{\partial p_{ss}}{\partial b_f} & -\frac{\partial p_{ss}}{\partial p_f} \end{bmatrix}$$

$$\approx \frac{1}{D}\begin{bmatrix} \frac{\Delta s_{ss}}{\Delta s_f} & -\frac{\Delta s_{ss}}{\Delta b_f} & -\frac{\Delta s_{ss}}{\Delta p_f} \\ \frac{\Delta b_{ss}}{\Delta s_f} & -\frac{\Delta b_{ss}}{\Delta b_f} & -\frac{\Delta b_{ss}}{\Delta p_f} \\ \frac{\Delta p_{ss}}{\Delta s_f} & -\frac{\Delta p_{ss}}{\Delta b_f} & -\frac{\Delta p_{ss}}{\Delta p_f} \end{bmatrix} \quad (30)$$

The above equations allow one to determine the control coefficients with simple steady-state perturbations. The detailed derivations are shown in Appendix A. To obtain the flux control coefficients, one needs to conduct three step changes in s_f, b_f, and p_f, each at a time, while keeping the rest of the operating conditions constant. One then takes the ratio of the changes in the metabolite steady states to the magnitudes of perturbations to calculate the flux control coefficients. This method offers a better estimation because it does not depend on linear approximation of the kinetic rate laws. It does, however, assume local linearity in s_{ss}, b_{ss}, and p_{ss} with respect to s_f, b_f, and p_f to determine the partial derivatives in the above equations. This method also offers simplicity by eliminating transient samplings as compared to the dynamic approach.

However, perturbations in b_f may be difficult to produce in practice, because they may involve cell recycle, which may alter the physiological state of the cells. In the process of removing the cells from the culture and reinjecting to the bioreactor, the cells may undergo significant physiological changes due to local limitation of oxygen and substrates. To circumvent this problem, we propose to combine the dynamic approach and the steady-state perturbation method to determine the control coefficients. Instead of conducting three perturbations, we now carry out two separate perturbations of s_f and p_f to obtain transient data and steady-state values for analysis. The first and third columns of matrices **Z** and Γ in Eqs. 29 and 30 are obtained using the perturbed steady-state approach. The second column, on the other hand, can be obtained by the dynamic approach.

Example 2. Here we use the steady-state perturbation and the combined approach methods to determine the control coefficients for system described in Example 1. As discussed above, the initial steady state is achieved with $D = 0.8$, $s_f = 45$, $b_f = 0$, and $p_f = 0$. We now perform the following perturbations: (1) increase s_f to 50; (2) increase b_f to 0.8; and (3) increase p_f to 2.6. The steady states corresponding to these perturbations are shown in Table 1. The flux and metabolite concentration control coefficients are then calculated according to Eqs. 29 and 30

Table 1. Results of Steady-State Perturbations in Example 2

	Steady State			
		New		
	Int.	$s_f = 50$	$b_f = 0.8$	$p_f = 2.6$
s_{ss}	16.84	18.65	15.66	17.87
b_{ss}	7.77	8.65	8.76	7.48
p_{ss}	25.74	28.64	27.00	27.39

$$\begin{bmatrix} Z_1^1 & Z_2^1 & Z_3^1 \\ Z_1^2 & Z_2^2 & Z_3^2 \\ Z_1^3 & Z_2^3 & Z_3^3 \end{bmatrix}_{\text{calculated}} = \begin{bmatrix} 0.64 & -1.48 & 0.40 \\ 0.18 & -0.24 & 0.11 \\ 0.58 & -1.58 & 0.37 \end{bmatrix} \quad (31)$$

and

$$\begin{bmatrix} \Gamma_1^S & \Gamma_2^S & \Gamma_3^S \\ \Gamma_1^B & \Gamma_2^B & \Gamma_3^B \\ \Gamma_1^P & \Gamma_2^P & \Gamma_3^P \end{bmatrix}_{\text{calculated}} = \begin{bmatrix} 0.45 & 1.85 & -0.50 \\ 0.23 & -1.55 & 0.14 \\ 0.73 & -1.98 & -0.79 \end{bmatrix} \quad (32)$$

These values agree very well with the exact solution shown in Eqs. 25 and 26.

As discussed above, we can use the combined approach to circumvent the perturbation of b_f. To do so, we perform two perturbations: increase s_f to 50 and increase p_f to 2.6. The concentrations of S, B, and P are then measured as a function of time. The steady-state values are used to calculate the first and third columns of the $\mathbf{Z}$ and Γ matrix in Eqs. 29 and 30. The second column is estimated from linear regression of the transient data from the s_f perturbation using Eqs. 17 and 19 and the coefficients calculated from the steady-state perturbations. The resulting control coefficients are

$$\begin{bmatrix} Z_1^1 & Z_2^1 & Z_3^1 \\ Z_1^2 & Z_2^2 & Z_3^2 \\ Z_1^3 & Z_2^3 & Z_3^3 \end{bmatrix}_{\text{calculated}} = \begin{bmatrix} 0.64 & -1.54 & 0.40 \\ 0.18 & -0.26 & 0.11 \\ 0.58 & -1.73 & 0.37 \end{bmatrix} \quad (33)$$

and

$$\begin{bmatrix} \Gamma_1^S & \Gamma_2^S & \Gamma_3^S \\ \Gamma_1^B & \Gamma_2^B & \Gamma_3^B \\ \Gamma_1^P & \Gamma_2^P & \Gamma_3^P \end{bmatrix}_{\text{calculated}} = \begin{bmatrix} 0.45 & 1.93 & -0.50 \\ 0.23 & -1.58 & 0.14 \\ 0.73 & -2.16 & -0.79 \end{bmatrix} \quad (34)$$

Note that the only assumption made in the steady-state perturbation approach is the finite difference approximation to the partial derivatives in Eqs. 29 and 30. In this example, the approximation works very well, because the relationships between steady-state concentrations of S, B and P and feed concentrations are almost linear. These linear relationships will vanish when the growth kinetics are highly nonlinear.

Determination of Elasticity Coefficients. Reder (1988) derived relationships that enable the calculation of elasticity coefficients from control coefficients. For this system, N is of maximal rank and these relationships can be expressed as

$$E\Gamma = \mathbf{Z} - I_6 \quad (35)$$

in which I_6 is the (6×6) identity matrix, and E is the matrix of the elasticity coefficients, defined in Eq. 3b. This equation enables the calculation of the elasticity coefficients from the control coefficients for systems in which N is of full rank. To tailor the equation for the bioreactor system, we partition the above equation as

$$\begin{bmatrix} E_1 \\ E_2 \end{bmatrix} [\Gamma_1 \quad \Gamma_2] = \begin{bmatrix} \mathbf{Z}_1 & \mathbf{Z}_2 \\ \mathbf{Z}_3 & \mathbf{Z}_4 \end{bmatrix} - I_6 \quad (36)$$

where all the submatrices are (3×3). From the structure of the continuous bioreactor, we have

$$E_1 = \begin{bmatrix} \frac{\partial \nu_1}{\partial s} & \frac{\partial \nu_1}{\partial b} & \frac{\partial \nu_1}{\partial p} \\ \frac{\partial \nu_2}{\partial s} & \frac{\partial \nu_2}{\partial b} & \frac{\partial \nu_2}{\partial p} \\ \frac{\partial \nu_3}{\partial s} & \frac{\partial \nu_3}{\partial b} & \frac{\partial \nu_3}{\partial p} \end{bmatrix} = \begin{bmatrix} -D & 0 & 0 \\ 0 & D & 0 \\ 0 & 0 & D \end{bmatrix} \quad (37)$$

From Eq. 36, we have

$$E_2 = \begin{bmatrix} \frac{\partial \nu_4}{\partial s} & \frac{\partial \nu_4}{\partial b} & \frac{\partial \nu_4}{\partial p} \\ \frac{\partial \nu_5}{\partial s} & \frac{\partial \nu_5}{\partial b} & \frac{\partial \nu_5}{\partial p} \\ \frac{\partial \nu_6}{\partial s} & \frac{\partial \nu_6}{\partial b} & \frac{\partial \nu_6}{\partial p} \end{bmatrix} = \mathbf{Z}_3 \Gamma_1^{-1} \quad (38)$$

Note that $\mathbf{Z}_2 = \mathbf{Z}_1$, which can be determined by the use of the approaches described above (Eqs. 17 or 29). From the above equation, we see that the accuracy of elasticity coefficients relies on the accuracy of the control coefficients determined. These elasticity coefficients can then be used to calculate the pseudo-first-order kinetic constants for the specific rates of growth, substrate consumption, and product formation. The equations for these calculations are summarized in Table 2. From Eq. 36, we also obtain

$$\Gamma_1 = E_1^{-1}(\mathbf{Z}_1 - I_3) \quad (39)$$

This equation can be used in conjunction with the dilution rate (Eq. 35) to calculate the metabolite concentration control coefficients from the flux control coefficients.

Example 3. We continue to consider the system discussed in Examples 1 and 2. Now that the flux and the metabolite concentration control coefficients have been calculated, the elasticity coefficients can be determined from Eq. 38 as

Table 2. Equations for Calculating Pseudo-First-Order Constants of the Specific Rates (Q_s, Q_p, and μ) from Elasticity Coefficients*

$\frac{\partial Q_s}{\partial s} = \frac{1}{b_{ss}} \frac{\partial \nu_4}{\partial s}$	$\frac{\partial \mu}{\partial s} = \frac{1}{b_{ss}} \frac{\partial \nu_5}{\partial s}$	$\frac{\partial Q_p}{\partial s} = \frac{1}{b_{ss}} \frac{\partial \nu_6}{\partial s}$
$\frac{\partial Q_s}{\partial b} = \frac{1}{b_{ss}} \left(\frac{\partial \nu_4}{\partial b} - (Q_s)_{ss} \right)$	$\frac{\partial \mu}{\partial b} = \frac{1}{b_{ss}} \left(\frac{\partial \nu_5}{\partial b} - \mu_{ss} \right)$	$\frac{\partial Q_p}{\partial b} = \frac{1}{b_{ss}} \left(\frac{\partial \nu_6}{\partial b} - (Q_p)_{ss} \right)$
$\frac{\partial Q_s}{\partial p} = \frac{1}{b_{ss}} \frac{\partial \nu_4}{\partial p}$	$\frac{\partial \mu}{\partial p} = \frac{1}{b_{ss}} \frac{\partial \nu_5}{\partial p}$	$\frac{\partial Q_P}{\partial p} = \frac{1}{b_{ss}} \frac{\partial \nu_6}{\partial p}$

*All partial derivatives are evaluated at the steady state.

$$E_2 = \begin{bmatrix} \frac{\partial \nu_4}{\partial s} & \frac{\partial \nu_4}{\partial b} & \frac{\partial \nu_4}{\partial p} \\ \frac{\partial \nu_5}{\partial s} & \frac{\partial \nu_5}{\partial b} & \frac{\partial \nu_5}{\partial p} \\ \frac{\partial \nu_6}{\partial s} & \frac{\partial \nu_6}{\partial b} & \frac{\partial \nu_6}{\partial p} \end{bmatrix} = Z_3 \Gamma_1^{-1}$$

$$= \begin{bmatrix} 0.89 & 2.90 & -0.55 \\ 0.30 & 0.80 & -0.18 \\ 0.74 & 2.65 & -0.46 \end{bmatrix}_{\text{Exact}} \quad (40)$$

$$E_2 \approx \begin{bmatrix} 0.84 & 2.53 & -0.47 \\ 0.28 & 0.72 & -0.17 \\ 0.71 & 2.39 & -0.40 \end{bmatrix}_{\text{Example 1}} \quad (41)$$

$$E_2 \approx \begin{bmatrix} 0.97 & 2.90 & -0.61 \\ 0.33 & 0.81 & -0.21 \\ 0.80 & 2.64 & -0.52 \end{bmatrix}_{\text{Example 2}} \quad (42)$$

These values are then used in equations in Table 2 to calculate the pseudo-first-order kinetic constants (Table 3), which agree well with the exact values determined from the known kinetics. Therefore, the effects of s, b, and p on the culture kinetics are determined.

Effect of Measurement Noise. In general, if random error exists, steady-state measurements must be checked repeatedly to ensure that the system actually reaches the steady state. One can then use the average of the measurements in the calculations. Therefore, the steady-state perturbation approach will not be corrupted much by random noise. On the other hand, the dynamic approach is more sensitive to random noise and the error in the calculation of control coefficients will propagate to the elasticity coefficients and the pseudo-first-order constants of the specific rates. The combination of the steady-state perturbation and dynamic methods greatly reduces the sensitivity to random noise in the estimation of the control coefficients. Table 4 shows the results of the pseudo-first-order constants of the system discussed in Examples 1 and 2 calculated by using the combined steady-state perturbation and dynamic approaches. The measurements were corrupted by 0%, 20%, and 50% random noise normalized to the size of steady-state perturbation. In this case, random error up to 50% still yielded reasonable results for practical purposes. In the calculation of metabolite concentration control coefficients using corrupted transient data, we found that Eq. 39 often gives better results than Eq. 18, because the former is less sensitive to error.

Improving Operating Conditions. The main operating variables in bioreactor systems are the feed concentrations of S, B, and P, and the dilution rate. To improve the product output, one would like to know the effect of the feed and dilution rate on the output variables of interest, that is, $(\partial p_{ss}/\partial M)_{ss}$ and $(\partial J_3/\partial M)_{ss}$, in which M stands for s_f, p_f, b_f, or D. With this information, one can then improve the bioreactor performance by increasing or decreasing these operating variables. The partial derivatives with respect to the feed concentrations can be obtained experimentally as discussed above and they are related to the control coefficients by Eqs. 29 and 30. The partial derivatives with respect to D can be calculated by use of the following equations

$$\left(\frac{\partial J_3}{\partial D} \right)_{ss} = \frac{1}{D} (J_1 Z_1^3 + J_2 Z_2^3 + J_3 Z_3^3) \quad (43)$$

$$\left(\frac{\partial p_{ss}}{\partial D} \right)_{ss} = \frac{1}{D} (J_1 \Gamma_1^P + J_2 \Gamma_2^P + J_3 \Gamma_3^P) \quad (44)$$

These equations are a direct consequence of the general definition of the control matrices and can also be derived by use

Table 3. Pseudo-First-Order Constants for Specific Rates in Example 3*

Exact Solution		
$\frac{\partial Q_s}{\partial s} = 0.11$	$\frac{\partial \mu}{\partial s} = 0.04$	$\frac{\partial Q_p}{\partial s} = 0.10$
$\frac{\partial Q_s}{\partial b} = 0.00$	$\frac{\partial \mu}{\partial b} = 0.00$	$\frac{\partial Q_p}{\partial b} = 0.00$
$\frac{\partial Q_s}{\partial p} = -0.07$	$\frac{\partial \mu}{\partial p} = -0.02$	$\frac{\partial Q_p}{\partial p} = -0.06$
Dynamic Approach		
$\frac{\partial Q_s}{\partial s} = 0.11$	$\frac{\partial \mu}{\partial s} = 0.04$	$\frac{\partial Q_p}{\partial s} = 0.09$
$\frac{\partial Q_s}{\partial b} = -0.05$	$\frac{\partial \mu}{\partial b} = -0.01$	$\frac{\partial Q_p}{\partial b} = -0.03$
$\frac{\partial Q_s}{\partial p} = -0.06$	$\frac{\partial \mu}{\partial p} = -0.02$	$\frac{\partial Q_p}{\partial p} = -0.05$
Steady-State Perturbation Approach		
$\frac{\partial Q_s}{\partial s} = 0.12$	$\frac{\partial \mu}{\partial s} = 0.04$	$\frac{\partial Q_p}{\partial s} = 0.10$
$\frac{\partial Q_s}{\partial b} = 0.00$	$\frac{\partial \mu}{\partial b} = 0.00$	$\frac{\partial Q_p}{\partial b} = 0.00$
$\frac{\partial Q_s}{\partial p} = -0.08$	$\frac{\partial \mu}{\partial p} = -0.03$	$\frac{\partial Q_p}{\partial p} = -0.07$

*Derivatives are evaluated at the steady state.

Table 4. Effect of Error in Measurements on Determining Pseudo-First-Order Constants Using the Steady-State Perturbation Approach for Figure 2*

0% Error		
$\frac{\partial Q_s}{\partial s} = 0.12$	$\frac{\partial \mu}{\partial s} = 0.04$	$\frac{\partial Q_p}{\partial s} = 0.10$
$\frac{\partial Q_s}{\partial b} = 0.00$	$\frac{\partial \mu}{\partial b} = 0.00$	$\frac{\partial Q_p}{\partial b} = 0.00$
$\frac{\partial Q_s}{\partial p} = -0.08$	$\frac{\partial \mu}{\partial p} = -0.03$	$\frac{\partial Q_p}{\partial p} = -0.07$
20% Error		
$\frac{\partial Q_s}{\partial s} = 0.11$	$\frac{\partial \mu}{\partial s} = 0.04$	$\frac{\partial Q_p}{\partial s} = 0.10$
$\frac{\partial Q_s}{\partial b} = 0.00$	$\frac{\partial \mu}{\partial b} = 0.00$	$\frac{\partial Q_p}{\partial b} = 0.00$
$\frac{\partial Q_s}{\partial p} = -0.07$	$\frac{\partial \mu}{\partial p} = -0.03$	$\frac{\partial Q_p}{\partial p} = -0.06$
50% Error		
$\frac{\partial Q_s}{\partial s} = 0.06$	$\frac{\partial \mu}{\partial s} = 0.04$	$\frac{\partial Q_p}{\partial s} = 0.09$
$\frac{\partial Q_s}{\partial b} = 0.00$	$\frac{\partial \mu}{\partial b} = 0.00$	$\frac{\partial Q_p}{\partial b} = 0.00$
$\frac{\partial Q_s}{\partial p} = -0.04$	$\frac{\partial \mu}{\partial p} = -0.02$	$\frac{\partial Q_p}{\partial p} = -0.06$

*The error is normalized to the size of the steady-state differences between perturbations; derivatives are evaluated at the steady state.

of the Euler's Theorem; the derivations are presented in Appendices B and C, respectively. Note that the righthand sides of the above two equations include the flux and metabolite concentration control coefficients, respectively. Therefore, one can use the approaches discussed previously to determine the control coefficients and calculate the above quantities, which determine the direction to change D for better reactor performance.

Effects of Growth Kinetics on Control Coefficients. Having developed the methodologies for determining the control and elasticity coefficients, we now characterize the effects of growth kinetics on the control coefficients. Three cases are considered: (1) the specific growth rate is a function of substrate only $\mu = f(s)$; (2) the specific growth rate is a function of substrate and biomass concentrations $\mu = f(s,b)$; and (3) the specific growth rate is a function of substrate and product concentrations $\mu = f(s,p)$. In these cases we assume that

$$Q_s = \alpha_s \mu + \beta_s \tag{45}$$

$$Q_p = \alpha_p \mu + \beta_p \tag{46}$$

By solving analytically the steady-state values of s, b, and p, and with Eqs. 27 and 28, we obtain the properties summarized in Table 5. Note that the control coefficients with respect to ν_3 (Z_3^i, $i = 1, \ldots, 6$) are all equal to zero if the product does not affect the specific growth rate. If the specific growth rate is a function of the limiting substrate only, the flux control coefficient Z_1^1 is equal to unity. This result holds regardless of any activating or inhibitory effects of the limiting substrate on the specific growth rate. Moreover, Z_1^2 and Z_1^3 are equal to the biomass yield and product yield, respectively. If the specific growth rate is a function of s and

Table 5. Influence of Growth Kinetics on Flux Control Coefficients*

	Case 1 $\mu = f(s)$	Case 2 $\mu = f(s,b)$		Case 3 $\mu = f(s,p)$	
		$b-$	$b+$	$p-$	$p+$
Z_1^1	$=1$	<1	>1	<1	>1
Z_1^2	$=\frac{b_{ss}}{s_f - s_{ss}}$	$<\frac{b_{ss}}{s_f - s_{ss}}$	$>\frac{b_{ss}}{s_f - s_{ss}}$	$<\frac{b_{ss}}{s_f - s_{ss}}$	$>\frac{b_{ss}}{s_f - s_{ss}}$
Z_1^3	$=\frac{p_{ss}}{s_f - s_{ss}}$	$<\frac{p_{ss}}{s_f - s_{ss}}$	$>\frac{p_{ss}}{s_f - s_{ss}}$	$<\frac{p_{ss}}{s_f - s_{ss}}$	$>\frac{p_{ss}}{s_f - s_{ss}}$
Z_3^i $i=1,2,3$	$=0$	$=0$	$=0$	>1	<1

*Case 1: μ is a function of s only; Case 2: μ is a function of s and b; Case 3: μ is a function of s and p. The specific rates of substrate consumption and production formation take the form of Eqs. 45 and 46. The negative and positive signs beside b and p denote inhibition and activation, respectively.

b, where the biomass concentration may either inhibit or activate the cell growth, then the control coefficients (Z_1^1, Z_1^2, and Z_1^3) will be less or greater than case (1), respectively. Similarly, if the specific growth rate is affected by the product concentration, the control coefficients (Z_1^1, Z_1^2, and Z_1^3) will be less than or greater than case (1), depending on whether the product inhibits or activates the cell growth. Moreover, the control coefficients with respect to ν_3 will be nonzero: they will be greater than zero when product inhibition exists, and less than zero when the product enhances cell growth. These results are valid if Q_s and Q_p are described by Eqs. 45 and 46; they provide qualitative insights regarding the effects of growth kinetics on the control coefficients.

Discussion

The results presented here extend MCA to chemostat bioreactors. In addition to determining the rate-controlling steps, we found that the control coefficients are useful in estimating the pseudo-first-order kinetic constants. We developed two approaches for determining kinetic parameters in chemostat bioreactors using simple experiments. The dynamic approach requires the measurements of transient data (s, b, and p) following a perturbation. This approach is based on a linear approximation of kinetics around the steady state, and the results are relatively sensitive to nonlinearity. To improve the estimation of control coefficients, we developed a steady-state perturbation method, which requires the measurements of steady states only. This method is less sensitive to nonlinearity and involves simpler measurements. However, this method requires perturbations in s_f, b_f, and p_f, of which perturbing b_f is relatively difficult. Therefore, the combination of the dynamic and the steady-state perturbation method is a better approach for most practical cases. The combination of the steady-state perturbation and dynamic approaches circumvents the problem in perturbing b_f and is less sensitive to nonlinearity.

The control coefficients indicate the relative importance of the three macroscopic processes to system responses. For example, if the control coefficient Z_1^3 is dominant, increasing the substrate uptake rate by genetic or environmental manipulations may increase the production formation flux. In addition, the control coefficients also help to determine the func-

tional dependence of the kinetic equations. Although cases analyzed in Table 5 are restricted for systems described by Eqs. 45 and 46, more general cases can be derived in a similar way.

The control coefficients can also be used to calculate the elasticity coefficients, which then give the pseudo-first-order kinetic constants of the specific rates of growth, substrate consumption, and product formation. These constants contain useful kinetic information in design and operation of bioreactors. They reveal quantitatively whether the specific rates are inhibited or enhanced by s, b, or p. Such information is traditionally obtained either quantitatively in batch experiments or quantitatively and tediously in chemostat cultures. The approaches described here offer a quantitative and simple approach for determining the kinetic properties.

Although the derivation of equations is mathematically intensive, the application is straightforward and is amenable to automation. It is possible to develop an on-line perturbation method to check the kinetic properties of the cells and to improve the performance of the reactor. This is particularly useful in industrial applications such as ethanol production, where continuous cultures have been used in large scales. The perturbation can be performed automatically and periodically to monitor and analyze the process. A large deviation of the kinetic parameters from the standard values indicates contamination or changes in the genetic and physiological state. Moreover, the control coefficients calculated can be used to improve the process performance by adjusting variables such as the dilution rate and the substrate feed concentration. Although most bioreaction processes are not conducted in chemostats, they can be used in the research and development stage for kinetic studies. The results reported here provide a theoretical basis for designing experiments and extracting information from data.

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Literature Cited

Burns, J. A., A. Cornish-Bowden, A. K. Groen, R. Heinrich, H. Kacser, J. Porteous, S. M. Rapoport, T. A. Rapoport, J. W. Stucki, J. M. Tager, R. J. A. Wanders, and H. V. Westerhoff, "Control Analysis of Metabolic Systems," *Trends Biochem. Sci.*, **10**, 16 (1985).

Caracotsios, M., and W. E. Stewart, "Sensitivity Analysis of Initial Value Problems with Mixed ODEs and Algebraic Equations," *Comput. Chem. Eng.*, **9**, 359 (1985).

Delgado, J., and J. C. Liao, "Identifying Rate-Limiting Steps in Metabolic Pathways Without Kinetic Parameters," *Biotechnol. Prog.*, **7**, 15 (1991).

Delgado, J., and J. C. Liao, "Determination of Flux Control Coefficients Using Transient Metabolite Concentrations," *Biochem. J.*, **282**, 919 (1992a).

Delgado, J., and J. C. Liao, "Metabolic Control Analysis Using Transient Metabolite Concentrations: Determination of Metabolite Concentration Control Coefficients," *Biochem. J.*, **285**, 965 (1992b).

Delgado, J., and J. C. Liao, "Control of Metabolic Pathways by Time-Scale Separation," *BioSystems*, **36**, 55 (1995).

Fell, D. A., "Metabolic Control Analysis: a Survey of its Theoretical and Experimental Development," *Biochem. J.*, **286**, 313 (1992).

Heinrich, R., and T. A. Rapoport, "A Linear Steady-State Treatment of Enzymatic Chains: General Properties, Control and Effector Strength," *Eur. J. Biochem.*, **42**, 89 (1974).

Kacser, H., and J. A. Burns, "The Control of Flux," *Rate Control of Biological Processes*, D. D. Davies, ed., Cambridge University Press. Cambridge, U.K., p. 65 (1973).

Kell, D. B., and H. V. Westerhoff, "Metabolic Control Theory: its Role in Microbiology and Biotechnology," *FEMS Microbiol. Rev.*, **39**, 305 (1986).

Liao, J. C., and J. Delgado, "Advances in Metabolic Control Analysis," *Biotechnol. Prog.*, **9**, 221 (1993).

Reder, C., "Metabolic Control Theory: a Structural Approach," *J. Theor. Biol.*, **135**, 175 (1988).

Savageau, M. A., "Parameter Sensitivity as a Criterion for Evaluating and Comparing the Performance of Biochemical Systems," *Nature*, **229**, 542 (1971).

Savageau, M. A., *Biochemical Systems Analysis*, Addison-Wesley, Reading, MA (1976).

Schuster, S., and R. Heinrich, "The Definitions of Metabolic Control Analysis Revisited," *BioSystems*, **27**, 1 (1992).

Small, J. R., "Design and Analysis of Chemostat Experiments Using Metabolic Control Analysis: a Top Down Approach," *Microbiol.*, **140**, 2439 (1994).

Westerhoff, H. V., W. Van Heeswijk, D. Kahn, and D. B. Kell, "Quantitative Approaches to the Analysis of the Control and Regulation of Microbial Metabolism," *Antonie Leeuwenhoek*, **60**, 193 (1991).

Appendix A: Derivation of Eqs. 29 and 30

Recall that

$$Z_{\nu_j}^{J_i} = \left(\frac{\partial J_i}{\partial M_j}\right)\left(\frac{\partial \nu_j}{\partial M_j}\right)^{-1} \tag{A1}$$

in which M_j denotes the manipulated parameters that affects ν_j only and the derivatives are evaluated at the steady state. In this case, $M_1 = s_f$, $M_2 = b_f$, and $M_3 = p_f$. From the definition of ν_1 (Eq. 8a), we have

$$\left(\frac{\partial \nu_1}{\partial s_f}\right)_{ss} = D \tag{A2}$$

At the steady state, we have

$$J_1 = (s_f - s_{ss})D \tag{A3}$$

and thus

$$\left(\frac{\partial J_1}{\partial s_f}\right)_{ss} = D\left(1 - \frac{\partial s_{ss}}{\partial s_f}\right)_{ss} \tag{A4}$$

Dividing Eq. A4 by Eq. A2 gives

$$Z_{\nu_1}^{J_1} = \left(1 - \frac{\partial s_{ss}}{\partial s_f}\right)_{ss} \tag{A5}$$

The other elements in Eqs. 29 and 30 are derived similarly.

Appendix B: Derivation of Eqs. 43 and 44

These equations will be derived using the general definition of the control coefficients. The flux control coefficients can be defined as (Reder, 1988)

$$\frac{\partial \boldsymbol{J}}{\partial \boldsymbol{\theta}} = \mathbf{Z}\frac{\partial \boldsymbol{v}}{\partial \boldsymbol{\theta}} \tag{B1}$$

which corresponds to the matrix version of Eq. 1b in the text. If we let θ be the dilution rate D, Eq. B1 can be written as

$$\left[\frac{\partial J_1}{\partial D}\ \frac{\partial J_2}{\partial D}\ \frac{\partial J_3}{\partial D}\right] = \mathbf{Z}\begin{bmatrix}\frac{\partial \nu_1}{\partial D}\\ \frac{\partial \nu_2}{\partial D}\\ \frac{\partial \nu_3}{\partial D}\end{bmatrix} \tag{B2}$$

Thus, for the steady-state flux of product we obtain

$$\frac{\partial J_3}{\partial D} = Z_1^3\frac{\partial \nu_1}{\partial D} + Z_2^3\frac{\partial \nu_2}{\partial D} + Z_3^3\frac{\partial \nu_3}{\partial D} \tag{B3}$$

Taking the derivative with respect to D in Eqs. 8a to 8c and evaluating at the steady state, we have that

$$\frac{\partial \nu_1}{\partial D} = s_f - s_{ss} = \frac{J_1}{D}$$

$$\frac{\partial \nu_2}{\partial D} = b_{ss} - b_f = \frac{J_2}{D}$$

$$\frac{\partial \nu_3}{\partial D} = p_{ss} - p_f = \frac{J_3}{D} \tag{B4}$$

Substituting into Eq. B3 we obtain Eq. 43 in the text. Equation 44 in the text can be proved following the same procedure and using the definition

$$\frac{\partial \boldsymbol{\sigma}}{\partial \boldsymbol{\theta}} = \boldsymbol{\Gamma}\frac{\partial \boldsymbol{v}}{\partial \boldsymbol{\theta}} \tag{B5}$$

for the metabolite concentration control coefficients, which is the matrix form of Eq. 2b.

Appendix C

Derivation of Eqs. 43 and 44 using Euler's theorem

These equations will be derived using Euler's theorem, which states that if

$$f(a,b,\lambda x,\lambda y) = \lambda^h f(a,b,x,y) \tag{C1}$$

then

$$\frac{\partial f}{\partial x}x + \frac{\partial f}{\partial y}y = hf \tag{C2}$$

To apply this theorem, we recognize that the steady-state flux functions are first-degree homogeneous functions ($h = 1$) of all the ν_j

$$J_i(\lambda\nu_1, \lambda\nu_2, \lambda\nu_3, \lambda\nu_4, \lambda\nu_5, \lambda\nu_6) = \lambda J_i(\nu_1, \nu_2, \nu_3, \nu_4, \nu_5, \nu_6) \tag{C3}$$

Therefore, we have

$$\left(\nu_1\frac{\partial J_i}{\partial \nu_1} + \nu_2\frac{\partial J_i}{\partial \nu_2} + \nu_3\frac{\partial J_i}{\partial \nu_3} + \nu_4\frac{\partial J_i}{\partial \nu_4} + \nu_5\frac{\partial J_i}{\partial \nu_5} + \nu_6\frac{\partial J_i}{\partial \nu_6}\right) = J_i \tag{C4}$$

Note that increasing D by a factor of k changes ν_1, ν_2, and ν_3 by the same factor k. Therefore, a simultaneous change in ν_1, ν_2, and ν_3 can be achieved by changing D. Thus, we can relate various fluxes to the dilution rate D as

$$J_i(\lambda D, \lambda\nu_4, \lambda\nu_5, \lambda\nu_6) = \lambda J_i(D, \nu_4, \nu_5, \nu_6) \tag{C5}$$

Thus,

$$\left(D\frac{\partial J_i}{\partial D} + \nu_4\frac{\partial J_i}{\partial \nu_4} + \nu_5\frac{\partial J_i}{\partial \nu_5} + \nu_6\frac{\partial J_i}{\partial \nu_6}\right) = J_i \tag{C6}$$

Comparing Eq. C4 with Eq. C6, we obtain

$$D\frac{\partial J_i}{\partial D} = \nu_1\frac{\partial J_i}{\partial \nu_1} + \nu_2\frac{\partial J_i}{\partial \nu_2} + \nu_3\frac{\partial J_i}{\partial \nu_3} \tag{C7}$$

which proves Eq. 43 in the text.

To prove Eq. 44, we recognize that the steady-state product concentration is a zero-degree homogeneous function ($h = 0$) of all the reaction rates

$$P_{ss}(\lambda\nu_1, \lambda\nu_2, \lambda\nu_3, \lambda\nu_4, \lambda\partial_5, \lambda\nu_6) = p_{ss}(\nu_1, \nu_2, \nu_3, \nu_4, \nu_5, \nu_6) \tag{C8}$$

Therefore, we have

$$\left(\nu_1\frac{\partial p_{ss}}{\partial \nu_1} + \nu_2\frac{\partial p_{ss}}{\partial \nu_2} + \nu_3\frac{\partial p_{ss}}{\partial \nu_3} + \nu_4\frac{\partial p_{ss}}{\partial \nu_4} + \nu_5\frac{\nu p_{ss}}{\partial \nu_5} + \nu_6\frac{\partial p_{ss}}{\partial \nu_6}\right) = 0 \tag{C9}$$

Using the above concept again, we can relate various fluxes to the dilution rate D as

$$p_{ss}(\lambda D, \lambda\nu_4, \lambda\nu_5, \lambda\nu_6) = p_{ss}(D, \nu_4, \nu_5, \nu_6) \tag{C10}$$

Thus,

$$\left(D\frac{\partial p_{ss}}{\partial D} + \nu_4\frac{\partial p_{ss}}{\partial \nu_4} + \nu_5\frac{\partial p_{ss}}{\partial \nu_5} + \nu_6\frac{\partial p_{ss}}{\partial \nu_6}\right) = 0 \tag{C11}$$

Comparing Eqs. C4 and C6, we obtain

$$D\frac{\partial p_{ss}}{\partial D} = \nu_1\frac{\partial p_{ss}}{\partial \nu_1} + \nu_2\frac{\partial p_{ss}}{\partial \nu_2} + \nu_3\frac{\partial p_{ss}}{\partial \nu_3} \tag{C12}$$

which proves Eq. 44.

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