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REVIEW

Developing the Control Criterion for a Continuous Culture of Microorganisms

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Abstract—A short survey and a critical analysis of previously proposed criteria for growth control of populations of microorganisms in the chemostat are presented. Based on the analysis of a mathematical model of the steady state of a microbial population in the chemostat, an adequate control criterion is suggested, along with a method to identify the corresponding control factors. The new control criterion is expressed as a product of the factor transformation coefficient and the sensitivity coefficient (SC) of the biomass with respect to the change in the factor at the chemostat inlet (hereinafter, the biomass SC). The control criterion determines the strength of the control exerted by a factor. The method of determining control factors consists in experimental determination of the real SCs for factors and the biomass and calculation on this basis of the corresponding ideal SCs, assuming constant factor transformation coefficients. The ideal SCs are shown to add up to integer values, a constraint that we call *quantization* relationships. Such relationships are used to test the completeness of the list of control factors. The proposed method was applied to both our own and literature data.

Key words: microorganism population, control factor, chemostat, control criterion, sensitivity coefficients.

The population dynamics of microorganisms belonging to one trophic level is determined above all by physicochemical characteristics of their habitat (substrates, metabolites, temperature, light conditions, etc.). This stems from the fact that major characteristics of microorganisms' activity (the specific growth rate, factor transformation coefficients, etc.) are determined by the strength (the concentration or intensity) of the factors involved. The factor transformation coefficient is defined as the ratio between the change in the factor strength and the change in the biomass density in a closed space. For a substrate, the transformation coefficient is the reciprocal of the yield.

In terms of the relationship between the specific growth rate of the population (μ), the factor level (A) and the factor transformation coefficient (a), the environmental factors can be classified as follows:

stimulator ($\partial\mu/\partial A > 0$, $a < 0$): stimulates growth and is consumed;

autostimulator ($\partial\mu/\partial A > 0$, $a > 0$): stimulates growth and is released;

inhibitor ($\partial\mu/\partial A < 0$, $a < 0$): inhibits growth and is consumed;

autoinhibitor ($\partial\mu/\partial A < 0$, $a > 0$): inhibits growth and is produced;

stimulator–modifier ($\partial\mu/\partial A > 0$, $a = 0$): only stimulates growth;

inhibitor–modifier ($\partial\mu/\partial A < 0$, $a = 0$): only inhibits growth.

The first four types of factors are characterized by nonvanishing transformation coefficients and, therefore, vary with the population density. Such factors are called *regulators*. The last two types (modifiers) do not depend on the population density and are not considered in this study.

This objective of this work is to advance a new method for identification of regulators, including limiting factors, for real populations of microorganisms and to develop an adequate criterion of population growth control. The control criterion represents a quantitative measure of population functioning, describing the strength of the control exerted by the given factor, or, in other words, the dependence of the population dynamics upon the regulator level.

The term *control* should not be confused with the term *regulation*. According to Odum, regulation is a "drive to maintain the steady-state population size" [1]. The term control is used here to denote a type of control with a feedback coupling between the controlled value and the controlling factor [2]. This occurs in the chemostat because the specific growth rate depends upon environmental factors, which, in turn, depend upon the population density. It follows that regulation is the outcome of the control process, which may fail to result in regulation. For a population in the chemostat, regulation (i.e., attaining the steady state) is not possible unless certain conditions are fulfilled (specifically, the maximum specific growth rate must be greater than the flow rate under perfect mixing). Hereinafter, we shall

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generally employ the term *control*, whereas the term *regulation* will be used only when referring to the steady state.

The issue of population growth control is closely connected with that of limiting factors. Without going into the history of how the limiting factor definition evolved, let us briefly outline the relationship between the phenomena of limitation and control. The former is an external manifestation of control exerted when a variable (e.g., the biomass) or a controlled value (e.g., the rate of a biochemical reaction) in a biological system is subject to a constraint. In the first case, we have so-called stoichiometric limitation, and, in the second case, kinetic limitation [3]. Any of the above factors, including modifiers, can act as a limiting factor, provided it is subject to a constraint.

The major issue in controlled cultivation of microorganisms is determining the control scheme effective in the microbial community. In this study, the term *control scheme* is used to denote the set of regulators (control factors) and the dependences of the population's specific growth rate and transformation coefficients upon the regulator levels. Solving this problem will be a major step towards solving many other applied and basic problems of population microbiology: formulating the laws governing stable community functioning, controlling the community composition, optimizing the production of the desired product, etc. Drawing up the list of control factors and their ranking is the initial step towards this goal.

In our view, continuous cultivation in the chemostat is still the most acceptable way to study the control scheme in populations and communities of microorganisms. The interest in continuous cultivation has declined to a certain degree over the last decade. This might be partly due to a lack of theoretical generalizations of the enormous body of accumulated experimental evidence. The goal of this paper is to help bridge this gap.

THE EXISTING CONTROL CRITERIA IN THE CHEMOSTAT AND THEIR CRITICISM

Choosing an adequate criterion of population growth control in the chemostat constitutes an important part of the regulator identification procedure. Such a criterion must meet two requirements: it must be quantitative and it must be normalized, which implies it must be nondimensional. The criteria used in most studies fail to satisfy these requirements. The authors often state that a certain factor is responsible for growth control but fall short of determining its control strength. The normalization requirement means that the criterion value may vary within a certain limited range, the same for all similar objects (e.g., from –1 to 1 or from 0 to 1). A criterion that meets these requirements would allow different control factors to be ranked, insignificant factors neglected, and different objects compared.

The following criteria of microorganism growth control in the chemostat can be found in the literature (methods of limitation determination):

1. The control criterion most widespread in the literature, a low substrate content of the medium (less than the half-saturation constant). In our view, this criterion is not at all constructive since it relies on prior knowledge of how the specific growth rate depends upon the regulator level, which, in effect, is the ultimate goal of the study.

2. The proportional change of the steady-state population density with the control factor level in the freshly supplied medium [4–6]. One shortcoming of this criterion is that it is not at all normalized, as discussed below.

3. The sensitivity coefficient of the regulator's steady-state level with respect to its change at the input (also known as the autostabilization coefficient) [7–10]. This criterion is both quantitative and normalized but of limited applicability, as discussed below.

4. The method of small perturbations in the steady state. The method involves introducing microdoses of the supposed control factor into the fermenter and monitoring low-inertia parameter values, like the regulator content [11], the titration rate in medium pH stabilization, or rate of oxygen utilization [12, 13]. The advantage of the method is its fast response, yet no normalized control criterion has been proposed by advocates of this method.

RELATIONSHIPS BETWEEN CONTROL CRITERIA IN THE CHEMOSTAT AND IDENTIFICATION OF THE MOST ADEQUATE CRITERION

Identifying the most adequate control criterion is based on the analysis of a steady state of a microbial population in the chemostat, described by the following set of equations [8]:

$$\begin{cases} \mu(A_1, \ldots, A_m) = D; \\ A_j - A_j^0 = a_j(A_1, \ldots, A_m)X; \quad j = 1, \ldots, m, \end{cases} \tag{1}$$

where D is the flow rate of the medium X is the steady-state population density A_j^0 and A_j are the levels of the jth factor in the influent medium and in the fermenter, respectively μ is the specific growth rate of the population a_j is the coefficient of the jth factor transformation by the population and m is the number of factors. In the generic case, the transformation coefficients are functions of factor levels in the fermenter, $a_j = a_j(A_1, \ldots, A_m)$, which is equivalent to their parametric dependence upon their input levels, $a_j = a_j(A_1^0, \ldots, A_m^0)$. In terms of the control theory, the strength of growth control by a given factor is determined by the value of $\partial X/\partial A$. However, the level of a factor in the chemostat cannot be a

control parameter because it cannot vary independently of the levels of other factors. One advantage of the chemostat that we shall make use of is the possibility to vary the level of any factor in the influent medium. Therefore, let us take the input level A_j^0 of the jth factor as the control parameter and write out the derivative of the jth factor with respect to A_j^0 (the subscripts are dropped):

$$\frac{\partial A}{\partial A^0} = 1 + a\frac{\partial X}{\partial A^0} + X\frac{\partial a}{\partial A^0}. \quad (2)$$

Equation (2) relates three different sensitivity coefficients (SCs) with respect to A^0: the SC of the factor itself $\partial A/\partial A^0$, the SC of the biomass density $\partial X/\partial A^0$ (the biomass SC), and the SC of the transformation coefficient $\partial a/\partial A^0$. The biomass SC is the most adequate measure of the strength of population growth control by the given factor. This quantity is used as a control criterion in many papers concerned with continuous cultures. However, the actual values of $\partial X/\partial A^0$ for factors of different nature can vary by orders of magnitude, which hinders quantitative evaluation of their control strengths. At the same time, as we are going to show, the value of $a\partial X/\partial A^0$ can vary within a fairly narrow range and can serve as an adequate control criterion. In addition, $a\partial X/\partial A^0$, unlike $\partial X/\partial A^0$, is nondimensional. Hereinafter, when using the term *biomass SC*, we shall refer to the quantity $a\partial X/\partial A^0$.

If the transformation coefficient a is constant, the relationship between the SCs of the biomass and the factor becomes straightforward,

$$\frac{\partial A}{\partial A^0} = 1 + a\frac{\partial X}{\partial A^0}, \quad (3)$$

and the factor SC is an equally good control criterion as the biomass SC. In this case, the biomass and factor SCs may be called ideal SCs.

To determine the variation range of ideal SCs, one has to know how the specific growth rate changes with the factor level. In the neighborhood of the steady state, this dependence can be represented as a linear function of the levels of the m factors: $\mu = \mu_0 + \sum_{j=1}^{m} b_j A_j$, where $b_j = \partial\mu/\partial A_j$ is the population affinity with respect to the jth factor. Differentiating the solution to equation set (1) with respect to A_k^0, we can obtain the expressions for the corresponding ideal SCs [9, 10]

$$a_k\frac{\partial X}{\partial A_k^0} = -a_k b_k / \sum_{j=1}^{m} a_j b_j, \quad (4)$$

$$\frac{\partial A_k}{\partial A_k^0} = \left(\sum_{j=1}^{m} a_j b_j - a_k b_k\right) / \sum_{j=1}^{m} a_j b_j. \quad (5)$$

Table 1. Stable steady-state conditions for a chemostat population and possible values assumed by ideal SCs with respect to the first factor in populations controlled by two factors

a_2b_2 \ a_1b_1	>0 (type 1)	<0 (type 2)
>0 (type 1)	(1) unstable steady state	(2) $\|a_1b_1\| > \|a_2b_2\|$ factor SC < 0 biomass SC < –1
<0 (type 2)	(3) $\|a_1b_1\| < \|a_2b_2\|$ factor SC > 1 biomass SC > 0	(4) always stable factor SC from 0 to 1 factor SC from –1 to 0

Note: For the second factor, everything is symmetric. In the case of more than two factors, a_2b_2 should be replaced by $\sum_{j=2}^{m} a_j b_j$.

The denominator in Eqs. (4) and (5) is the population feedback coefficient [9, 10]. The steady state in the chemostat shall not be Lyapunov stable unless the feedback coefficient is negative [8]. Table 1 gives ranges of values of ideal SCs depending on the value of ab along with the steady-state stability conditions. The factors in this table are classified into two types in terms of the values of ab [8]. If $ab > 0$, the factor is an autostimulator or inhibitor and belongs to type 1. If $ab < 0$, the factor is either a stimulator or autoinhibitor and belongs to type 2.

Populations of microorganisms are normally controlled by autostimulators or autoinhibitors (cell 4 in Table 1) such that, at a maximum control strength, the ideal factor SC is equal to 0 and the ideal biomass SC is –1, whereas, at minimum control strength, they are 1 and 0, respectively. When the control strength attains its maximum for the given factor, the specific growth rate in this state comes to be determined by this factor alone. When the control strength is zero, the specific growth rate of the population does not depend upon the given factor.

High absolute values of ideal SCs in cells 2 and 3 of Table 1 (for different factor types) can be observed when the feedback coefficient nears zero from the left and the system approaches an unstable steady state. Such situations are hard to realize in an experiment and we are not aware of any evidence in the literature as regards high absolute values of the ideal factor and biomass SCs.

The situation where the factor SC is close to zero is known as the autostabilization of the factor. This phenomenon was given much attention in the literature [7–9], but the point that such factors are constrained in their type was never brought up. Autostabilization in its classical meaning applies only to stimulators and autoinhibitors, i.e., to factors in cell 4 of Table 1.

If the transformation coefficients are constant, the ideal SCs for a community comprising n species (for a single population, $n = 1$) and controlled by m factors have a very important property of adding up to an integer total; i.e.,

$$\sum_{j=1}^{m} \partial A_j / \partial A_j^0 = m - n, \quad (6.1)$$

$$\sum_{i=1}^{n}\sum_{j=1}^{m} a_{ji} \partial X_i / \delta A_j^0 = -n. \quad (6.2)$$

The proof of (6.1) is given in [9], and relationship (6.2) can be readily obtained from Eq. (3) by taking into account (6.1). We called Eqs. (6) *quantization relationships*, and these have both important theoretical and applied significance. For example, they may allow one to verify that the list of control factors drawn up is actually complete. Whenever relationships (6) hold for a number of steady states, the probability for all control factors to be accounted for is very high. A detailed discussion of different applications of quantization relationships falls outside the scope of this paper.

When the transformation coefficients are not constant, relationships (6) are not generally valid. The practical experience of microorganism culturing indicates that the transformation coefficients are most often not constant and can change severalfold with culture conditions. Let us now see how the inconstancy of transformation coefficients affects the values of real (actual) SCs and the fulfillment of quantization relationships. For this purpose, consider the steady-state equations in the chemostat (1). The first equation of this set describes the functional binding between the steady-state levels of control factors:

$$A_j = f_j(A_1, \ldots, A_{j-1}, \ldots, A_{j+1}, \ldots, A_m). \quad (7)$$

Let us now derive the expression for the real SC of the *j*th factor by differentiating equation (7) with respect to A_j^0,

$$\frac{\partial A_j}{\partial A_j^0} = \sum_{k=1, k\neq j}^{m} \frac{\partial f_j}{A_k} \frac{\partial A_k}{\partial A_j^0}. \quad (8)$$

The steady-state levels of the *j*th and *k*th control factors are related by

$$A_k = A_k^0 + \frac{a_k}{a_j}(A_j - A_j^0). \quad (9)$$

From (9), we can find $\partial A_k / \partial A_j^0$, and, by substituting it into (8), we arrive at the relationship for the real SCs of all factors,

$$\frac{\partial A_j}{\partial A_j^0} = \sum_{k=1, k\neq j}^{m} \frac{\partial f_j}{\partial A_k} \times \left[a_j X \frac{\partial a_k}{\partial A_j^0} - a_k X \frac{\partial a_j}{\partial A_j^0} - a_k\right] \Big/ \left[a_j - \sum_{k=1, k\neq j}^{m} \frac{\partial f_j}{\partial A_k} a_k\right]; \quad (10)$$

$$j = 1, \ldots, m.$$

If all transformation coefficients are constant, the relationships for ideal factor SCs are given by

$$\frac{\partial A_j}{\partial A_j^0} = \sum_{k=1, k\neq j}^{m} \frac{\partial f_j}{\partial A_k} a_k \Big/ \left[\sum_{k=1, k\neq j}^{m} \frac{\partial f_j}{\partial A_k} a_k - a_j\right]; \quad (11)$$

$$j = 1, \ldots, m.$$

The corresponding relationships for the biomass SC can be readily obtained from (2) (the derivation is omitted for brevity).

The most common case in the practice of continuous cultivation is the presence of one or two control factors.

With two control factors, relationship (7) takes on the form of two connected equations: $A_1 = f_1(A_2)$ and $A_2 = f_2(A_1)$, where f_1 and f_2 are two mutually reciprocal functions satisfying $\partial f_1/\partial A_2 = (\partial f_2/\partial A_1)^{-1}$. Denoting for brevity $\partial f_1/\partial A_2$ by f', we arrive at the following equations for the real SCs that can be conveniently analyzed:

$$\frac{\partial A_1}{\partial A_1^0} = \left[Xf'\left(a_1\frac{\partial a_2}{\partial A_1^0} - a_2\frac{\partial a_1}{\partial A_1^0}\right) - f'a_2\right]/(a_1 - f'a_2), \quad (12.1)$$

$$\frac{\partial A_2}{\partial A_2^0} = \left[X\left(a_1\frac{\partial a_2}{\partial A_2^0} - a_2\frac{\partial a_1}{\partial A_2^0}\right) + a_1\right]/(a_1 - f'a_2), \quad (12.2)$$

$$a_1\frac{\partial X}{\partial A_1^0} = \left[Xa_1\left(f'\frac{\partial a_2}{\partial A_1^0} - \frac{\partial a_1}{\partial A_1^0}\right) - a_1\right]/(a_1 - f'a_2), \quad (12.3)$$

$$a_2\frac{\partial X}{\partial A_2^0} = \left[Xa_2\left(f'\frac{\partial a_2}{\partial A_2^0} - \frac{\partial a_1}{\partial A_2^0}\right) + f'a_2\right]/(a_1 - f'a_2). \quad (12.4)$$

The corresponding relationships for the ideal SCs are as follows:

$$\frac{\partial A_1}{\partial A_1^0} = \frac{-f'a_2}{a_1 - f'a_2}, \quad (13.1)$$

$$\frac{\partial A_2}{\partial A_2^0} = \frac{a_1}{a_1 - f'a_2}, \quad (13.2)$$

$$a_1\frac{\partial X}{\partial A_1^0} = \frac{-a_1}{a_1 - f'a_2} = -\frac{\partial A_2}{\partial A_2^0} = \frac{\partial A_1}{\partial A_1^0} - 1, \quad (13.3)$$

$$a_2\frac{\partial X}{\partial A_2^0} = \frac{f'a_2}{a_1 - f'a_2} = -\frac{\partial A_1}{\partial A_1^0} = \frac{\partial A_2}{\partial A_2^0} - 1. \quad (13.4)$$

If the first factor is the sole regulator in the system, then $f' = 0$. In this case, the real and ideal SCs of the factor are zero, the ideal biomass SC equals –1, and the real biomass SC can assume any value (see (12.3)).

The values of the real SCs can be determined directly from experimental data (the setup of the experiment and the method of SC calculation are described below). The same data will also be sufficient to determine uniquely all terms on the right-hand sides of Eqs.(10)–(13) except $\partial f_j/\partial A_k$. Mathematical analysis of

these equations shows that, in the case of three or more control factors, the ideal SCs of factors and for that the values of $\partial f_j/\partial A_k$ cannot be derived from the values of real SCs unless the function $\mu(A_1, \ldots, A_m)$ is known. However, in the case of just two factors, the ratio between the real and the ideal SCs of the jth factor does not depend upon this function and is given by

$$SC_{real}/SC_{ideal} = 1 + X\left(\frac{\partial a_j}{\partial A_j^0} - \frac{a_j}{a_k}\frac{\partial a_k}{\partial A_j^0}\right); \quad j, k = 1, 2. \tag{14}$$

Relationship (14) makes it possible to determine the ideal SCs of the factors from experimental data. The corresponding relationship for the biomass SC with respect to the jth factor includes the unknown values $\partial f_j/\partial A_k$,

$$SC_{real}/SC_{ideal} = 1 + X\left(\frac{\partial a_j}{\partial A_j^0} - \frac{\partial f_j}{\partial A_k}\frac{\partial a_k}{\partial A_j^0}\right); \quad j, k = 1, 2. \tag{15}$$

However, the ideal biomass SCs with respect to the two likely control factors can be readily found from Eq. (3).

Let us turn once again to the problem of developing an adequate control criterion. As implied by equation set (1), control relations in the chemostat arise from the dependence of the population specific growth rate upon factor levels and the dependence, in the generic case, of transformation coefficients upon the same factor levels. The first dependence constitutes the basis of regulation at the kinetic level, whereas the second dependence gives rise to stoichiometric regulation (not to be confused with kinetic and stoichiometric limitations [3]).

The strength of kinetic control is equally well reflected by ideal biomass and factor SCs.

The overall control strength is most adequately reflected by the real SC of the biomass. First of all, the absolute value of the biomass SC, unlike that of the factor SC, is a direct function of control strength. Second, as follows from Eq. (2), in the case of nonconstant transformation coefficients, the factor SC fails to reflect adequately the control strength. In addition, if the number of populations is equal to the number of factors ($m = n$) and transformation coefficients are not constant, then all real and ideal SCs of factors will vanish and all ideal biomass SCs will equal –1, while the real biomass SCs with respect to different factors will not be equal in the general case (the proof of this statement is outside the scope of this paper). This means that different factors will differ also in their control strengths, just as occurs in reality.

The strength of stoichiometric control is expressed by the *stoichiometric control coefficient* K_{stoich}, which determines the ratio between the real and ideal biomass SCs (see (15)),

$$SC_{real} = SC_{ideal}(1 + K_{stoich}). \tag{16}$$

A factor can exert purely kinetic or purely stoichiometric control or act simultaneously as a kinetic and a stoichiometric regulator. Formally, the regulation type is determined by the ratio of the ideal and real SCs of the biomass (SC_{ideal}/SC_{real}). If $SC_{ideal}/SC_{real} = 1$, then the control exerted by the factor is purely kinetic; if $SC_{ideal}/SC_{real} = 0$, then it is purely stoichiometric; and otherwise the factor is responsible for both kinetic and stoichiometric control. It should be emphasized that the fact that $\partial a_j/\partial A_j^0$ vanishes does not necessarily mean that the factor j is not a stoichiometric regulator. Because of the dependence $a_k = a_k(A_1^0, \ldots, A_m^0)$, this factor can exert stoichiometric control over population growth via the transformation coefficient of another (the kth) factor.

Let us now summarize the main points. If the transformation coefficients are constant, there exist two peer control criteria: the sensitivity coefficients of the factors and the biomass. The real and ideal values of these coefficients are equal. When the transformation coefficients are not constant, the control strength is determined by the real biomass SC. In this case, the ideal SCs (of the biomass and the factors) can be used to verify, by means of quantization relationships, that the list of control factors drawn up is indeed complete.

It often happens that the data available to an experimenter relate to just one control factor, although a second control factor is likely to be involved. In such a situation, one cannot use relationships (3) and (14) to evaluate the ideal SCs because one is unable to compute $\partial a_k/\partial A_j^0$ ($k \neq j$). Even so, the outlined theory makes it possible to deduce the likely population control scheme. Let us now demonstrate how this can be done.

Tables 2 and 3 list possible values of real SCs depending on the population control scheme. Table 2 relates to the case of kinetic control by the first factor alone ($\mu = \mu(A_1)$) and contains the expressions for SCs in full. Table 3 relates to the case of kinetic control by two factors ($\mu = \mu(A_1, A_2)$), but because the corresponding relations are so cumbersome, only the qualitative characteristics of the SCs are given. These relationships can be obtained using Eqs. (12). With the aid of Tables 2 and 3 and using the available values of real SCs for the known control factor, the likely population control scheme can be hypothesized. The data of Tables 2 and 3 are generalized in Table 4, which lists all possible combinations of real SC values along with the corresponding control schemes. Table 4 can be readily used in practice, as illustrated by the following example.

Suppose that the transformation coefficient of the studied factor is constant; i.e., $\partial a/\partial A_0 = 0$, the real factor SC is zero, and the real biomass SC equals –1. In this case, the possible schemes are 1, 2, 5, and 6 (factor 1). Schemes 1 and 2 imply that there are no control factors other than the one studied (the real biomass SC with respect to the second factor equals 0). Schemes 5 and 6

Table 2. Possible values assumed by real SCs in the case of kinetic control by the first factor and different types of stoichiometric control by the two factors

	Control scheme	Factor	Control type	Real factor SC	Real biomass SC
1	$\mu(A_1)$, a_1 = const,	**1**	k	0	–1
	a_2 = const, $a_2(A_1^0)$	**2**	–	1	0
2	$\mu(A_1)$, a_1 = const,	**1**	k	0	–1
	$a_2(A_2^0)$, $a_2(A_1^0, A_2^0)$	**2**	–	$1 + X\frac{\partial a_2}{\partial A_2^0}$	0
3	$\mu(A_1)$, $a_1(A_1^0)$, a_2 = const,	**1**	k + s	0	$-1 - X\frac{\partial a_1}{\partial A_1^0}$
	$a_2(A_1^0)$	**2**	–	1	0
4	$\mu(A_1)$, $a_1(A_1^0)$, $a_2(A_2^0)$, $a_2(A_1^0, A_2^0)$	**1**	k + s	0	$-1 - X\frac{\partial a_1}{\partial A_1^0}$
		2	–	$1 + X\frac{\partial a_2}{\partial A_2^0}$	0
5	$\mu(A_1)$, $a_1(A_2^0)$, a_2 = const,	**1**	k	0	–1
	$a_2(A_1^0)$	**2**	s	$1 - X\frac{a_2}{a_1}\frac{\partial a_1}{\partial A_2^0}$	$-X\frac{a_2}{a_1}\frac{\partial a_1}{\partial A_2^0}$
6	$\mu(A_1)$, $a_1(A_2^0)$, $a_2(A_2^0)$, $a_2(A_1^0, A_2^0)$	**1**	k	0	–1
		2	s	$1 + X\left(\frac{\partial a_2}{\partial A_2^0} - \frac{a_2}{a_1}\frac{\partial a_1}{\partial A_2^0}\right)$	$-X\frac{a_2}{a_1}\frac{\partial a_1}{\partial A_2^0}$
7	$\mu(A_1)$, $a_1(A_1^0, A_2^0)$, a_2 = const, $a_2(A_1^0)$	**1**	k + s	0	$-1 - X\frac{\partial a_1}{\partial A_1^0}$
		2	s	$1 - X\frac{a_2}{a_1}\frac{\partial a_1}{\partial A_2^0}$	$-X\frac{a_2}{a_1}\frac{\partial a_1}{\partial A_2^0}$
8	$\mu(A_1)$, $a_1(A_1^0, A_2^0)$, $a_2(A_2^0)$, $a_2(A_1^0, A_2^0)$	**1**	k + s	0	$-1 - X\frac{\partial a_1}{\partial A_1^0}$
		2	s	$1 + X\left(\frac{\partial a_2}{\partial A_2^0} - \frac{a_2}{a_1}\frac{\partial a_1}{\partial A_2^0}\right)$	$-X\frac{a_2}{a_1}\frac{\partial a_1}{\partial A_2^0}$

Note: The ideal SCs for all schemes are equal to the real SCs for scheme 1; k" denotes kinetic control, "s" denotes stoichiometric control, and "–" means lack of control.

imply the presence of an additional stoichiometric control factor (the real biomass SC with respect to the second factor does not vanish). The studied factor acts only as a kinetic regulator. The available data do not warrant more accurate identification of the scheme. The control schemes that correspond to other SC values can be distinguished in a similar manner (see the next section).

Concluding the theoretical part of the paper, let us briefly outline the key steps in control factor identification. The first step is to make a list of possible regulators based on the knowledge of the nutritional and physiological requirements of the population. The second step is to run experiments to determine SCs with respect to the corresponding factors. The third step involves analysis of the real SCs and evaluation of ideal SCs in accordance with relationships (3) and (14). The last step is to verify that the quantization relationships are indeed fulfilled (preferably, in several steady states). If this is reliably so, then all the control factors were identified and the strength of control exerted by

Table 3. Values assumed by real SCs in the case of kinetic control by two factors

No.	Control scheme	Factor	Control type	Real factor SC	Real biomass SC
9	$\mu(A_1, A_2)$, a_1 = const, a_2 = const	**1**	k	≠1, ≠0	$SC_f - 1$
		2	k	≠1, ≠0	$SC_f - 1$
10	$\mu(A_1, A_2)$, a_1 = const, $a_2(A_2^0)$	**1**	k	≠1, ≠0	$SC_f - 1$
		2	k + s	any value	$\neq SC_f - 1$
11	$\mu(A_1, A_2)$, a_1 = const, $a_2(A_1^0)$	**1**	k + s	any value	$\neq SC_f - 1$
		2	k	≠1, ≠0	$SC_f - 1$
12	$\mu(A_1, A_2)$, $a_1(A_1^0)$, $a_2(A_1^0)$	**1**	k + s	any value	$\neq SC_f - 1$
		2	k	≠1, ≠0	$SC_f - 1$
13	$\mu(A_1, A_2)$, a_1 = const, $a_2(A_1^0, A_2^0)$	**1**	k + s	any value	$\neq SC_f - 1$
		2	k + s	any value	$\neq SC_f - 1$

Note: SC_f denotes factor SC; other notation is as in Table 2. The ideal SCs for all schemes are equal to the real SCs for scheme 9. Factor numbers can be interchanged. The control types and qualitative characteristics of SCs for schemes (14) $a_1(A_1^0)$, $a_2(A_2^0)$; (15) $a_1(A_1^0)$, $a_2(A_1^0, A_2^0)$; (16) $a_1(A_2^0)$, $a_2(A_1^0)$; (17) $a_1(A_2^0)$, $a_2(A_1^0, A_2^0)$; and (18) $a_1(A_1^0, A_2^0)$, $a_2(A_1^0, A_2^0)$ are identical to those of scheme (13).

Table 4. Determining a likely control scheme of a population on the basis of real SCs with respect to one factor

No.	$\partial a/\partial A_0$	Real factor SC	Real biomass SC	Control scheme
1	0	0	–1	1(1), 2(1), 5(1), 6(1)
2	0	1	0	1(2), 3(2)
3	0	≠1, ≠0	$SC_f - 1$	5(2), 7(2), 9(a), 10(1), 11(2), 12(2)
4	0	≠1, ≠0	$\neq SC_f - 1$	11(1), 13(1), 16(a)
5	≠0	$1 + X\frac{\partial a}{\partial A^0}$	0	2(2), 4(2)
6	≠0	0	$-1 - X\frac{\partial a}{\partial A^0}$	3(1), 4(1), 7(1), 8(1)
7	≠0	any value	$\neq SC_f - 1$	6(2), 8(2), 10(2), 13(2), 14(a), 12(1), 15(a), 17(a), 18(a)

Note: The numbers in brackets refer to factor numbers in Tables 2 and 3; "a" means any factor; and SC_f is the factor SC.

each factor was determined. If these relationships are not reliably satisfied, then it can be concluded that all factors were not taken into account and the relative strength of control by the given factor cannot be deduced from the real SC of the biomass with respect to this factor. Neither is it possible to tell how many factors remain unaccounted for. This is because the ideal SCs of the known factors might be functions of levels of unknown factors. This is a limitation of the method.

Finally, it is worth pointing out that some factors are not suitable for studying by this method. As implied by the definition of an SC, the researcher must be able to adjust the level of the factor at the chemostat inlet. Therefore, metabolites that cannot be identified and independently supplied with the incoming medium are not amenable to the described method.

APPLYING THE CONTROL CRITERION TO EXPERIMENTAL DATA

1. The minimal experiment to calculate SCs with respect to two control factors. In order to determine the SCs with respect to two likely control factors, at least three steady states with different input factor levels have to be analyzed. The optimal settings of the experiment are given in Table 5 (to avoid using double subscripts, the factors are denoted by *N* and *P,* with subscripts now indicating the number of the steady state). The values of SCs are determined in steady state 2. In order to accurately determine the SC values, the input factor levels must be optimally varied. On the one hand, the difference in the input factor levels should be small enough to replace derivatives with finite differences. On the other hand, it should be large enough for

Table 5. A minimal set of steady-state characteristics of a population needed for determination of SCs with respect to two assumed control factors *N* and *P*

Operation regime no.	*N*, input	*N*, output	*P*, input	*P*, output	Biomass	Transformation coefficient for *N*	Transformation coefficient for *P*
1	N_1^0	N_1	$P_1^0 = P_2^0$	P_1	X_1	$a_1^N = \frac{N_1 - N_1^0}{X_1}$	$a_1^P = \frac{P_1 - P_1^0}{X_1}$
2	$N_2^0 = N_3^0$	N_2	$P_2^0 = P_1^0$	P_2	X_2	$a_2^N = \frac{N_2 - N_2^0}{X_2}$	$a_2^P = \frac{P_2 - P_2^0}{X_2}$
3	$N_3^0 = N_2^0$	N_3	P_3^0	P_3	X_3	$a_3^N = \frac{N_3 - N_3^0}{X_3}$	$a_3^P = \frac{P_3 - P_3^0}{X_3}$

changes in the biomass density of the population and factor levels in the chemostat to be reliably recorded. Our recommendation would be to vary input levels of the factors by 10–50%, depending on the sensitivity of available measurement methods.

The steps to be taken in calculating the SC values with respect to factor *N* in terms of parameters of steady states 1 and 2 are as follows:

$$\partial a^N/\partial N^0 = b^{NN} = \frac{a_2^N - a_1^N}{N_2^0 - N_1^0}, \quad (17)$$

$$\partial a^P/\partial N^0 = b^{PN} = \frac{a_2^P - a_1^P}{N_2^0 - N_1^0}; \quad (18)$$

the real SC for factor *N*:

$$\partial N/\partial N^0 = \frac{N_2 - N_1}{N_2^0 - N_1^0}; \quad (19)$$

the real biomass SC for factor *N*:

$$a^N \partial X/\partial N^0 = a_2^N \frac{X_2 - X_1}{N_2^0 - N_1^0}; \quad (20)$$

Table 6. Steady-state characteristics of a chemostat culture of *Candida utilis* (phosphate as a control factor) as reported in [16]

Steady state no.	Biomass, g/l	Input phosphorus, g/l	Phosphorus in the medium, g/l	Transformation coefficient, g/g
1	3.25	0.164	0.06	–0.032
2	3.25	0.082	0.0007	–0.025
3	3.1	0.041	0.0007	–0.013
4	2.0	0.0205	0.0007	–0.010
5	0.75	0.007	0.0007	–0.008
6	5.8	0.164	0.03	–0.023

the ideal SC for factor *N*:

$$N = \text{real}/\left(1 + X_2\left[b^{NN} - \frac{a^N}{a^P} b^{PN}\right]\right); \quad (21)$$

the ideal biomass SC with respect to factor *N*:

$$(\text{ideal SC for factor } N) - 1. \quad (22)$$

The SCs with respect to factor *P* can be calculated in a similar way in terms of experimental data for steady states 2 and 3 (*N* and *P* should be interchanged in all the above relationships and the steady-state subscripts should be changed to the corresponding numbers).

In order to determine the SC with respect to a single expected control factor (e.g., *N*), just two steady states need be analyzed and then the real values of SCs have to be calculated by expressions (17), (19), and (20). The values of ideal SCs cannot be found using (21) and (22) because the value of b^{PN} remains unknown. If the quantization relationships are satisfied by the real SCs, then *N* is the sole control factor. Otherwise, another control factor has to be identified.

Applying this method to real experimental systems described in the literature is not a simple matter due to a lack of required data and a low accuracy of measurements. Nevertheless, an attempt was made to test our approach by applying it to several suitable experimental systems, including our own.

2. The case of available data relating to only one control factor. Tables 6 and 7 contain parameter values for steady states of a chemostat culture of *Candida utilis* controlled by phosphate, along with the estimated SC values. The source data were borrowed from [16]. For operation regimes 1–5, the ideal SCs can be calculated by relationships (14) and (15), wherefrom it follows that, if the real SC for a factor or the biomass is zero, then the corresponding ideal SC will also be zero. For operation regime 5–6, the ideal SCs cannot be calculated because, in the given regime, phosphorus is not the sole control factor. The analysis of real SC values with the aid of Table 4 leads to the following conclusions about the likely control scheme in the studied

Table 7. SC values calculated in this study on the basis of data reported in [16]

Regime	$\partial a/\partial A_0$, l/g	Real factor SC	Ideal factor SC	Real biomass SC	Ideal biomass SC
1–2	–0.085	0.723	1	0	0
2–3	–0.292	0	0	–0.069	–1
3–4	–0.151	0	0	–0.614	–1
4–5	–0.111	0	0	–0.847	–1
5–6	–0.093	0.186	not determined	–0.505	not determined

Note: The SC values were calculated for two nearby steady states and correspond to some intermediate state.

Table 8. Steady-state characteristics of a chemostat culture of *Candida utilis* (phosphate and glucose as control factors) as reported in [17]

State no.	Biomass, g/l	Glucose content of input, g/l	Glucose content of medium, g/l	Transformation coefficient for glucose, g/g	Phosphate content of input, g/l	Phosphate content of medium, g/l	Transformation coefficient for phosphate, g/g
1	3.47	12	3.4	–2.48	0.054	0.0011	–0.0152
2	3.28	20	9.6	–3.17	0.054	0.0011	–0.0161
3	1.79	20	13.8	–3.46	0.027	0.0011	–0.0145
4	2.07	5	0.36	–2.24	0.027	0.0011	–0.0125

population. In operation regime 1–2, the SC values correspond to row 5 of Table 4, i.e., to schemes 2 and 4 (factor 2). Therefore, in this operation regime, phosphate is not a control factor. In regimes 2–5, the SC values correspond to row 6 of Table 4, i.e., to schemes 3, 4, 7, and 8 (factor 1). Schemes 3 and 4 imply that phosphorus is the sole kinetic and stoichiometric control factor. Schemes 7 and 8 envisage the presence of a second stoichiometric control factor. However, the strength of control exerted by phosphorus (limitation by substrate deficit) increases with decreasing phosphate content at the input. This is reflected in the increase of the absolute value of the real biomass SC from 0.069 to 0.847. This fact, however, does not tell us anything about the real SC of the factor, which equals zero in states 2–5, but shows clearly the advantage of using the biomass SC as a quantitative control criterion. The SC values in operation regime 5–6 correspond to line 7 of Table 4. Therefore, two variants are possible: (1) phosphate is both a kinetic and a stoichiometric control factor but not the sole factor, and (2) phosphate is only a stoichiometric control factor. The additional factor can be either a kinetic regulator or both a kinetic and a stoichiometric one. The paper cited lacks sufficient data to refine the control scheme and identify additional control factors. It is worth noting that, in the study analyzed, the concentrations of input factors were adjusted by amounts that were too large and it was impossible to tell the point where the control factor changed or an additional factor came into play.

3. The case of available data relating to two control factors. Tables 8 and 9 list steady-state characteristics of a chemostat culture of *Candida utilis*, controlled by phosphate and glucose along with the values of the real and ideal SCs that we calculated. The data were borrowed from [17]. The analysis of these SC values leads to the following conclusions. The quantization relationships are fulfilled in all operation regimes: the ideal SCs of the biomass and the factors add up to –1 and 1, respectively. Therefore, the kinetic control factors are fully accounted for and phosphate is the sole kinetic regulator. The SC values in the region studied follow scheme 8 (with phosphate as the first factor and glucose as the second). This conclusion is drawn on the assumption that the real values of the biomass SC with respect to glucose are indeed not zero. Therefore, kinetic and stoichiometric control by phosphate and stoichiometric control by glucose must be effective, the latter accounting for about 10% of the overall control strength.

The authors of the paper considered, which studied how the biomass responded to changes in concentrations of input factors, concluded that growth was controlled by the combined action of the limiting (phosphorus) and the inhibiting (glucose) factors. Our analysis indicates, however, that, under the given culture conditions, kinetic control is exerted by phosphate alone. In other words, the specific growth rate of yeasts is a function of phosphate content alone and *there is no inhibiting effect of glucose on the specific growth rate.* The role of glucose is constrained to stoichiometric control effected by an influence on the transformation coefficients of both factors. Such a conclusion does not immediately follow from the data reported in the cited paper, which evidences the fruitfulness of the method described.

Table 9. SC values calculated in this study from the data reported in [17]

Factor	$\partial a_1/\partial A_1^0$, l/g	$\partial a_2/\partial A_1^0$, l/g	Real factor SC	Ideal factor SC	Real biomass SC	Ideal biomass SC
			Regime 2			
Phosphate	−0.0614	10.85	0	0	−0.798	−1
Glucose	−0.0865	−0.00011	0.775	0.984 (≈1)	0.075	0.0156 (≈0)
			Regime 3			
Phosphate	−0.0614	10.85	0	0	−0.798	−1
Glucose	−0.0815	−0.00013	0.896	1.018 (≈1)	0.041	−0.0185 (≈0)

Note: The SCs for regime 2 were calculated on the basis of data for steady states 1, 2, and 3; for regime 3, the SCs were calculated using data for steady states 2, 3, and 4.

Table 10. Values of SCs for a chemostat culture of *Candida utilis* (with glucose and medium pH as control factors) calculated from our data [14]

Steady state	Glucose content of input, g/l	Input pH	Real SC with respect to glucose	Real SC with respect to pH	Ideal SC with respect to glucose	Ideal SC with respect to pH
8	1	2.38	$\frac{0.018}{-0.857}$	$\frac{1.046}{0.0}$	$\frac{0.014}{-0.986}$	$\frac{1.00}{0.00}$
10	2	2.37	$\frac{0.004}{-1.54}$	$\frac{0.642}{0.042}$	$\frac{0.003}{-0.997}$	$\frac{0.985}{-0.015}$
13	2	2.14	$\frac{0.046}{-1.21}$	$\frac{0.71}{-0.40}$	$\frac{0.032}{-0.968}$	$\frac{1.00}{0.0}$
16	2.5	2.14	$\frac{0.102}{-1.59}$	$\frac{0.65}{-0.132}$	$\frac{0.073}{-0.927}$	$\frac{0.973}{-0.027}$

Note: The numerators are factor SCs and the denominators are biomass SCs. The SC values in regime 8 were calculated from the data for steady states 6–9 [14]; in regime 10, from states 7, 10, and 11; in regime 13, from states 12–14; and in regime 16, from states 15–17.

In our previous work, experiments were conducted to determine the real SC values for a chemostat culture of *Candida utilis* yeasts controlled by glucose and the pH value of the medium. The corresponding steady-state characteristics can be found in [14]. Using the outlined method, the real and ideal SC values were calculated (Table 10) and the control scheme of the studied population was established. The quantization relationships were found to be fulfilled within the accuracy range in all chemostat regimes. The obtained SC values were in agreement with scheme 8 (with glucose as the first and pH as the second factor). This means that both kinetic and stoichiometric control were exerted by glucose and only stoichiometric control by pH. Hence, the specific growth rate of yeasts did not depend upon pH, which does not immediately follow from the experimental data.

The outlined method was applied to other published experimental evidence. In all cases studied involving no more than two control factors, the application of the method made it possible to refine the population control scheme.

A still wider application of the new population control criterion would depend on the development of theoretical foundations of the method to accommodate an arbitrary number of factors. In our view, there are also good prospects for the use of the so-called *mixed* SC factors, i.e., the SC of the steady-state level of one factor with respect to the change in the input level of another factor. Specifically, mixed SCs make it possible to derive the specific growth rate of the population as a function of control factor levels. We also hope to eventually develop a method for identifying control factors in multispecies communities of microorganisms.

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