Control analysis of microbial interactions in continuous culture: a simulation study

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Metabolic Control Analysis (MCA) has been applied to flux of substrates and products during the growth of a single species and of two interacting species in a chemostat. Single-species growth was described by classical chemostat kinetics and the two-species interaction was commensalism, the first species converting the inflowing limiting substrate to a product which provided the limiting substrate for the second species. For the single species situation, control of flux to product is shared by the dilution rate and bacterial specific growth rate, and control can be quantified in terms of two flux control coefficients C_D^J and $C_{\mu_m}^J$, representing the fractional changes in flux resulting from fractional changes in the dilution rate and maximum specific growth rate, respectively. At low dilution rates, dilution rate exerts greater control, whilst $C_{\mu_m}^J$ exceeds C_D^J at high dilution rates. In the two-species commensal interaction, additional control on flux to product is exerted by species 2 and may be quantified in a further flux control coefficient $C_{\mu_m}^J$. Control exerted by a particular species in this interaction increases as factors, e.g. maximum specific growth rate and saturation constant for growth, change to decrease its specific growth rate. Control over flux by a species is also increased by addition of an inhibitor specific to that species and a method is proposed for determining experimentally the flux control coefficient for a species which can be inhibited in this manner.

Introduction

Micro-organisms are rarely found in nature as pure cultures, usually forming complex communities of interacting species. These interactions vary in strength and effect, ranging from mutually detrimental interactions involved in competition for resources, mutually beneficial symbiotic interactions and combinations of beneficial, detrimental and neutral interactions, e.g. commensalism, amensalism, parasitism and predation. Biochemical aspects of many such interactions are well understood, particularly for two species interactions, and investigations have frequently adopted a physiological and quantitative approach, often involving a combination of theoretical and experimental models (Megee *et al.*, 1972; Bazin & Saunders, 1973; Williams, 1980; Saunders, 1983). The theoretical models usually consist of sets of

differential equations describing rates of change in the concentrations of biomass, substrates, products and compounds which inhibit or enhance growth rate or yield. They are frequently constructed to describe growth and interactions in continuous culture systems, which provide the best experimental models for studying such interactions.

Whilst this approach has proved valuable for investigating interactions between a limited number of species (2–3), the complexity of multispecies communities prevents critical testing, in particular of quantitative behaviour. Values must be provided for growth parameters of each interacting species and kinetic functions, and associated kinetic constants, must be found for effects of factors mediating interactions. The level of complexity at which a particular system becomes unmanageable depends on information available on the components of the system and the range and type of interactions. Generally, however, with more than three interacting organisms it is not possible to use such a modelling approach to test critically the quantitative aspects of the system, nor the precise mechanisms and

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Abbreviation: MCA, Metabolic Control Analysis.

kinetics of interactions. Complex multispecies models therefore frequently represent examples of over-modelling and complexity, containing subcomponents which are impossible to test. This applies equally to models describing the cycling of nutrients in natural environments. The relative importance of particular species, processes and interactions is difficult to determine and predictions regarding the effect of changes in numbers of a particular species, or in the rate of a particular process, must be treated with caution.

Metabolic Control Analysis

An alternative approach to quantifying the importance of individual groups within complex communities is the application of Metabolic Control Theory or Analysis (MCA), first proposed independently by Kacser & Burns (1973, 1979) and Heinrich & Rapoport (1974, 1983), and reviewed by Kacser (1983, 1988), Porteous (1985) and Fell (1992). MCA considers the flux of material through a metabolic system consisting of n interlinked enzymes at steady state. The effect of enzyme i on overall system flux, J , and on metabolite concentrations is defined in terms of control coefficients. The flux control coefficient, $C_{E_i}^J$, is the fractional change in flux resulting from a fractional change in the concentration, E_i , of enzyme i :

$$C_{E_i}^J = \frac{\delta J}{J} \bigg/ \frac{\delta E_i}{E_i} \quad (1)$$

The metabolite control coefficient, $C_{E_i}^{s_j}$, defines the fractional change in concentration, s_j , of metabolite j , resulting from a fractional change in E_i :

$$C_{E_i}^{s_j} = \frac{\delta s_j}{s_j} \bigg/ \frac{\delta E_i}{E_i} \quad (2)$$

Both control and metabolite coefficients may be positive or negative and fulfil two summation properties:

$$\sum_{i=1}^n C_{E_i}^J = 1 \quad (3)$$

$$\sum_{i=1}^n C_{E_i}^{s_j} = 0 \quad (4)$$

The first of these states that all flux control coefficients sum to unity. In a system involving many enzymes, control is shared among all enzymes. In a large system, therefore, most enzymes will tend to have low flux control coefficients. A single step can only be said to be 'rate-limiting' if the control coefficient for the enzyme involved has a value close to unity and consequently other control coefficients are negligible or negative. In branched systems, both positive and negative flux control coefficients exist and therefore values of $C_{E_i}^J > 1$ are

possible. The second summation property states that changes in metabolite concentrations throughout the system balance and cancel.

Control coefficients therefore represent systemic or global properties, quantifying changes in system behaviour resulting from changes in enzyme concentrations when the enzyme is embedded within the system. In this study we propose the application of MCA to the regulation and control of flux of material through microbial communities. Its use will be illustrated first with reference to growth of a single species in a chemostat, and then extended to two-species interactions. This involves consideration of microbial cells or populations of cells as equivalent to enzymes, and of external metabolite concentrations as those affecting microbial, rather than enzyme activity. The basic approach involves sensitivity analysis to determine the extent to which individual parameters control flux through the system. Whilst this approach is directly applicable to microbial growth and interactions, the assumptions on which MCA is based differ. The significance of these differences is considered in the Discussion.

Application to single-species growth in continuous culture

Classical chemostat theory (see Pirt, 1975) assumes that growth of a single species, 1, is limited by a component of the medium supplied at a concentration s_{r1} and present in the culture vessel at a concentration s_1 . Metabolism of s_1 results in formation of a product, concentration s_2 , and of biomass, concentration x_1 . This process may be modelled by three differential equations describing the rates of change of biomass, substrate and product concentrations:

$$\frac{dx_1}{dt} = \mu_1 x_1 - D x_1 \quad (5)$$

$$\frac{ds_1}{dt} = D s_{r1} - \frac{\mu_1 x_1}{Y_1} - D s_1 \quad (6)$$

$$\frac{ds_2}{dt} = k_1 \mu_1 x_1 - D s_2 \quad (7)$$

where D represents dilution rate, k_1 is the specific rate of product formation and Y_1 is the yield coefficient, representing the amount of biomass formed per unit substrate converted and assumed to be constant. Specific growth rate, μ_1 , is assumed to be limited by the concentration of a single substrate, as described by the Monod equation:

$$\mu_1 = \frac{\mu_{m1} s_1}{K_{s1} + s_1} \quad (8)$$

Table 1. Flux and metabolite control coefficients during single- and two-species growth in continuous culture

Single-species growth ($\mu_{m1} = 1 \text{ h}^{-1}$, $Y_1 \cong 0.5$, $s_{r1} = 50$, $K_{s1} = 5$ and $D = 0.5 \text{ h}^{-1}$)			
	Effect on s_1	Effect on s_2	Effect on flux to s_2 , J_2
Modulate D	2 ($C_D^{s_1}$)	-18 ($C_D^{s_2}$)	0.78 ($C_D^{J_2}$)
Modulate μ_{m1}	-2 ($C_{\mu_{m1}}^{s_1}$)	18 ($C_{\mu_{m1}}^{s_2}$)	0.22 ($C_{\mu_{m1}}^{J_2}$)

Two-species growth ($\mu_{m1} = 1 \text{ h}^{-1}$, $K_{s1} = 5$, $Y_1 = 0.5$, $\mu_{m2} = 1.25 \text{ h}^{-1}$, $K_{s2} = 5$, $Y_2 = 0.5$, $s_{r1} = 50$ and $D = 0.5 \text{ h}^{-1}$)				
	Effect on s_1	Effect on s_2	Effect on s_3	Effect on flux to s_3 , J_4
Modulate D	1.67 ($C_D^{s_1}$)	2 ($C_D^{s_2}$)	-0.37 ($C_D^{s_3}$)	0.63 ($C_D^{J_4}$)
Modulate μ_{m1}	-1.67 ($C_{\mu_{m1}}^{s_1}$)	0 ($C_{\mu_{m1}}^{s_2}$)	0.13 ($C_{\mu_{m1}}^{s_3}$)	0.13 ($C_{\mu_{m1}}^{J_4}$)
Modulate μ_{m2}	0 ($C_{\mu_{m2}}^{s_1}$)	-2 ($C_{\mu_{m2}}^{s_2}$)	0.24 ($C_{\mu_{m2}}^{s_3}$)	0.24 ($C_{\mu_{m2}}^{J_4}$)

where μ_{m1} is the maximum specific growth rate and K_{s1} the saturation constant for growth of species 1. In the steady state:

$$\bar{s}_1 = \frac{DK_{s1}}{\mu_{m1} - D} \quad (9)$$

$$\bar{s}_2 = k_1 \bar{x}_1 \quad (10)$$

$$\bar{x}_1 = Y_1(s_{r1} - \bar{s}_1) \quad (11)$$

To apply MCA, this system must be treated as a three step reaction, the first being supply of s_1 at a specific rate, D , with subsequent conversion of s_1 to s_2 and x_1 and removal of all material by outflow at a specific rate, D .

where v_i represents the velocity of each reaction. In the steady state these are $v_1 = Ds_{r1}$, $v_2 = k_1\mu_1\bar{x}_1$ and $v_3 = \mu_1\bar{x}_1$. (Subsequent analysis refers to steady state concentrations and bars above symbols for biomass and substrate concentrations have been omitted for clarity.)

We are interested in quantifying the relative importance of the two parameters, D and μ_{m1} , in controlling the various fluxes. Two flux control coefficients can therefore be defined:

$$C_D^J = \frac{\delta J}{J} \frac{D}{\delta D} \quad (12)$$

$$C_{\mu_{m1}}^J = \frac{\delta J}{J} \frac{\delta \mu_{m1}}{\mu_{m1}} \quad (13)$$

where J is the flux through the step of interest.

Metabolite control coefficients may also be defined for s_1 and s_2 :

$$C_D^{s_1} = \frac{\delta s_1}{s_1} \frac{D}{\delta D} \quad (14)$$

$$C_{\mu_{m1}}^{s_1} = \frac{\delta s_1}{s_1} \frac{\delta \mu_{m1}}{\mu_{m1}} \quad (15)$$

$$C_D^{s_2} = \frac{\delta s_2}{s_2} \frac{D}{\delta D} \quad (16)$$

$$C_{\mu_{m1}}^{s_2} = \frac{\delta s_2}{s_2} \frac{\delta \mu_{m1}}{\mu_{m1}} \quad (17)$$

The system equations put the following limits on the relative values of the control coefficients:

$$C_D^{x_1} = C_D^{s_2} = -C_D^{s_1} \frac{s_1}{s_{r1} - s_1} \quad (18)$$

$$C_{\mu_{m1}}^{x_1} = C_{\mu_{m1}}^{s_2} = -C_{\mu_{m1}}^{s_1} \frac{s_1}{\mu_{m1}s_{r1} - s_1} \quad (19)$$

$$C_D^{J_2} = 1 + C_D^{x_1} \quad (20)$$

$$C_{\mu_{m1}}^{J_2} = C_{\mu_{m1}}^{x_1} \quad (21)$$

where J_2 is the flux to s_2 . By differentiation of equation (9) and suitable scaling we obtain:

$$C_D^{s_1} = \frac{\mu_{m1}}{\mu_{m1} - D} \quad (22)$$

$$C_{\mu_{m1}}^{s_1} = -\frac{\mu_{m1}}{\mu_{m1} - D} \quad (23)$$

Hence, from the values of equations (22) and (23) we can determine the values of all the other coefficients of interest. Examples of such values are provided in Table 1 for realistic growth constants and a single dilution rate, 0.5 h^{-1} , demonstrating the summation properties de-

Fig. 1. Changes, with dilution rate, D , in values of flux control coefficients, C_D^J (—,), and $C_{\mu_{m1}}^J$ (---, — · — · —), established in steady states in a chemostat inoculated with an organism with $\mu_{m1} = 1 \text{ h}^{-1}$, $Y_1 = 0.5$, $s_{r1} = 50$ and $K_{s1} = 5$ (—, ---) or $K_{s1} = 1$ (....., — · — · —). Curves were generated using equations (18)–(23).

Fig. 2. The effect of K_{s1} values on the dilution rate at which control is shared equally by D and μ_{m1} . Growth constants as in Fig. 1.

scribed in equations (3) and (4), and evident from equations (18)–(23). The effect of dilution rate on distribution of control was investigated by calculating flux control coefficients for steady states at dilution rates from zero to the critical dilution rate, D_{crit} (Fig. 1). At low dilution rates, D exerts greatest control, and control is shared equally by D and μ_{m1} at $D = 0.81$ and 0.62 h^{-1} for the growth constants used in Fig. 1 and for values of K_{s1} of 1 and 5, respectively. A decrease in K_{s1} increases the specific growth rate at any particular substrate concentration. This has the effect of increasing the proportion of control exerted by D and shifts both curves to the right, i.e. increasing the cross-over dilution rate at which control is shared equally by D and μ_{m1} . The dilution rate at which flux control coefficients are equal is relatively insensitive to the value of μ_{m1} but varies significantly with K_{s1} (Fig. 2). At K_{s1} values which are much less than s_{r1} , increases in K_{s1} significantly decrease

the dilution rate at which control is distributed equally between D and μ_{m1} . As K_{s1} increases, however, its influence on the cross-over dilution rate decreases and dilution rate at which control is shared equally decreases asymptotically to zero as K_{s1} approaches infinity. The manner in which K_{s1} affects the cross-over also depends on s_{r1} , as can be seen from equations (18) and (19). The distribution of control is independent of the value of the yield coefficient.

Thus at low dilution rates, steady state substrate concentrations are low and supply of fresh substrate will have a bigger effect on flux than changing μ_{m1} because μ_1 will be low and dependent more on K_{s1} . At high dilution rates, specific growth rate is determined to a greater extent by μ_{m1} , increasing its control, substrate concentrations are greater and increased supply of substrate has less effect on flux.

At high values of D , C_D^J becomes negative, and $C_{\mu_{m1}}^J$ exceeds 1. This is because, in addition to supplying fresh substrate, flow through the chemostat removes biomass. At high dilution rates this negative effect on flux will predominate over the beneficial effects of increased nutrient supply. Thus, if $s_{r1} \gg K_{s1}$, D exerts greater control than μ_{m1} over most of the range of possible dilution rates. As D approaches the critical dilution rate (equivalent to μ_{m1}) the analysis breaks down due to mathematical deficiencies in the model, which is only valid for $D < \frac{\mu_{m1}}{1 + K_{s1} s_{r1}}$. At higher dilution rates, negative product concentrations are predicted.

Metabolite control coefficients for the two control parameters are equal in magnitude but of opposite sign [equations (22) and (23), Table 1]. C_D^J is always positive, as s_1 always increases with increased substrate supply, while $C_{\mu_{m1}}^J$ is always negative. At low dilution rates, coefficients for s_1 are greater than those for s_2 , reflecting the influence of substrate limitation on specific growth rate.

For a single species chemostat, therefore, the application of MCA predicts control of flux to s_2 to be quantified via the two coefficients, C_D^J and $C_{\mu_{m1}}^J$ and provides information on whether, for a particular dilution rate, increasing D or μ_{m1} will have the greater effect on increasing flux to product. It also demonstrates that an increase in K_{s1} will decrease the control exerted by D and identifies which aspects are most sensitive to changing environmental conditions and to inhibition.

Application to two-species interaction

Extension of this analysis to multispecies interactions is illustrated by a commensal interaction between two micro-organisms, 1 and 2, interacting in a chemostat. Species 2 utilizes the product of species 1 (concentration s_2) to form a product of concentration s_3 . This requires

modification of equation (7) and addition of equations for changes in biomass concentration of species 2, x_2 , and changes in s_3 :

$$\frac{dx_2}{dt} = \mu_2 x_2 - D x_2 \quad (24)$$

$$\frac{ds_2}{dt} = k_1 \mu_1 x_1 - \frac{\mu_2 x_2}{Y_2} - D s_2 \quad (25)$$

$$\frac{ds_3}{dt} = k_2 \mu_2 x_2 - D s_3 \quad (26)$$

where μ_2 is the specific growth rate of species 2, described by equation (8) with maximum specific growth rate μ_{m2} and saturation constant K_{s2} . In the steady state, substrate concentrations are given by the equations:

$$\bar{s}_1 = \frac{DK_{s1}}{\mu_{m1} - D} \quad (27)$$

$$\bar{s}_2 = \frac{DK_{s2}}{\mu_{m2} - D} \quad (28)$$

$$\bar{s}_3 = k_2 \bar{x}_2 \quad (29)$$

The reaction scheme now involves 5 steps:

where $v_4 = k_2 \mu_2 \bar{x}_2$ and $v_5 = \mu_2 \bar{x}_2$.

The control coefficients with respect to s_1 , x_1 and flux to product s_3 , J_4 , are identical to those for the single-species system, since the presence of species 2 has no effect on species 1. The additional system equations for the second organism put similar constraints on the remaining control coefficients:

$$C_D^{x_2} = C_D^{s_3} = C_D^{x_1} \frac{s_2^{\max}}{s_2^{\max} - s_2} - C_D^{s_2} \frac{s_2}{s_2^{\max} - s_2} \quad (30)$$

$$C_{\mu_{m2}}^{x_2} = C_{\mu_{m2}}^{s_3} = -C_{\mu_{m2}}^{s_2} \frac{s_2}{s_2^{\max} - s_2} \quad (31)$$

$$C_{\mu_{m1}}^{x_2} = C_{\mu_{m1}}^{s_3} = C_{\mu_{m1}}^{x_1} \frac{s_2^{\max}}{s_2^{\max} - s_2} \quad (32)$$

$$C_D^{J_4} = 1 + C_D^{x_2} \quad (33)$$

$$C_{\mu_{m1}}^{J_4} = C_{\mu_{m1}}^{x_2} \quad (34)$$

$$C_{\mu_{m2}}^{J_4} = -C_{\mu_{m2}}^{x_2} \quad (35)$$

$s_2^{\max}$ is the maximum possible value of s_2 , i.e. its concentration in the absence of species 2, and can be expressed as $k_1 x_1$. By differentiation of equation (28):

$$C_D^{s_2} = \frac{\mu_{m2}}{\mu_{m2} - D} \quad (36)$$

$$C_{\mu_{m2}}^{s_2} = -\frac{\mu_{m2}}{\mu_{m2} - D} \quad (37)$$

The relationship between control coefficients is similar to that for a single species. $C_D^{J_4}$ decreases with increasing dilution rate, whilst $C_{\mu_{m1}}^{J_4}$ and $C_{\mu_{m2}}^{J_4}$ show corresponding increases (Fig. 3a). If growth constants for both organisms are identical, $C_{\mu_{m1}}^{J_4}$ and $C_{\mu_{m2}}^{J_4}$ are also identical for the whole range of dilution rates, and the three control coefficients sum to unity (Table 1). If K_s values are identical but maximum specific growth rates differ, the value of $C_{\mu_{m1}}^{J_4}$ will be greater for the organism with lower μ_m at all dilution rates (Fig. 3b). Similarly, for equal μ_m values, the value of $C_{\mu_{m2}}^{J_4}$ will be greater for the organism with higher K_s (Fig. 3c). Each of these changes in μ_m and K_s would lead to a decrease in specific growth rate at any particular concentration of limiting substrate, and control of flux by an organism may therefore be considered to depend on the value of μ , mediated through μ_m and K_s . In a chemostat at steady state, however, μ is constant and equal to D . Consequently, the observed effects of changes in μ_m and K_s on control of flux arise through resultant changes in steady state biomass concentration.

If the organism with the higher μ_m also has the higher K_s value, curves for $C_{\mu_{m1}}^{J_4}$ and $C_{\mu_{m2}}^{J_4}$ cross over (Fig. 3d). Under these conditions, the organism exerting greater control depends on the dilution rate, although again this effect is mediated through respective values of specific growth rate. The dilution rate at which curves intersect is that at which substrate saturation curves also intersect. As with the single species system, control coefficients are independent of the relative values of yield coefficients for each organism.

Although not illustrated here, extension of the pathway to include several species results in similar relationships between control coefficients, and distribution of control among the components of the pathway is determined by respective values of specific growth rate, calculated from growth constants μ_m and K_s . If all other growth constants are equal, the organism with the highest μ_m or lowest K_s will exert least control over flux. Improvements in overall productivity are therefore best achieved through manipulation of other organisms in the pathway. The important advantage of application of flux control theory is its ability to quantify the control exerted by each species, enabling identification of those steps to which attention should be directed.

Fig. 3. Changes in values of flux control coefficients, C_D^J (—), $C_{\mu_1}^J$ (.....) and $C_{\mu_2}^J$ (---) in steady states established in a chemostat inoculated with two organisms. (a) $\mu_{m1} = 1 \text{ h}^{-1}$, $K_{s1} = 5$, $Y_1 = 0.5$, $\mu_{m2} = 1 \text{ h}^{-1}$, $K_{s2} = 5$, $Y_2 = 0.5$ and $s_{r1} = 50$; (b) as (a) except $\mu_{m1} = 1.25 \text{ h}^{-1}$; (c) as (a) but $K_{s1} = 1$; and (d) as (c) but $\mu_{m1} = 1.25 \text{ h}^{-1}$. Curves were generated using equations (18)–(23) and (30)–(37).

Fig. 4. Effects of an inhibitor of the maximum specific growth rate of species 1 on the relative flux to s_3 , J_3 (—, A) and on the maximum specific growth rate, μ_{m1} (....., B) in a chemostat inoculated with two organisms. Growth constants as in Fig. 3a, with $K_i = 0.1$. Curves were generated using equations (27), (28) and (43).

Measurement of control coefficients

If the kinetics of interactions are fully characterized, and values of growth constants are known for each interacting species, computer simulation of the interactions, similar to that described above, will be sufficient for calculation of control coefficients. In practice this is usually not possible. Kinetics may deviate from those of classical chemostat theory, growth constants may be

difficult to determine and the nature of interactions may be difficult to characterize and quantify. An alternative technique for calculation of control coefficients involves use of inhibitors specific for individual organisms within a pathway. The qualitative effect of inhibitors on the control exerted by a particular species may be understood in terms of their effects on specific growth rates of the inhibited organisms, such that any factor affecting specific growth rate of an organism in a negative sense will increase control exerted by that organism. Thus, for the two-species interaction described above, an inhibitor specific to species 1 will increase the flux control coefficient of that organism. Quantification of such effects experimentally enables determination of control coefficients.

If a steady state is established for the two-species system described above, and inflowing medium is then supplemented with an inhibitor of species 1, the steady state values for the various fluxes will change. The sensitivity of a flux to an external inhibitor is quantified as a response coefficient, R_I^J , equivalent to the fractional change in flux resulting from a fractional change in inhibitor concentration, I :

$$R_I^J = \frac{\delta J}{J} \bigg/ \frac{\delta I}{I} \quad (38)$$

It is possible to partition this response coefficient into the product of two other coefficients. If, for example, the inhibitor specifically inhibits the maximum specific growth rate of species 1, the response of flux to s_3 , J_4 , to this inhibitor may be expressed as:

$$R_{I_1}^{J_4} = C_{\mu_{m1}}^{J_4} \varepsilon_{I_1}^{\mu_{m1}} \quad (39)$$

where $\varepsilon_{I_1}^{\mu_{m1}}$ is termed an elasticity coefficient defined as:

$$\varepsilon_{I_1}^{\mu_{m1}} = \frac{\delta \mu_{m1}}{\mu_{m1}} \bigg/ \frac{\delta I}{I} \quad (40)$$

Elasticity coefficients are local coefficients, $\varepsilon_{I_1}^{\mu_{m1}}$ being a measure of the sensitivity of μ_{m1} to the inhibitor determined with all other effectors of μ_{m1} held constant to their values in the chemostat. The value of the flux control coefficient $C_{\mu_{m1}}^{J_4}$ may be determined by rearrangement of equation (39):

$$C_{\mu_{m1}}^{J_4} = (R_{I_1}^{J_4} / \varepsilon_{I_1}^{\mu_{m1}})_{\text{lim} \rightarrow 0} \quad (41)$$

This limit is taken as we are interested in the value of the control coefficient in the absence of the inhibitor. Expanding this expression using the definitions above gives:

$$C_{\mu_{m1}}^{J_4} = \frac{(\delta J / \delta I) / J^0}{(\delta \mu_{m1} / \delta I) / \mu_{m1}} \quad (42)$$

The numerator of this expression can be determined from changes in flux within the chemostat following supply of the inhibitor. The denominator can either be determined from monoculture experiments, in which the effect of the inhibitor of the maximum specific growth rate of species 1 can be measured in pure culture, or may be calculated more directly if the relationship between specific growth rate and inhibitor concentration is known. For example, if the inhibitor acts in a non-competitive manner, the effect of inhibitor may be represented by the following equation:

$$\mu_{m1}^I = \frac{\mu_{m1}^0}{(1 + I/K_i)} \quad (43)$$

where μ_{m1}^I and μ_{m1}^0 are the values of μ_{m1} at inhibitor concentrations of I and zero, respectively, and K_i is the inhibitor constant. The denominator of equation (42) then reduces to $(-1/K_i)$ and the flux control coefficient can be given by:

$$C_{\mu_{m1}}^{J_4} = \frac{\delta J K_i}{\delta I J} \quad (44)$$

Thus if K_i is known, $C_{\mu_{m1}}^{J_4}$ may be calculated solely from chemostat experiments.

Fig. 4 provides an illustration of this method of calculating flux control coefficients experimentally for the two-species interaction analysed above with a specific inhibitor of the maximum specific growth rate of species

1. Curve A illustrates the response of the flux to s_3 , J_4 , due to increasing amounts of inhibitor in the inflowing medium. Curve B illustrates the plot of μ_{m1} against inhibitor concentration which could be obtained from batch culture experiments. The value of the flux control coefficient $C_{\mu_{m1}}^{J_4}$ may be determined from the ratio of the initial slope of curve A and the initial slope of curve B. If the mechanism shown in equation (43) is known to be valid, and the value of K_i is also known, then the value of $C_{\mu_{m1}}^{J_4}$ can be estimated directly using equation (44), i.e. from the chemostat inhibitor experiment alone, where the effect of different concentrations in the inflowing medium on flux to s_3 would be determined. Experimental measurement of flux control coefficients by this method is therefore facilitated by use of a non-competitive inhibitor, whose effects on μ_m can readily be determined in batch growth experiments at different inhibitor concentrations. Calculation of control coefficients would also be possible using a competitive inhibitor, which affected K_s values. Experimental determination of effects of the inhibitor would, however, be necessary at concentrations established at the steady states, and submaximal specific growth rates, which would be more difficult experimentally.

Discussion

Previous applications of MCA have considered flux through metabolic pathways catalysed by enzymes at fixed levels, or at constant relative concentrations in exponentially growing batch systems. This study is the first to apply MCA to microbial interactions, treating micro-organisms as equivalent to enzymes and their substrate and product concentrations equivalent to enzyme metabolites. In both cases, the same basic approach is adopted, using sensitivity analysis to identify the degree to which different parameters control flux through the system. There are, however, differences with regard to the assumptions on which the analyses are based. In particular, MCA normally assumes enzyme concentrations to be fixed, in the absence of induction or repression, and independent of the reactions which they catalyse (however, see Fell, 1992). The amount of biomass of a particular species will depend on the amount of substrate which that species has converted, and will consequently depend on dilution rate, inflowing substrate concentration and the growth constants of the organism. Steady state biomass concentration must therefore be considered a variable, rather than a parameter. The analysis described here considers only flux to metabolic products, and not biomass, and differences in basic assumptions regarding biomass concentrations raise no problems. A broader application of MCA will, however, require more detailed con-

sideration of feedback effects, equivalent to those operating in enzyme induction and repression. It has also been assumed that k_1 and k_2 , and the yield coefficients, Y_1 and Y_2 , as used in equations (9)–(11) and (27)–(29) are true constants, in that their values are not affected significantly by changes in D or $\mu_{\max}$. Again, a more detailed consideration is required if these assumptions are relaxed.

This study is also the first to consider control of flux during microbial growth and interactions in continuous culture. Flux control coefficients are defined for the specific rate of supply of fresh nutrient, and removal of products, and for the specific growth rate of each organism. Although illustrated for relatively simple interactions, the general principles hold for more complex interactions. Thus, the distribution of control varies with dilution rate, being dominated by C_D' at low dilution rates and switching to factors determining maximum specific growth rate at higher dilution rates. Factors which reduce specific growth rate of an organism increase the flux control coefficient associated with that organism.

The application of MCA has been illustrated using a mathematical model of a chemostat, based on traditional chemostat kinetic equations. Where a system can be accurately modelled in this way, and all relevant growth constants measured experimentally, flux control coefficients can be calculated by simulation of modulations, as described here. The major advantage of MCA in such circumstances is in quantifying the distribution of control among different organisms within a community. This enables identification of steps in a process which are most sensitive in terms of their effect on overall flux through the system. To achieve this by traditional simulation of chemostat equations would require considerably greater effort and would not represent sensitivity quantitatively. Most interactions involving several species will not be accurately described by traditional equations, and kinetic constants will be difficult to determine experimentally. MCA analysis does not rely on knowledge of such underlying kinetics and experimental determination of control coefficients, e.g. by measuring substrate and product concentrations following application of specific inhibitors, provides quantitative information on distribution of control not available using traditional techniques.

Two areas of application of this work are envisaged. The first is in analysis of microbially mediated nutrient cycling processes in natural environments. In situations where biomass concentrations are constant, with respect to the time scale over which studies are carried out, direct application of MCA of enzyme-catalysed metabolic pathways is possible. In growing systems, extension of the chemostat analysis described here is more applicable,

taking into account microbial growth and supply and removal of substrates and products. Traditional models of such interactions in natural environments are usually of a complexity which prevents critical and rigorous testing because of uncertainties in values of kinetic constants, and difficulties in their experimental determination. It is therefore difficult to have confidence in their ability to predict the effects of perturbations in environmental conditions and of pollution on flux through the nutrient cycles. MCA does not require detailed information on mechanisms of interactions, and experimental measurement of flux control coefficients will enable prediction of which steps in the process are most sensitive to perturbations and which can be affected without altering overall flux. In addition, quantitative analysis of the effects of specific inhibitors on the distribution of the control of flux within interacting systems, whilst providing a means of determination of flux control coefficients experimentally, also enables prediction of quantitative effects of inhibition of individual steps on flux, i.e. which steps are most sensitive to disturbance. This information is essential both for environmental impact studies and for manipulation of nutrient cycle processes.

The second application is in mixed culture fermentations, where the aim may be to improve productivity. Again, such interactions may be too complex for detailed mechanistic modelling but determining of flux control coefficients will provide information on which aspects of the system require modification to enable significant improvement in product formation. A simplistic example is illustrated with reference to Fig. 1. If a chemostat is operated at a dilution rate of 0.1, increasing the maximum specific growth rate, e.g. by increasing temperature or selection for faster growing mutants, will have relatively little effect on product formation, as flux control is dominated by the rate of substrate supply. At higher dilution rates, however, such modifications would lead to significant improvements in productivity. The benefits of traditional MCA for optimization of biotechnological processes have been discussed by Kell & Westerhof *et al.* (1986).

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