ELSEVIER

Biophysical Chemistry 73 (1998) 179–188

Biophysical Chemistry

The Synthesizing Unit as model for the stoichiometric fusion and branching of metabolic fluxes

S.A.L.M. Kooijman*

Department of Theoretical Biology, Vrije Universiteit, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Received 22 January 1998; received in revised form 30 March 1998; accepted 30 April 1998

Abstract

The Synthesizing Unit (SU) binds given numbers of substrate molecules of several types of substrate to produce a product molecule or set of product molecules. Irreversible binding results in relatively simple and explicit expressions for the rate of product formation. Reversible binding can be implemented with relative ease in the carrier-SU complex, where the products of a set of carriers (a special type of SU) serve as substrate for an SU or set of SUs. A simple and parameter sparse approximation is presented for the production rate of a generalized compound, i.e. a rich mixture of compounds that does not change in composition. An analysis of Droop's data on the growth of a haptophyte on phosphate and vitamin B_{12} reserves illustrates the application of SUs.

Keywords: Multi type Poisson processes; Erlangian distribution; Enzyme kinetics; Metabolic control analysis; Multiple nutrient limitation of algal growth

1. Introduction

The modeling of metabolic networks frequently involves the fusion and branching of mass fluxes, such that the stoichiometric requirements for the formation of chemicals are observed. (See [14] for a lucid introduction to the subject.) A popular way to achieve this is to identify the limiting substrate flux for the formation of the product(s), and let this substrate flux fully specify the product flux. Especially in situations where the substrate fluxes change in time, this specification of the product flux can become rather cumbersome, and not fully realistic in view of the stochastic nature of processes at the molecular level. Models with switches are almost always difficult to analyze [18]. If two substrate fluxes are about equally restrictive for the product formation, stochastic fluctuations will ensure that both substrate fluxes will restrict product formation simultaneously, which means that the minimum model cannot apply in detail, as will be explained.

The aim of this paper is to present a mechanis-

* E-mail: bas@bio.vu.nl

0301-4622/98/$19.00
PII S0301-4622(98)00162-8

tically inspired solution to this problem in the form of the Synthesizing Unit, which can be considered as a straightforward but simple generalization of the classical enzyme concept. More specifically, a quantification will be given for the rate at which a complex enzyme-mediated chemical transformation proceeds, that is consistent with standard Michaelis Menten kinetics. This rate is, generally, a vector-valued function of the concentrations of substrates and products, rather than a scalar one, where the rates at which each substrate disappears, or product appears, are linked via mass and energy conservation laws and subjected to stoichiometric constraints. The latter constraints make the job far from easy, because it introduces complex forms of mutual dependencies. Substrate molecules can only participate in the transformation if they arrive at the Synthesizing Unit, in one way or another. Simple diffusion and/or convection models imply that the arrival rate is proportional to the concentration. This paper will, however, not focus on transport models describing the relationship between concentrations and arrival rates. On the assumption that the transport of product molecules away from the Synthesizing Unit is fast enough to neglect interference of the products with the transformation, the task to quantify the transformation reduces to describe how it depends on the arrival rates of substrate molecules. The substrate concentrations and, therefore, the arrival rates, may vary in time.

2. The Synthesizing Unit

2.1. The one substrate-one copy SU: 1-SU

In its simplest form, the Synthesizing Unit (SU) is an enzyme or a complex of enzymes that binds a substrate molecule to deliver a product molecule or a set of product molecules. For simplicity's sake, I will assume that the substrate molecules arrive according to a Poisson process, that the binding occurs with a fixed probability p if the SU is in its binding stage, and that the production stage lasts an exponentially distributed time interval. During the production process, no substrate molecules are accepted by the SU, so the binding probability $\underline{p}$ for each arriving substrate molecule follows a renewal process [5], alternating between the values p and 0, when the SU is binding and producing, respectively. I will call this SU a one substrate-one copy SU, and briefly discuss its properties to introduce the more interesting multi substrate-multi copy SU.

Let $\phi_{\underline{t}_Y}(t) = \dot{J}_m \exp\{-\dot{J}_m t\}$ denote the probability density function (pdf) of the production period, and $\phi_{\underline{t}_1}(t) = \dot{J}_X \exp\{-\dot{J}_X t\}$ the pdf of the binding period of substrate molecules arriving at rate $\dot{J}_X^* = \dot{J}_X / p$ where p denotes the binding probability per arriving substrate molecule. The cycle period of the SU, $\underline{t}_0$, concatenates one binding period and the subsequent production period. The inverse of its expected value, $J_Y = 1/\mathscr{E}\underline{t}_0$, equals the mean production rate, which I will call the intensity, defined as the ratio of the cumulative number of events in a period and the length of the period, for a large period.

When substrate molecules are sent to a one substrate-one copy SU, according to a Poisson process with intensity $\dot{J}_X^*$, it returns a Poisson process of rejected substrate molecules, with an intensity that alternates between values $(1-p)\dot{J}_X^*$ and $\dot{J}_X^*$, and a renewal process of product molecules, with intensity $\dot{J}_Y = (\dot{J}_m^{-1} + \dot{J}_X^{-1})^{-1}$. The mean intensity of the rejected substrate molecules amounts to $\dot{J}_X^* - \dot{J}_Y$. Note that for very high intensities of the arrival process, the production process satiates to the value $\dot{J}_Y = \dot{J}_m$.

The processes of rejected substrate molecules and produced molecules are mutually dependent, but I will not work out the structure in detail, because the practical interest is not in the performance of a single SU, but in a large set of independently operating SUs. The central limit theorem for the addition of independent stochastic point processes implies that the rejected substrate molecules and the product molecules of a sufficiently large set of s independent SUs converge to independent Poisson processes with constant intensities $\dot{J}_X^* - \dot{J}_Y$ and $\dot{J}_Y = ((s\dot{J}_m)^{-1} + \dot{J}_X^{-1})^{-1}$, respectively. An increase in the amount of SUs has the effect of decreasing the production period; the reduction of the intensity of arriving substrate molecules per SU cancels against the increase of the binding probability. Other implementations of the step to group performance are

conceivable, but require details of the spatial organization of the SUs.

2.2. The multi substrate-multi copy SU: $\{n_i\}_1^n$-SU

We can generalize the one substrate-one copy SU, to more copies by requiring that the moment at which the production stage of the SU is entered, $\underline{t}_1$, equals the moment of the nth binding, $\underline{t}_{X_n}$, so $\underline{t}_1 = \underline{t}_{X_n}$. Such an SU can be called a one substrate-multi copy SU, or n-SU. The binding period follows the Erlangian distribution $\phi_{\underline{t}_1}(t) = \frac{\dot{J}_X(\dot{J}_X t)^{n-1}}{(n-1)!}\exp\{-\dot{J}_X t\}$ which has a mean value of $\mathscr{E}\underline{t}_1 = n\dot{J}_X^{-1}$. It results from adding n independently exponentially distributed random variables with parameter $\dot{J}_X$. (The change in intensities of the arrival process during one binding period must be negligibly small, but this is usually the case in practice.) The production process is a renewal process with intensity $\dot{J}_Y = (\dot{J}_m^{-1} + n\dot{J}_X^{-1})^{-1}$. A large set of s SUs will produce a Poisson stream of product molecules with intensity $\dot{J}_Y = ((s\dot{J}_m)^{-1} + n\dot{J}_X^{-1})^{-1}$, and a Poisson stream of rejected substrate molecules of intensity $\dot{J}_X^* - n\dot{J}_Y$.

The model does not specify the details of the production process. The SU might have n different binding sites, or just a single one in combination with a fast process of precursor production, while the precursor molecules remain in the local environment of the SU that is under its control.

Now we are ready for the more interesting multi substrate-multi copy SU, which requires n different substrate types for the production of a single molecule, or set of molecules, Y: the n_1, $n_2, \cdots, n_n$-SU. The kinetics of the production process is based on the idea that the SU can only enter the production stage if all required substrate molecules are bound. I will discuss two different extensions to multi substrates: sequential and parallel binding.

2.2.1. Sequential binding

When the SU binds n different types of substrate sequentially, in a random order, the expected waiting time to the binding of n_i molecules of type i is $n_i/\dot{J}_{X_i}$. The order of the types is not relevant, but when the SU is binding type i, it continues to do so till all required molecules for the production of one product molecule are bound. This directly leads to the expected binding period, by simply adding the binding periods for the different types

$$\mathscr{E}\underline{t}_1 = \sum_{i=1}^{n} n_i/\dot{J}_{X_i} \tag{1}$$

The mean production rate becomes $\dot{J}_Y = (\dot{J}_m^{-1} + \sum_i n_i/\dot{J}_{X_i})^{-1}$.

The interest in this mechanism is mainly in its mathematical simplicity, and its interesting properties (Martin Boer, personal communication). The parallel binding period will turn out to equal the sequential binding period minus the gain in time (compare (1) and (5)). Suppose that the substrate fluxes are proportional to the substrate concentrations X_i, as a result of some convection or diffusion process. The production rate can then be rewritten as $\dot{J}_Y = \dot{J}_m(1 + \sum_i X_{Ki}/X_i)^{-1} = \dot{J}_m f_n$, where X_{Ki} denotes the saturation constant, which quantifies the affinity of the SU for substrate i, including the transport rate from the (local) environment to the SU, and the factor f_n is the scaled functional response for n types of possibly limiting substrates, which takes values between 0 and 1. (The term 'functional response' originates from ecology, and stands for the feeding rate of a predator as function of the concentration of prey.) The recurrent relationship $f_n = \frac{X_n f_{n-1}}{X_n + X_{Kn} f_{n-1}}$ applies, for $f_0 = 1$ and $n = 1, 2, \cdots$, which leads to $f_n = \prod_i X_i(\prod_i X_i + \sum_i X_{Ki}\prod_{j\neq i} X_j)^{-1}$.

2.2.2. Parallel binding

Suppose that the binding of substrate of one type does not interfere with the binding of substrate of another type. The SU will not bind substrate i molecules, if it already bound n_i molecules of that substrate, but still has to bind other types of substrate, or if the SU is in the production stage. Fig. 1 illustrates the behaviour of a 1,1-SU. Let $\underline{t}_{X_i}$ denote the moment of the binding of the n_ith molecule of substrate type i, and $\underline{t}_1 =$

$\max_i\{\underline{t}_{X_i}\}$ the moment when all required substrate molecules are bound, and the production stage is entered. The distribution function of binding period $\underline{t}_1$ relates to that of the $\underline{t}_{X_i}$ as

$$\Phi_{\underline{t}_1}(t) = \prod_{i=1}^{n} \Phi_{\underline{t}_{X_i}}(t) = \prod_{i=1}^{n} \int_0^t \phi_{\underline{t}_{X_i}}(t_1)\mathrm{d}t_1 = \prod_{i=1}^{n} P\left(n_i, t\dot{J}_{X_i}\right) \tag{2}$$

where $P(n,t) = \frac{1}{\Gamma(n)} \int_0^t \exp\{-t_1\} t_1^{n-1} \mathrm{d}t_1 = 1 - \exp\{-t\} \sum_{j=0}^{n-1} \frac{n^j}{j!}$ is the incomplete gamma function. The expected value of the binding period is

$$\mathscr{E}\underline{t}_1 = \int_0^\infty \left(1 - \phi_{\underline{t}_1}(t)\right)\mathrm{d}t = \int_0^\infty \left(1 - \prod_{i=1}^{n} P\left(n_i, t\dot{J}_{X_i}\right)\right)\mathrm{d}t \tag{3}$$

and the expected value of the cycle period is $\mathscr{E}\underline{t}_0 = \dot{J}_m^{-1} + \mathscr{E}\underline{t}_1$. The mean production rate, therefore, occurs at intensity $\dot{J}_Y = (\dot{J}_m^{-1} + \mathscr{E}\underline{t}_1)^{-1}$ for a single SU, and $\dot{J}_Y = ((s\dot{J}_m)^{-1} + \mathscr{E}\underline{t}_1)^{-1}$ for a set of s SUs. The intensity of the rejected substrate molecules of type i amounts to $\dot{J}^*_{X_i} - n_i \dot{J}_Y$, where $\dot{J}^*_{X_i} = \dot{J}_{X_i}/p_i$ denotes the intensity of the arrival process of molecules of substrate i, which

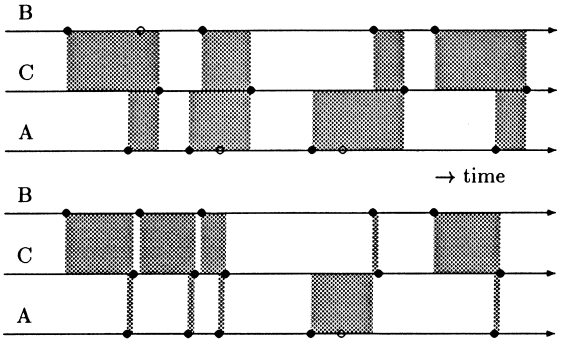

Fig. 1. These pictures illustrate the production by an efficient relatively slow (upper) and a very fast (lower) 1,1-SU. The arrival events of substrate molecules A and B, and the production events of product molecules C are indicated with filled and open dots on three time-axes. Filled dots stand for acceptance, open ones for rejection. The grey areas indicate periods during which the SU is blocked for the two substrates. Note that the fast SU still has substantial blocked periods.

are bound with probability p_i if the SU is in the binding stage.

Using the mentioned series expansion for the incomplete gamma function, and integrating (3) analytically, we arrive for two possibly limiting nutrients ($n = 2$) at

$$\mathscr{E}\underline{t}_1 = \frac{n_1}{\dot{J}_{X_1}} + \frac{n_2}{\dot{J}_{X_2}} - \sum_{i=0}^{n_1-1} \sum_{j=0}^{n_2-1} \frac{(i+j)!}{i!j!} \times \frac{\dot{J}_{X_1}^i \dot{J}_{X_2}^j}{\left(\dot{J}_{X_1} + \dot{J}_{X_2}\right)^{i+j+1}} \tag{4}$$

and for three possibly limiting nutrients at

$$\mathscr{E}\underline{t}_1 = \sum_{i=1}^{3} \frac{n_i}{\dot{J}_{X_i}} - \sum_{i_2 > i_1 = 1}^{3} \sum_{i=0}^{n_{i_1}-1} \sum_{j=0}^{n_{i_2}-1} \times \frac{(i+j)!}{i!j!} \frac{\dot{J}_{X_1}^i \dot{J}_{X_2}^j}{\left(\dot{J}_{X_{i1}} + \dot{J}_{X_{i2}}\right)^{i+j+1}} + \sum_{i_3 > i_2 > i_1 = 1}^{3} \sum_{i=0}^{n_{i_1}-1} \sum_{i=0}^{n_{i_2}-1} \sum_{k=0}^{n_{i_3}-1} \frac{(i+j+k)!}{i!j!k!} \times \frac{\dot{J}_{X_{i1}}^i \dot{J}_{X_{i2}}^j \dot{J}_{X_{i3}}^k}{\left(\dot{J}_{X_{i1}} + \dot{J}_{X_{i2}} + \dot{J}_{X_{i3}}\right)^{i+j+k+1}} \tag{5}$$

from which it is obvious how this expression generalizes for a larger number of possibly limiting substrates. There is no need to evaluate the integral in (3), when it comes to practical computations. Note that the first summation in the last (i.e. third) summation term only contains one element. The first summation in the middle summation term contains three elements.

Fig. 2 illustrates that the 1,1-SU behaves close to a minimum operator for small substrate supply fluxes. This can be quantified using the metabolic control analysis [7], which shows that the flux control coefficients

$$\frac{\partial \ln \dot{J}_Y}{\partial \dot{J}_{X_i}}$$

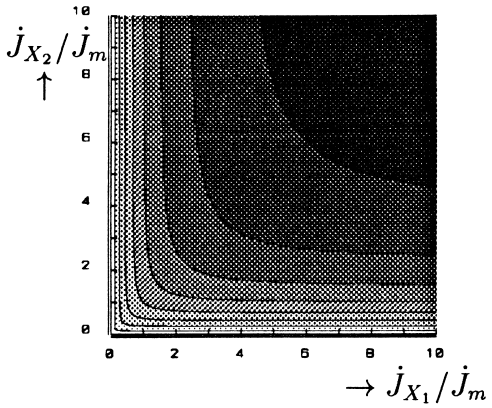

Fig. 2. The 0.1(0.1)0.7 contours of the scaled production flux $\dot{J}_Y/\dot{J}_m$ as functions of the substrate supply fluxes $\dot{J}^*_{X_1}$ and $\dot{J}^*_{X_2}$ for a 1,1-SU. The production flux for a 1,1-SU simplifies to $\dot{J}_Y = (\dot{J}_m^{-1} + \dot{J}_{X_1}^{-1} + \dot{J}_{X_2}^{-1} - (\dot{J}_{X_1} + \dot{J}_{X_2})^{-1})^{-1}$.

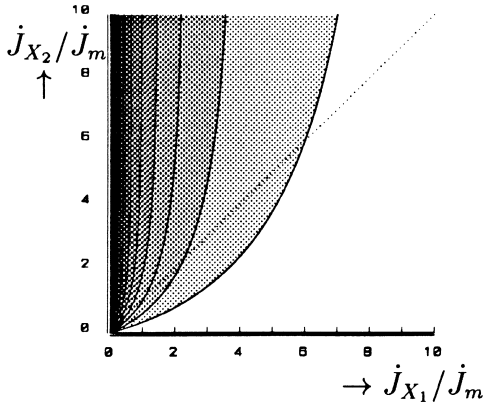

Fig. 3. The 0.1(0.1)0.9 contours (right to left) of the flux control coefficients d ln $\dot{J}_Y$ (d ln $\dot{J}^*_{X_1})^{-1}$ of the substrate flux $\dot{J}^*_{X_1}$ on the production flux $\dot{J}_Y$ for a 1,1-SU. The flux control coefficients for substrate $\dot{J}_{X_2}$ can be obtained by interchanging the labels on the axes. The strippled line marks $\dot{J}_{X_1} = \dot{J}_{X_2}$.

rapidly decrease for increasing substrate concentrations, see Fig. 3. The elasticity coefficients, which quantify the effect of a change in the SU concentration on the production flux, are

$$\frac{\partial \ln \dot{J}_Y}{\partial \ln s} = \frac{\dot{J}_Y}{s\dot{J}_m}$$

When a 1,1-SU would bind sequentially, the production rate is $\dot{J}_Y = (\dot{J}_m^{-1} + \dot{J}_{X_1}^{-1} + \dot{J}_{X_2}^{-1})^{-1}$, which is obviously lower than using parallel binding.

An SU can be efficient (binding probabilities close to 1), and fast ($\dot{J}_m$ very large), but it still rejects substrate molecules, even if they arrive in the proper relative frequencies for synthesis. This is due to stochastic fluctuations, as illustrated in Fig. 1 for a 1,1-SU. Although A and B arrive in the same mean intensity, while only one molecule of each is necessary to produce a molecule C, the 1,1-SU is blocked for each substrate during about one third of the time. This holds for low and high intensities. If A would be more abundant than B, the latter would seldom be rejected.

The supply fluxes of substrates to the SU can result from convection or diffusion processes, which makes it likely that they are proportional to the concentration X_i of substrate in the local environment of the SU and the number of SUs. The 1-SU then behaves quantitatively according to the familiar Michaelis Menten kinetics [8,11]. Most texts on these kinetics [15,16] assume a reversible binding to the enzyme, however. For the 1-SU such an extension hardly complicates the model. The 1,1-SU requires nine binding and dissociation rates to quantify the production process [1,10], but reversible binding becomes really complex for the multi substrate-multi copy enzymes. It requires the specification of the kinetics of all possible combinations of partially filled enzyme-substrate complexes [13], which is not only cumbersome, but also involves a huge amount of parameters. The Carrier-Synthesizing Unit complex, which is discussed in the next section, allows reversible binding with relative ease.

3. The Carrier-Synthesizing Unit complex

3.1. The one product CSU complex: $s\{c_i\}_1^n$-CSU

An interesting application of the SU model is in combination with carriers, see e.g. [17]. A carrier is taken to be 1-SU, with a specialized function: it receives substrate molecules from outside the cell (or organelle), and delivers products to a set of s SUs inside the cell. Moreover, the subs-

trate flux to the carriers is taken to be proportional to the concentration of the substrate in the environment, X_i. I first discuss the situation where we have one type of $\{n_i\}_1^n$-SUs which are served by c_i carriers for substrate type i, $i = 1, 2, \cdots, n$. The CSU complex produces a single product or set of products. The product of the carriers might be identical to the substrate, in which case the function of the carrier is just to import substrate from the environment into the cell. It is of little concern here, because the substrates of the carriers will be related directly to the product(s) of the SUs, leaving precursors in the black box of the CSU complex. I will assume that the substrate-carrier association/dissociation is in pseudo-steady state, i.e. it is fast compared to changes of substrate concentrations in the environment.

In the description of the fluxes, I will again take the macroscopic view of mass fluxes to and from the pool of c_i carriers of type i and to and from the pool of s SUs. This is because the total number of carriers and SUs is assumed to be large, fixed and unknown in many practical applications, while a description at the molecular level is complex because of the dependence structure of the production and rejected substrate fluxes, and the detailed spatial organization. A subtle difference with the previous section is that the flux to the s SUs was treated there as given, so independent of the number of SUs, while the flux to the SUs is here proportional to the number of SUs. The concentration in the environment is now taken to be constant, rather than the flux to the CSU complex, which now depends on the number of carriers and SUs and their properties.

The binding rate of substrate molecules to the carriers is proportional to the fraction θ_i of carriers of type i in the binding stage, see Fig. 4. The substrate flux to the carriers in the binding stage can be written as $p_i X_i c_i \theta_i$, for some constant specific binding probability rate $\dot{p}_i$. Reversible binding to the carriers hardly makes the problem more complex, and I assume that the carriers lose bound substrate molecules at a rate $\dot{J}_{Z_i} c_i (1 - \theta_i)$ (i.e. they return the substrate unchanged to the environment), and deliver their products to the s SUs at rate $\dot{J}_{X_i} c_i (1 - \theta_i)$. The products of the carriers that are not bound as substrates by the

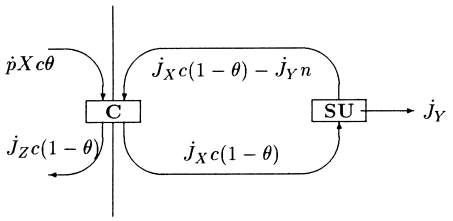

Fig. 4. The *sc*-CSU complex binds one type of substrate in the environment reversibly and delivers one product at rate $\dot{J}_Y$ to the cellular metabolism. The exchange between the carrier and the SU is indicated. The fraction θ of carriers in the binding stage follows from the assumption of steady rate.

SUs are returned to the free carriers, which occurs at rate $\dot{J}_{X_i} c_i (1 - \theta_i) - \dot{J}_Y n_i$. If the CSU complex is in steady state, the fraction of carriers in the binding stage does not change, i.e.

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_i = \dot{J}_Y n_i / c_i + (1 - \theta_i)\dot{J}_{Z_i} - \theta_i \dot{p}_i X_i = 0 \qquad (6)$$

from which follows that the fraction of carriers of type i in the binding stage equals

$$\theta_i = \frac{\dot{J}_{Z_i} + \dot{J}_Y n_i / c_i}{\dot{J}_{Z_i} + \dot{p}_i X_i} \qquad (7)$$

The flux to the SUs, therefore, amounts to

$$\dot{J}_{X_i} \frac{c_i \dot{p}_i X_i - \dot{J}_Y n_i}{\dot{J}_{Z_i} + \dot{p}_i X_i},$$

which must be substituted for $\dot{J}_{X_i}$ into (3) to yield (implicitly) the production flux $\dot{J}_Y$, as function of the substrate concentration X_i. The flux of substrate molecules taken up by the CSU equals $n_i \dot{J}_Y$.

For a single type of substrate and a large value for $s\dot{J}_m$, the product flux simplifies to

$$\dot{J}_Y = \frac{c\dot{J}_X \dot{p} X}{\dot{J}_Z + n\dot{J}_X + \dot{p}X}$$

which is hyperbolic in the substrate concentration X, and, therefore, follows the well-known Michaelis Menten kinetics. If the production period for the set of s SUs is not negligibly small, the quadratic equation in $\dot{J}_Y$ results, and the

CSU complex deviates from the Michaelis Menten kinetics.

3.2. The multi product CSU complex: $\{s_i\}_1^{n_s}\{c_i\}_1^n$-CSU

The one product CSU can be generalized to the more realistic multiple product CSU, where we have not one, but more types of $\{n_i\}_1^n$-SUs, which compete for the same set of carriers $\{c_i\}_1^n$. Each type of SU has its own stoichiometric requirements, which means that their products cannot be pooled. Fluctuations in the substrate concentrations X_i can result in shifts in the relative rates of product formation.

The total flux from the carriers of type i to all types j of SUs amounts to $\sum_{j=1}^{n_s} \dot{J}_{X_{ij}} c_i(1-\theta_i)$ and the total flux of rejected substrate molecules to the carriers is $\sum_{j=1}^{n_s} \dot{J}_{X_{ij}} c_i(1-\theta_i) - \dot{J}_{Yj} n_{ij}$, where $\dot{J}_{X_{ij}}$ denotes the specific affinity of substrate type i for SU type j, and $\dot{J}_{Y_j}$ denotes the flux of product type j. The change in the fraction of carriers of type i in the binding stage is

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_i = \left(\sum_{j=1}^{n_s} \dot{J}_{Y_j} n_{ij}/c_i\right) + (1-\theta_i)\dot{J}_{Z_i} - \theta_i \dot{p}_{X_i} X_i \tag{8}$$

which leads to the fraction in steady state

$$\theta_i = \frac{\dot{J}_{Z_i} + \sum_j \dot{J}_{Y_j} n_{ij}/c_i}{\dot{J}_{Z_i} + \dot{p}_i X_i} \tag{9}$$

The flux to the SUs of type j, therefore, amounts to

$$\dot{J}_{X_i} \frac{c_i \dot{p}_i X_i - \sum_j \dot{J}_{Y_j} n_{ij}}{\dot{J}_{Z_i} + \dot{p}_i X_i},$$

which must be substituted for $\dot{J}_{X_i}$ into (3) to yield the production flux $\dot{J}_{Y_j}$ of type j, as function of the substrate concentration X_i. The flux of substrate molecule taken up by the CSU equals $\sum_j n_{ij} \dot{J}_{Y_j}$.

4. The production of generalized compounds

As might be expected, an increase in substrate concentration almost cancels against an increase in stoichiometric requirements, so $\dot{J}_Y$ is rather insensitive for multiplication of both $\dot{J}_{X_i}$ and n_i with an arbitrary factor. This allows the use of SUs to quantify the production of generalized compounds, i.e. rich mixtures of compounds in fixed mixing ratios, such as biomass that is under homeostatic control. The chemical coefficients for the various elements of such generalized compounds are usually expressed relative to carbon and assumed to be constant. The product flux of a $\{n_i\}_1^n$-SU approximates that of a 1, 1, $\cdots$, 1-SU, when we replace $\dot{J}_{X_i}$ by $\dot{J}_{X_i}/n_i$, resulting in

$$\dot{J}_Y = \left(\dot{J}_m^{-1} + \sum_{i_1=1}^{n}\left(\frac{\dot{J}_{X_{i1}}}{n_{i_1}}\right)^{-1} - \sum_{i_2>i_1=1}^{n}\left(\sum_{j=1}^{2}\frac{\dot{J}_{X_{ij}}}{n_{i_j}}\right)^{-1} + \sum_{i_3>i_2>i_1=1}^{n}\left(\sum_{j=1}^{3}\frac{\dot{J}_{X_{ij}}}{n_{i_j}}\right)^{-1} - \cdots - (-1)^n \sum_{i_n>\cdots>i_1=1}^{n}\left(\sum_{j=1}^{n}\frac{J_{X_{ij}}}{n_{i_j}}\right)^{-1}\right)^{-1} \tag{10}$$

As is obvious from the derivation, the constraints $n_i \dot{J}_Y < \dot{J}_{X_i}$ apply for all $i = 1, 2, \cdots, n$. If $\dot{J}_Y(\dot{J}_{X_i}, \dot{J}_{X_2}|n_1,n_2)$ denotes the production rate of a n_1,n_2-SU with substrate arrival rates $\dot{J}_{X_1}$ and $\dot{J}_{X_2}$, we have $\dot{J}_Y(\dot{J}_{X_1},\dot{J}_{X_2}|n_1,n_2) > \dot{J}_Y(\dot{J}_{X_1}/n_1,\dot{J}_{X_2}/n_2|1,1)$ for $n_i > 1$. The error is typically less than 10%.

5. Application

A useful application of SUs is e.g. in modeling algal growth that is subjected to simultaneous nutrient limitations. I will illustrate this by extending the Dynamic Energy Budget model [9] for the uptake and use of substrates by organisms to more than one substrate and more than one reserve in a simple situation of a dividing unicellular organism with negligible maintenance costs for the substrates of interest. Let two types of substrate be taken up by the cell via simple carri-

ers (so the uptake rate is a hyperbolic function of the substrate concentration). The products are added separately to two internal reserves (pools of metabolites, which are usually stored in polymer form). These reserves are mobilized at rates proportional to the reserves density, i.e. the ratio of the reserves and the structural mass of the cell, and the two fluxes of mobilized reserves are used by a n_1,n_2-SU to synthesize structural cell mass. (This reserve dynamics can be derived using homeostasis and partitioning arguments, but the derivation is not presented here because it is outside the scope of this paper. I use the term 'density' rather than 'concentration', because mixing is not required at the molecular level.) The structural mass, and reserves, will contain material derived from other nutrients, but the behaviour of SUs shows that this will not affect growth, as long as these nutrients are abundant enough.

Droop [6] presented data on the cellular contents of labelled P and Co, as tracers of phosphate and vitamin B_{12}, in the haptophyte *Pavlova* in a chemostat at steady state. By using various levels of these nutrients in the feed, and different throughput rates, he managed to obtain widely different internal reserves and growth combinations, which I will use to test the realism of the SU for growth.

Let $m_{Ei} = M_{Ei}/M_V$ denote the reserve density, i.e. the ratio of the reserve mass M_{Ei} and the structural mass M_V, where $i = 1,2$. The flux that is mobilized from the reserve, the catabolic flux, equals

$$\dot{J}_{Ci} = M_{Ei}\left(\dot{k}_{Ei} - \frac{\mathrm{d}}{\mathrm{d}t}\ln M_V\right) = M_{Ei}\left(\dot{k}_{Ei} - \dot{J}_G/M_V\right) = M_{Ei}\left(\dot{k}_{Ei} - \dot{r}_G\right) \quad (11)$$

where $\dot{k}_{Ei}$ denotes the rate constant of the first order process, and $\dot{r}_G$ the specific growth rate. The second term in (11) relates to the dilution by growth. I assume that the SU for growth is fast, i.e. $\dot{J}_m \to \infty$, and has high affinities for the reserve 'molecules', i.e. $p_i = 1$. This SU fuses the catabolic fluxes from the reserves stoichiometrically to produce structural biomass, but stoichiometric constraints imply that the growth SU can reject some of the arriving reserve 'molecules'. The growth rate is found from (10) for $n = 2$, and $i = 1, 2$, to be

$$\dot{J}_G = \frac{\mathrm{d}}{\mathrm{d}t}M_V = \left(\sum_i \left(\frac{\dot{J}_{Ci}}{n_{Vi}}\right)^{-1} - \left(\sum_i \frac{\dot{J}_{Ci}}{n_{Vi}}\right)^{-1}\right)^{-1}$$

$$\dot{r}_G = \frac{\dot{J}_G}{M_V} = \left(\sum_i \left(\left(\dot{k}_{Ei} - \dot{r}_G\right)\frac{m_{Ei}}{n_{Vi}}\right)^{-1} - \left(\sum_i \left(\dot{k}_{Ei} - \dot{r}_G\right)\frac{m_{Ei}}{n_{Vi}}\right)^{-1}\right)^{-1} \quad (12)$$

Note that the specification of the details of the assimilation processes and the fate of the rejected reserve fluxes is required to relate the extracellular nutrient levels to reserve densities. These details, however, do not affect the relationship (12) between growth rate $\dot{r}_G$ and reserve densities m_{Ei}. The assumption that the growth SU is (infinitely) fast does not imply a high maximum growth rate. This is because the dilution by growth restricts the input flux to the SU, while a maximum in the reserve density also restricts the input flux. The existence of such a maximum follows from the combination of first order dynamics and a maximum of the assimilation process.

Fig. 5 illustrates that the combination of first order kinetics for reserve densities and a fast two-substrate SU for growth is realistic. The cellular contents of the nutrients have been fitted to the data using relationship (12). These contents add the contributions of the reserves and the structural biomass, which amounts to $m_{Ei} + n_{Vi}$.

6. Discussion

A popular model for algal growth takes growth to be proportional to a product of terms that are hyperbolic in the nutrient concentrations [2,12]. Although this multiplicative model is attractive because of its simplicity, it is unrealistically restrictive (the use of SUs results in much higher growth rates given the same maximum growth rate), and it is sensitive to changes in abundant nutrients. The latter is a serious methodological drawback because the detailed nutritional re-

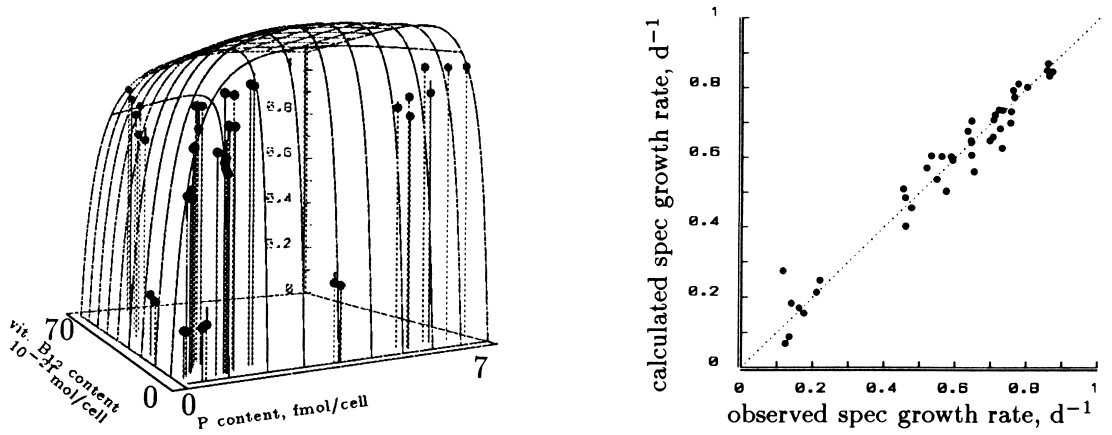

Fig. 5. The specific growth rate $\dot{r}_G$ of the haptophyte *Pavlova lutheri* as a function of the intracellular reserves of phosphorous (reserve 1) and vitamin B_{12} (reserve 2) at 20°C (left) and the relationship between the observed growth rate and the calculated one (right). Data from Droop [6]. The parameters (and standard deviations) are the reserve turnover rates $\dot{k}_{E1} = 1.19$ (0.09) d^{-1}, $\dot{k}_{E2} = 1.22$ (0.09) d^{-1}, stoichiometric requirements $n_{V1} = 0.39$ (0.05) fmol $cell^{-1}$, $n_{V2} = 2.35$ (0.27) 10^{-21} mol $cell^{-1}$.

quirements of algae are not known in practice. Research in algal growth kinetics is usually restricted to the effect of changes in one or two nutrients, such as nitrate and phosphate, assuming (implicitly) that all other required nutrients are abundantly available and do not affect growth. The multiplicative model requires, however, that these concentrations must be unrealistically high indeed, while models on the basis of SUs are much less restrictive. The multiplicative model frequently lacks a good fit to experimental data [6].

Another popular class model let the production rate depend on the limiting flux only, like e.g. Droop [6] proposed. This model is also simple, and can have quite a good fit to experimental data. The dynamics of the non-limiting nutrient are not yet determined, and should be modelled independently. This, however, causes consistency problems as well as problems in the analysis of the model behaviour in transient environments, particularly when growth does not depend directly on nutrient concentrations in the environment, but on reserve densities. If a particular reserve is high at a certain moment, the corresponding nutrient can be absent in the environment for quite a while, before it becomes limiting. The moment of the switch is not easy to evaluate. When the nutrient becomes limiting, it switches roles with the nutrient that was limiting before, and the nutrient obeys other dynamics. Apart from the questionable metabolic realism, these switches easily become problematic in situations where more species are around, with parameter values that differ among species.

The significance of the SU is that it is close to a minimum model, while it avoids the problems of switches; abundant nutrients do not affect growth, even if their abundance exceeds that of the limiting nutrient only by a modest amount, relative to their stoichiometric requirements. Chen and Christensen [3,4] modeled the growth rate by the distribution function of the multivariate logit and Weibull distribution, which has a minimum and a product model as special cases. The latter model results from the assumption that the probability on a cell division is proportional to the concentration of activated receptors, while a given number of substrate molecules are required to activate the receptor. The binding to the receptor is taken to be reversible and in steady state.

The application of SUs is, potentially, much wider than in modeling algal growth. The first purpose of the SU concept is to serve as a module in models for metabolic regulation of cells at the macrochemical level, and the quantitative role of cell organelles or biochemical modules, such as the respiration chain, in the cellular metabolism.

This field between the well-developed theory for enzyme kinetics at the very detailed molecular level and crude eco-physiological models for whole-cell performance is still poorly developed because of the complexity of integrating the rapidly expanding knowledge in molecular biology. The concept of homeostasis is very useful in this field, but implies stoichiometric constraints in the specification of production processes, which are difficult to handle in quantitative models. The simplicity of the SU concept might prove to be a valuable practical solution to this problem, which still has a clear interpretation at the molecular level, although the relationship with the molecular level might work out to be more complex in particular applications.

The SU can be considered as special case of the classical substrate-enzyme association-dissociation kinetics. What is new is the 'discovery' that a relatively simple and parameter sparse kinetics results, if the dissociation rates of substrate-enzyme complexes are negligibly small. High dissociation rates do not complicate the dynamics of 1-SUs (carriers), however, which suggests the application of CSUs in situations where dissociation rates are not small. The need to simplify and reduce the number of parameters is particularly felt in the analysis of large metabolic networks, which makes this field suitable for application of SUs.

Acknowledgements

I like to thank Martin Boer, Jacob van Dijk and Cor Zonneveld for helpful and stimulating discussions. This study has been supported by grant 013/1204.10 from the Dutch Government, National Research Programme on global air pollution and climate change.

References

[1] F.G. Bader, Analysis of double-substrate limited growth. Biotechnol. Bioeng. 20 (1978) 183–202.
[2] B. Baule, Zu mitscherlichs gestez der physiologischen beziehungen. Landw. Jahrb. 51 (1917) 363–385.
[3] C.-Y. Chen, E.R. Christensen, A unified theory for microbial growth under multiple nutrient limitation. Water Res. 19 (1985) 791–798.
[4] E.R. Christensen, Dose-response function in aquatic toxicity testing and the weibull model. Water Res. 18 (1984) 213–221.
[5] D.R. Cox, Renewal Theory, Methuen, London, 1962.
[6] M.R. Droop, The nutrient status of algal cells in continuous culture. J. Mar. Biol. Assoc. UK 54 (1974) 825–855.
[7] R. Heinrich, S. Schuster, The Regulation of Cellular Systems, Chapman and Hall, New York, 1996.
[8] M.V. Henri, Théorie générale de l'action de quelques diastases. Comp. Rend. Acad. Sci. 135 (1902) 916–919.
[9] S.A.L.M. Kooijman, Dynamic Energy Budgets in Biological Systems. Theory and Applications in Ecotoxiology, Cambridge University Press, 1993.
[10] D.E. Metzler, Biochemistry; The Chemical Reactions of Living Cells, Academic Press, New York, 1997.
[11] L. Michaelis, M.L. Menten, Die kinetik der invertwirkung. Biochem. Z. 49 (1913) 333–369.
[12] W.J. O'Brian, The dynamics of nutrient limitation of phytoplankton algae: a model reconsidered. Ecology 55 (1974) 135–141.
[13] D.V. Roberts, Enzyme Kinetics, Cambridge University Press, Cambridge, 1977.
[14] L.A. Segal (Ed.), Mathematical Models in Molecular and Cellular Biology, Cambridge University Press, Cambridge, 1980.
[15] L.A. Segal, Modeling Dynamic Phenomena in Molecular and Cellular Biology, Cambridge University Press, 1984.
[16] L.A. Segal, Biological Kinetics, vol. 12 of Cambridge Studies in Mathematical Biology, Cambridge University Press, Cambridge, 1991.
[17] T.F. Weiss, Cellular Biophysics, vol. 1, Transport, MIT Press, Cambridge, MA, 1996.
[18] C. Zonneveld, Modelling the kinetics of non-limiting nutrients in microalgae. J. Mar. Syst. 9 (1996) 121–136.