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Journal of Theoretical Biology 244 (2007) 576–587

Journal of Theoretical Biology

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A kinetic inhibition mechanism for maintenance

Caroline Tolla[a,*], Sebastiaan A.L.M. Kooijman[b], Jean-Christophe Poggiale[a]

[a]*Laboratoire de Microbiologie Géochimie et d'Ecologie Marine, UMR 6117—Centre d'Océanologie de Marseille, Case 901, Campus de Luminy, 13 288 Marseille Cedex 9, France*

[b]*Department of Theoretical Biology, Vrije Universiteit, Faculty of Earth & Life Sciences, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*

Received 17 February 2006; received in revised form 9 August 2006; accepted 6 September 2006

Available online 12 September 2006

Abstract

To fulfil their maintenance costs, most species use mobile pools of metabolites (reserve) in favourable conditions, but can also use less mobile pools (structure) under food-limiting conditions. While some empirical models always pay maintenance costs from structure, the presence of reserve inhibits the use of structure for maintenance purposes. The standard dynamic energy budgets (DEB) model captures this by simply supplementing all costs that could not be paid from reserve with structure. This is less realistic at the biochemical level, and involves a sudden use of structure that can complicate the analysis of the model properties. We here propose a new inhibition formulation for the preferential use of reserve above structure in maintenance that avoids sudden changes in the metabolites use. It is based on the application of the theory for synthesizing units, which can easily become rather complex for demand processes, such as the maintenance. We found, however, a simple explicit expression for the use of reserve and structure for maintenance purposes and compared the numerical behaviour with that of a classical model in oscillating conditions, by using parameters values from a fit of the models to data on yeasts in a batch culture. We conclude that our model can better handle variable environments. This new inhibition formulation has a wide applicability in modelling metabolic processes.

Keywords: Maintenance; Inhibition formulation; DEB theory; Synthesizing units

1. Introduction

In natural ecosystems, living organisms are submitted to varying environmental conditions which may lead them to support rather long periods of starvation. During these food limited periods, individuals can have complex adaptation strategies in order to survive (Gurney et al., 2003). For instance, allocation rules to the different processes (growth, reproduction, maintenance, etc.) can change to increase the survival period of the individual (Dawes, 1985). Growth usually continues in the first part of the starvation period. Some organisms are able to cease reproduction process. Moreover, in the situation of a prolonged starvation, a variety of possible biological behaviours can occur, such as dormancy (Archuleta et al., 2005) or migration, depending on the species and the environmental factors. Some bacteria can survive starving for many years (Morita, 1985; Postgate, 1990). Among the different strategies, some individuals are able to change their internal dynamics and only spend energy to maintain themselves (Lopez et al., 2006), sometimes leading to a shrinking. Indeed, many species can shrink in structural mass during starvation, as a way to pay their somatic maintenance costs (Dawes, 1976). Such a shrinking process has been observed in bacteria (Barcina et al., 1997) as well as in invertebrates (molluscs, Downing and Downing, 1993) and vertebrates (shrews, Genoud, 1988) or even humans (Lumey et al., 1995). Gurney et al. (2003) and Gurney and Nisbet (2004) studied the impact of starvation on the resource allocation and the adaptation to poor nutritional conditions in order to explain hyperphagia and compensatory growth phenomenon. In contexts like these, the details on how maintenance is actually paid do matter and the outcome is quite sensitive to the internal dynamics.

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*Corresponding author. Tel.: +33 491829126; fax: +33 491829641.
E-mail address: tolla@com.univ-mrs.fr (C. Tolla).

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doi:10.1016/j.jtbi.2006.09.001

Furthermore, these strategies have important consequences on the ecosystems functioning. In the context of the degradation of organic matter by microbial organisms for instance, the maintenance processes affect the fate of matter (Blagodatsky and Richter, 1998; Hess et al., 1996; Neill and Gignoux, 2006). Consequently, a good knowledge of the impact of starvation periods on organisms is needed to get insight in the understanding of ecosystem dynamics.

The maintenance is a set of processes allowing living organisms to stay alive (Kooijman, 2000, p. 89 and references therein). It is a cost excluding net production, i.e. growth and reproduction (Neidhardt et al., 1990). Maintenance comprises the turnover of structure, the activity (transport and movement), the energy needed to maintain concentrations gradients and the energy for defence systems. These processes are a necessity, and have priory with respect to other processes, whatever the environmental conditions are and take all their importance in starvation conditions (Hess et al., 1996; Kooijman, 2000). How an organism maintains itself in low-food environment in order to survive is a key problem, both from biological and ecological points of view.

Concerning the impact of starvation on resource allocation and subsequently the consequences for population dynamics, only few data is available that deals with the underlying processes. Mathematical models can thus help by suggesting assumptions on the mechanisms involved and may propose experiments to test these assumptions.

From the ecological point of view, models must remain rather simple when describing biological processes because of the large amount of interacting processes involved at the ecosystem level. However, the starvation periods have consequences at the individual level and it follows that the models need a trade-off between the realism of the biological processes and the relative simplicity required by ecosystem modelling. It is often admitted that a mechanistic approach is important for describing the microbial processes involved in biodegradation and fate of organic matter (see for instance Blagodatsky and Richter (1998) or Talin et al. (2003) and references therein). According to these general guidelines, we aim to develop a model that describes accurately the key processes during starvation, based on individual properties and which may be applied in ecosystem models.

Usually, the models do not take maintenance into account. The pioneer models describing maintenance (Marr and Ingraham, 1962; Marr et al., 1963; Pirt, 1965; Harvey et al., 1967), involve an intriguing problem around the payment of maintenance costs. They do not make the difference between death and maintenance. The difference becomes important because of the products that are involved, which serve as substrate for other organisms. The products of maintenance are mostly simple minerals (carbon dioxide, ammonia), and that of death is organic matter that might serve as energy or carbon substrate for others organisms. Furthermore, this continuous shrinking under non-starvation conditions is biochemically unrealistic.

Many more recent work aims to improve the modelling of maintenance (Blagodatsky and Richter, 1998; Gupta et al., 2005; Hess et al., 1996; Neill and Gignoux, 2006) and we propose a similar approach to deal with starvation. The dynamics energy budget (DEB) theory (Kooijman, 1993) provides an approach to deal with individual budget of energy linked with population dynamics. Like in other approaches (McGill et al., 1981), the DEB theory assumes that the biomass is partitioned in two compartments: one is rapidly available or temporary biomass (reserve) and the second one is less mobile matter or structural biomass (structure). This allows the models to easily handle growth rate related to changes in the chemical composition of biomass. But, the standard DEB model (Brandt, 2003; Brandt et al., 2004; Evers, 1991a; Kooijman, 2000; Kooijman et al., 1991), which pays maintenance from the reserve compartment is not adapted to starvation conditions. Indeed, when the growth rate per unit of structural biomass becomes negative, it leads to thermodynamic problems if parameters are kept constant. The maintenance costs via structure should be higher than via reserve since structure is produced from reserve; the second law of thermodynamics implies that there must always be overhead costs in the extra transformation.

Consequently, a model has been suggested (Kooijman, 2000) that has an extra maintenance parameter where maintenance is paid from reserve and payment is supplemented with structure if the mobilized reserve flux is too small. This absolute priority of reserve above structure as substrate for maintenance is not supported by direct measurements. Indirectly, models that account for this preference can explain experimental results (for instance see Kooijman and Troost, 2007). On the thermodynamics point of view, this assumption can be supported since the payment via structure has an extra step in synthesis. This DEB formulation involves a metabolic switch (see Eq. (1) for the specification of the switch (S) model), leading the organisms to use its structural biomass instead of its reserves as early as the reserve density is under a given threshold.

However, three problems may be noticed on this formulation. The first one concerns the suddenness of the switch, which might not be a biologically accurate description of these key processes. The second problem is that the S model is an empirical model that rests on an efficiency argument. We think that the mechanisms underlying the switches may help to understand the effect of starvation on populations biology and ecosystems dynamics. A mechanistic approach should provide a formulation able to reproduce switches closer to biological processes. Finally, the instantaneous switches used in the S model can easily give rise to inaccuracies in numerical simulations to the extent that they can dominate the result. This especially applies to individual-based population models (IBMs), where the number of switches is proportional to the number of individuals in the population. An accurate numerical scheme requires the evaluation of the exact moments at which point events occur, which can be computationally quite intensive if the number of events is large. This can become a problem if starvation periods are common, as frequently happens in the field (Gurney et al., 2003).

This manuscript is organized as follows. In the next section, we present a mechanistic model based on two assumptions in order to deal with starvation. The assumptions are (i) the preferential use of reserve to pay maintenance costs and (ii) the presence of switches when starvation leads to the depletion of the reserve density. We call this model the preference (P) model. Contrary to the empirical approach, this model could be tested from experiments. It turns out to be rather complex for applications in population dynamics; so, we provide a simplified version (SP) model which conserves the same properties. Section 3 compares the SP and S models and implements the maintenance modules in a population model. We then compare the previous population models with a simple population model involving maintenance, namely the Marr–Pirt (MP) model (Marr et al., 1963; Pirt, 1965). Finally, we investigate the responses of the different models to environmental perturbations leading to frequent starvation periods. Our results are discussed in the last section.

2. Models description

2.1. General assumptions

DEB theory partitions biomass in reserve and structure. Their amounts characterize the individual's state. We here focus on unicellulars, and do not pay attention to maturation and reproduction for the sake of simplicity. The reserve notion allows for the growth to be dependent on the internal state of the organism and not directly on the external concentrations of nutrients and substrates. Structure represents the part built from reserve and that cannot be easily remobilized. Resources are taken up from the environment and converted to reserve (assimilation); mobilized reserve that is not allocated to maintenance is converted to structure (growth); see Fig. 1. The DEB theory assumes that only the structure needs to be maintained, and the turnover of structure comprises a substantial part of the maintenance costs (Fig. 1). The maintenance rate is taken to be proportional to the amount of structure.

The rate at which reserve is mobilized only depends on the amounts of reserve and structure and the amount of reserve decreases during starvation. We now consider two extra-assumptions:

Assumption 1. Preference—maintenance is preferentially paid from reserve, rather than from structure since structure is less mobile than reserve. Consequently, maintenance costs are paid from reserve when the environmental conditions are good or from structure when the reserve is exhausted (Fig. 1).

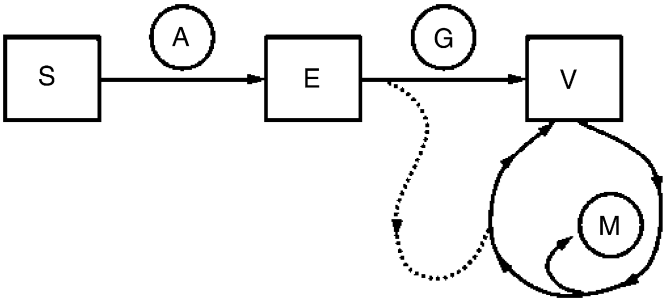

Fig. 1. Scheme of the mechanistic assumptions for the uptake and the use of resources in the DEB theory. The processes are A: assimilation, M: maintenance and G: growth, with the state variables S: substrates, E: reserve compartment and V: structural biomass. Maintenance is a part of the structure turnover (the loop). Its paid preferentially by reserve (dashed line) but if reserve is not enough its paid by structure. The MP model is a particular case of the DEB model considering that A is proportional to G and the dashed line equals to 0.

Assumption 2. Absolute priority—we also assume that this above preference of reserve on structure may be exclusive, that is whenever the reserve density is large enough, the maintenance is only paid from reserve.

Both assumptions have been used to build the empirical S model. This model permitted to explain some phenomena related to yield of biomass to uptake (Kooijman and Troost, 2007) which, else, would not be easily explained. Here, we suggest a mechanism for this formulation. This mechanism involves intra-cellular dynamics and up to now, only a very few data were available to validate it. However, it is a very simple mechanism and nevertheless, the obtained model is quite general. Indeed, it contains the properties of the previous models and, according to the parameter values, is able to represent the absolute priority of reserve on structure for maintenance. Nowadays, more and more data are obtained and shall be available on intra-cellular dynamics and we expect that this kind of data will be used to test our approach.

2.2. Mathematical formulations

The preferential use of reserve to pay maintenance costs can be compared to an inhibition process: the reserve inhibits the use of structure for the maintenance. Indeed, structure will be used for maintenance only when reserve will not be sufficient. We first recall the S model and then derive a "smooth" preference model. A description of variables and parameters is given in Table 1.

2.2.1. The switch (S) model

The S model (Kooijman, 2000) assumes that reserve allocation to maintenance has absolute priority above

Table 1
Nomenclature

Symbol	Dimension	Description
Variables		
S	$\#\mathrm{S\,l}^{-3}$	Substrate amount
E	$\#\mathrm{E\,l}^{-3}$	Amount of the global reserve of the microbial population
V	$\#\mathrm{V\,l}^{-3}$	Amount of the structural biomass of the microbial population
E	$\#\mathrm{E}.\#\mathrm{V}^{-1}$	Reserve density (amount of reserve per unit of structure)
P	$\#\mathrm{P\,l}^{-3}$	Amount of the maintenance product
Parameters		
Y_{*1*2}	$\#1.\#2^{-1}$	Yield coefficient: compound 1 needed by compound 2 degraded or compound 1 produced by compound 2 consumed
k_{*1*2}	t^{-1}	Handling rate of SU in 1 and 2 situation for maintenance
ρ_{*}/ρ_{*1*2}	—	Binding probability of * compound on SU/Binding probability of 1 compound on SU since 2 is already bound (inhibition process)
h_E	t^{-1}	Reserve turnover rate
$j_{*\Pi m}$	t^{-1}	Maximal rate for compound * use in the process Π
k_M	t^{-1}	Maintenance costs
α	—	Proportionality coefficient
Functions		
$j_{*I}{}^{\Pi}$	$\#1.\#\mathrm{V}^{-1}\,\mathrm{t}^{-1}$	Flux per unit of structure of compound *I (X, E, V and P) associated with the process Π ($\Pi =$ A: assimilation, G: growth, M: maintenance)
$J^{*}{}_{I}{}^{\Pi}$	$\#1.\mathrm{l}^{-3}\,\mathrm{t}^{-1}$	Volumetric flux of compound *I associated with process Π
θ_{ij}	—	Fraction of SU where the states i and j can be free or occupied by reserve or structure molecules, associated with maintenance
$\theta_{ij}*$	—	Steady-state fraction of SU where i and j can be free or occupied, associated with maintenance
I_S	$\#\mathrm{S\,l}^{-3}\,\mathrm{t}^{-1}$	Input substrate rate
e_S	$\#\mathrm{E}.\#\mathrm{V}^{-1}$	Threshold value of the reserve density setting off the switch

The following symbols are used for the dimensions: —, no dimension; #, amount (number, g or mol); l, length; t, time (Kooijman, 2000).

growth. If the flux of mobilized reserve is not sufficient to pay maintenance, the remaining cost is paid from structure. The required flux of maintenance is $k_M y_{EP}$ if paid from reserve or $k_M y_{VP}$ if paid from structure. So, the loss-fluxes to maintenance per unit of structure are

$$j_E^M = \min(j_E, k_M y_{EP}) \quad \text{and} \quad j_V^M = (k_M - j_E^M / y_{EP}) y_{VP}, \tag{1}$$

where j_E is the flux of mobilized reserve per unit of structure.

From Eq. (1), if j_E is larger than or equal to the maximum maintenance cost paid from reserve ($k_M y_{EP}$), all the maintenance costs are paid from reserve; if j_E is smaller, part of the maintenance costs are paid from structure. Note that if $j_E = 0$, all of the maintenance costs are paid from structure.

2.2.2. The preference (P) model and its simplification (SP)

We propose here a theoretical mechanism for maintenance. In order to put this mechanism in the context of the DEB theory, we use the concept of synthesizing units (SU, Kooijman, 1998, 2000). Roughly, the concept of SUs is based on an analogy with enzymatic kinetics but is more general because it relates substrate fluxes, rather than concentrations, to product fluxes. The principle is to represent the interaction between variables as in a chemical reaction; the SUs are generalized enzymes that catalyse the transformation. The link between the different states, represented here by arrows, corresponds to the transformation conditions, from one state to another one, defining the interactions within any system.

To explain the maintenance dynamics, we quantify the production of products (P) that are used for maintenance, and consider structure (V) and reserve (E) as substitutable substrates for these products using SU kinetics (Brandt et al., 2004). We say that the substrates are substitutable if they can independently be used in the transformation. Furthermore, the use of V is inhibited by E, as illustrated in Fig. 2(A). V does not affect the binding or transformation of E. However, the binding of E leads to the release of V. After P has been used for maintenance, the resulting metabolites (such as carbon dioxide and ammonia), are released in the environment and it does not interfere in the dynamics. Appendix A gives the mathematical formulation of the P model.

Although the described mechanism is simple, the resulting model is rather complex. As a consequence, there is a practical need for simplifications. Fig. 2(B) and Appendix B present the simplified preference (SP) model, where the dissociation rates k_E and k_{EV} are equal. This allows for rather simple explicit expressions for the use of reserve and structure for maintenance purposes

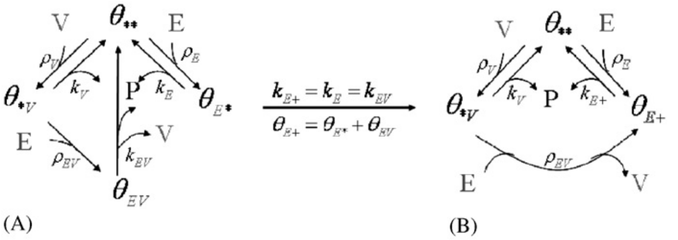

Fig. 2. Scheme of the SU states in the P model (A) and the SP model (B). Both models describe the interaction between transformations of reserve (*E*) and structure (*V*) for maintenance, with a preference for former. θ_{**} is the fraction of the free SU, θ_{*V} the SU fraction with a structure molecule bound, θ_{E*} the SU fraction with a reserve molecule bound and θ_{EV} the SU fraction with both reserve and structure molecules bound. k is the handling rate of SU for maintenance and ρ the binding probability on SU. Table 1 gives a precise description of parameters and variables. The arrow between (A) and (B) indicates the transformations to obtain the SP model from the P model.

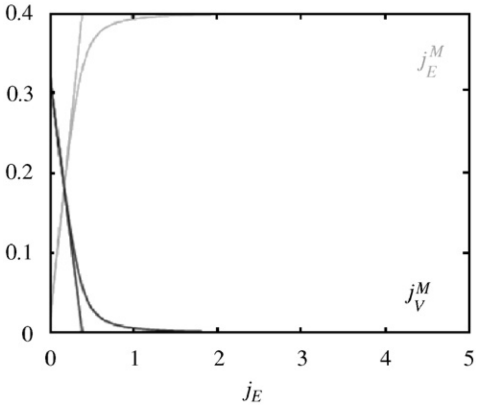

Fig. 3. Comparison between the S (lines) and the SP (curves) models through a simulation of the reserve (grey) and structure (black) fluxes per unit of structure allocated to maintenance as function of the available reserve with $\alpha = 0.1$, $k_M = 0.04\,\mathrm{t}^{-1}$, $y_{PV} = 0.12$, $y_{PE} = 0.1$ and $\rho_E = \rho_{EV} = 1$.

under the various nutritional conditions, with just a single parameter for the inhibition quantification, namely the parameter α.

3. Results

3.1. Fluxes comparison

Here, we compare the fluxes of reserve and structure allocated to maintenance for the S and the SP models, using a time-scale separation argument to make sure that the binding fractions of SUs are in quasi-steady state. Fig. 3 illustrates the fluxes per unit of structure allocated to maintenance of reserve j_E^M (grey) and structure j_V^M (black) as functions of the available reserve flux j_E. Since turnover is a main part of the maintenance costs, we assume that the flux of mobilized structure per unit of structure, j_V is also constant ($\rho_V j_V = k_M y_{VP}$, Appendix B); the part that is not used returns to the structure.

The behaviour of the SP model is controlled by parameter α: the proportionality ratio between dissociation rates of the SU-structure and the SU-reserve complexes (see Appendix A for more details). When α decreases to zero, the product release from the SU-structure complex is stopped, as long as there is some reserve used for the maintenance. In other words, α quantifies the ability of the organism to use a minimal amount of structure for maintenance when reserve is available. The S model can be seen as a particular extreme case of the SP model ($\alpha \to 0$). The variation of the binding probabilities, ρ_E and ρ_{EV}, affects the threshold value of the reserve density at which the switch occurs: if $\rho_{EV} < 1$, the switch of the SP model is at higher reserve mobilization rate than the S model. Thus, the S model is a particular case of the SP model and the SP model a particular case of the P model.

3.2. Parameters estimation in constant environment

Our intra-cellular model can easily be incorporated in a population dynamics model (see Appendix C for details). We considered DEB-based population models using the S and the SP sub-models. In this sub-section, we compare these models with a simple one (the MP model) using parameter values that we obtained by fitting the models to a set of data. In the next sub-section, we shall analyse their respective responses to varying environmental conditions.

Appendix C shows how the SP and the S modules can be implemented in the DEB growth model and presents the MP model. We use data from Ratledge et al. (1984), on a nitrogen limited growth of the yeast *Apiotrichum curvatum* in a batch culture. Fig. 4 shows the fits of the SP and the MP models; the parameters estimates are presented in Table 2. We assume that y_{PV} and k_M are identical for the SP and the MP models. Fig. 4 also shows simulations based on the S model, using the parameters of the SP model.

Contrary to the MP model, the SP model fits the data perfectly. Growth ceases in the MP model as soon as substrate is exhausted, while in fact it continues for a while, due to the use of reserve. The biomass-trajectory of the MP model is below that of the S and SP models, for the same substrate-trajectory. This observation comes from the condition that $y_{PV} < y_{PE} y_{EV}$ meaning that it is more expensive to pay maintenance via structure than via reserve. This is consistent, since the MP model assumes that maintenance is paid from structure only.

The model can be used to evaluate the fractions of SUs that are bound to reserve and/or structure. As long as there is some reserve, the fraction of SU-reserve complex is positive, but if the reserve is fully depleted, the SU-structure complex becomes dominant. The behaviour of the SUs in SP model is very sensitive for the values ρ_{EV} and

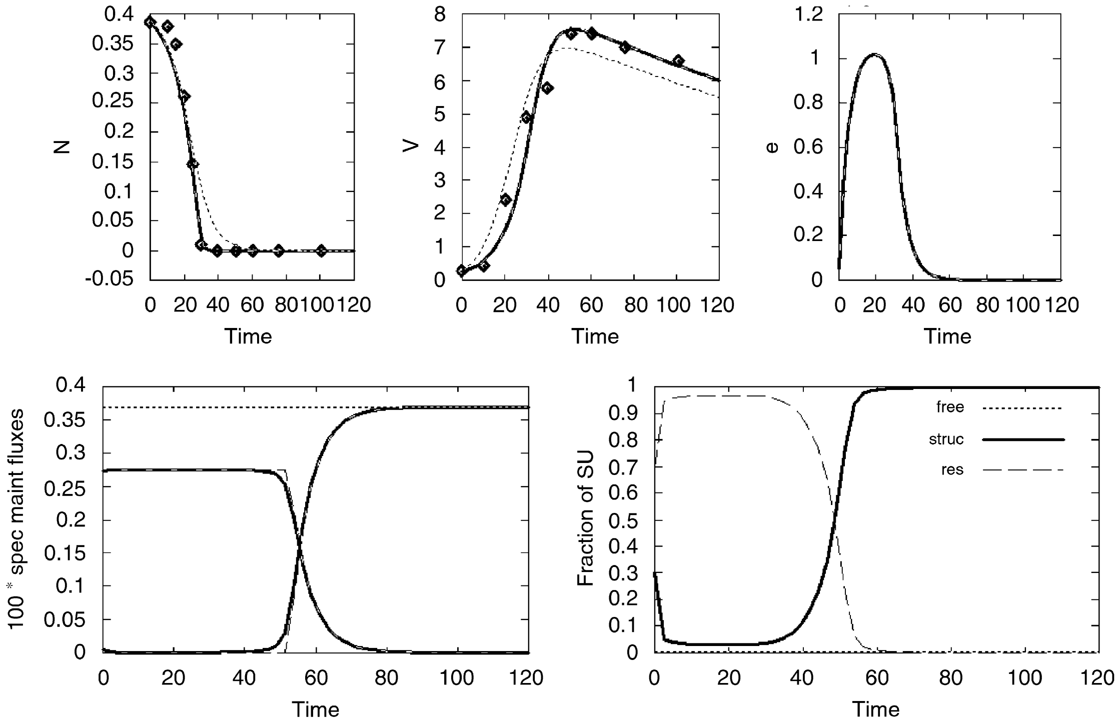

Fig. 4. Data (dots) from Ratledge et al. (1984) on the growth of the *Apiotrichum curvatum* yeast on nitrate in a batch culture versus time (h). The dotted curve is the fit of the MP model; the line is the fit of the SP modules and the dashed line the simulation of the S model, both implemented in a DEB-based model for unicellulars. The S and the SP models are superimposed. See Table 2 for parameters value and Appendix C for more details on models formulation. N is the substrate amount in the culture ($\mathrm{g\,N\,l^{-1}}$), e the reserve density ($\mathrm{g\,N\,g\,V^{-1}}$), V the structural biomass ($\mathrm{g\,V\,l^{-1}}$) and *spec. maint. fluxes* represents j_E^M ($\mathrm{g\,N\,g\,V^{-1}\,h^{-1}}$) and j_V^M ($\mathrm{h^{-1}}$).

Table 2
Estimated parameters values of the SP and the MP models from the set of data from Ratledge et al. (1984)

Parameters	Unit	Value	Origin
$N\ (t=0)$	$\mathrm{g\,N\,l^{-1}}$	0.387	Ratledge et al. (1984)
$V\ (t=0)$	$g\,Vl^{-1}$	0.28	Ratledge et al. (1984)
k_M	$\mathrm{h^{-1}}$	3.25×10^{-3}	Hanegraaf and Muller (2001)
y_{PV}	$\mathrm{g\,P\,g\,V^{-1}}$	$0.9y_{PE}\,y_{EV}$	Estimated
The SP model parameters			
$e\ (t=0)$	$\mathrm{g\,N\,g\,V^{-1}}$	0.051	Evers (1991b)
j_{NAm}	$\mathrm{g\,N\,g\,V^{-1}\,h^{-1}}$	0.013	Evers (1991b)
K_N	$\mathrm{g\,N\,l^{-1}}$	0.05	Evers (1991b)
y_{EV}	$\mathrm{g\,N\,g\,V^{-1}}$	0.829	Kooijman (2000)
y_{EN}	—	17.1637	Estimated
h_E	$\mathrm{h^{-1}}$	0.1821	Estimated
y_{PE}	$\mathrm{g\,P\,g\,N^{-1}}$	1.1797	Estimated
α	—	0.035	Estimated
$j_V' = \rho_{V}j_V$	$\mathrm{h^{-1}}$	1	Estimated
$\rho_E = \rho_{EV}$	—	1	Estimated
The MP model parameters			
j_{NAm}	$\mathrm{g\,N\,g\,V^{-1}\,h^{-1}}$	0.0703	Estimated
K_N	$\mathrm{g\,N\,l^{-1}}$	3.48	Estimated
y_{VN}	$\mathrm{g\,V\,g\,N^{-1}}$	19.57	Estimated

See Table 1 and Appendix C for parameters definition. Here, E represents the nitrogen reserve and gV the gram of structural biomass. The S model simulations are realized from the SP model parameters value.

α, while there is hardly an effect at the population level. This is illustrated in Fig. 4. We conclude that the values of α and ρ_{EV} are very important at the molecular level, but not at the population level. The implication is also that we need data at the molecular level to estimate these parameters appropriately.

3.3. Simulations in varying environments

In this section, we study the response of the SP model to different scenarios which represent simplified situations encountered in natural environments: (i) with a long starvation period, (ii) favourable conditions for population growth and (iii) with periodic starvations that can relate for instance to the seasonal phytoplanctonic bloom arriving at the surface of the sediment. In order to study the differences with a simple empirical model, we compare the solutions of the SP model with that of the MP model in the different environmental conditions. The initial values of structure and reserve and the parameters values are obtained from the previous fit (Table 2).

To analyse model properties, we compare the following cases: (i) a batch culture without any supply during the simulation in order to study the effects of an occurrence of a long starvation period (Eq. (2), $A=0$ so that $I_N(t)=0$, Fig. 5) and (ii) a fed-batch culture with a constant substrate supply in order to represent favourable conditions for growth (Eq. (2), $P=0$ so that I_N is a constant, Fig. 6); and (iii) with a periodically oscillating substrate supply that allows favourable and starvation conditions alternately (Fig. 7). For this purpose we use the following supply of

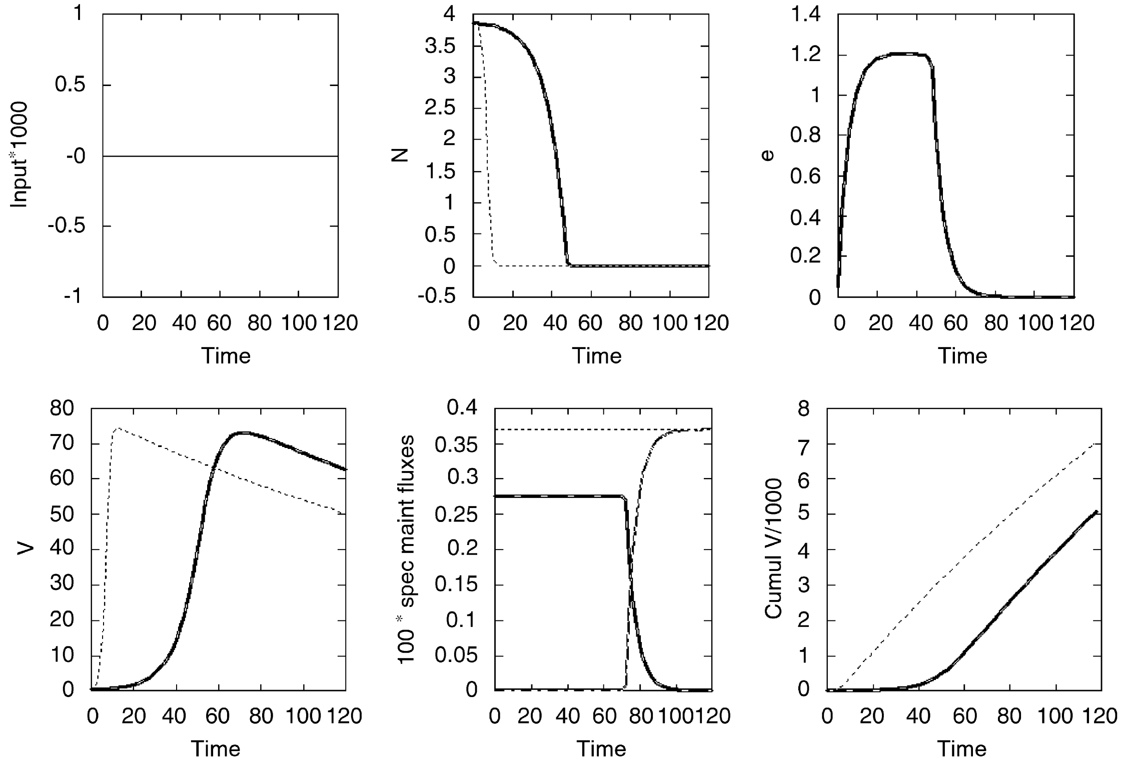

Fig. 5. Simulation of the SP (line), S (dash line) and the MP (dot line) models in a batch culture ($I_N = 0$) with initial substrate amount of 3.87 g N l^{-1} versus time (h). The S and SP models are superimposed. See Table 2 for parameters values. N is the substrate amount in the culture (g N l^{-1}), e the reserve density (g N g V^{-1}), V the structural biomass (g V l^{-1}) and *spec. maint. fluxes* represents j_E^M (g N g V^{-1} h^{-1}) and j_V^M (h^{-1}), *Cumul V* the cumulative structural biomass (g V l^{-1}) and *Input* = I_N (g N l^{-1} h^{-1}).

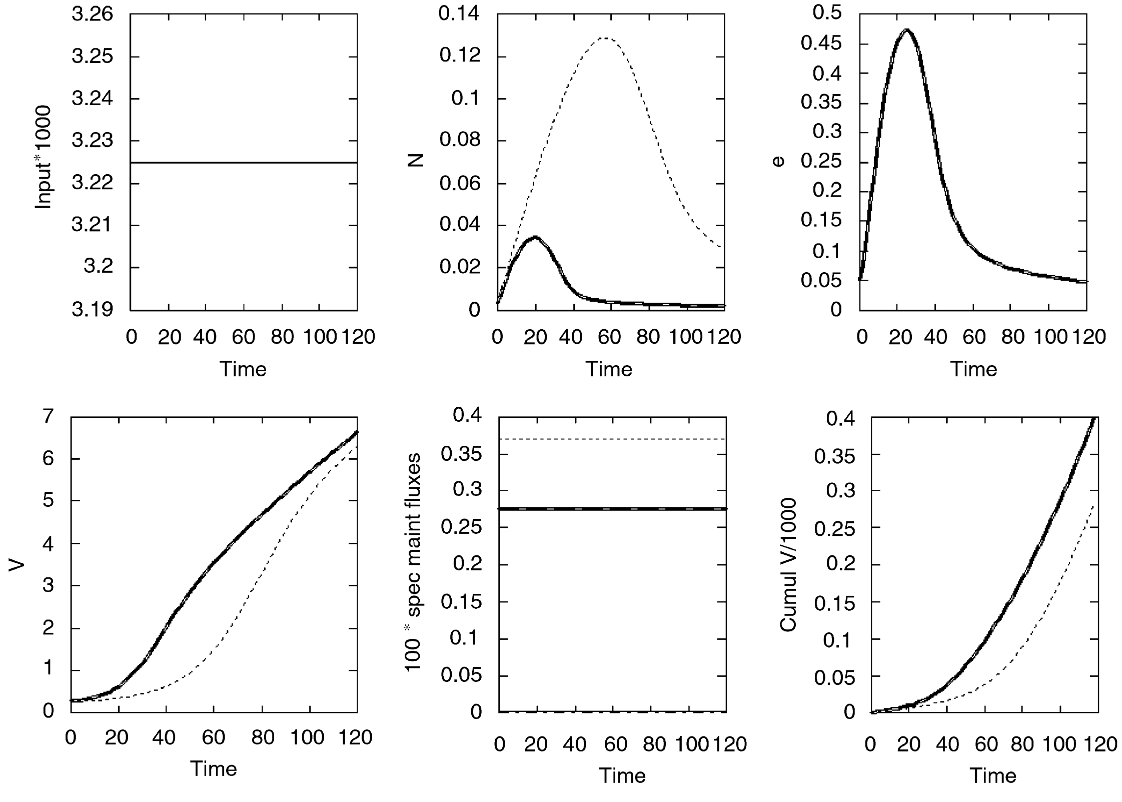

Fig. 6. Simulation of the SP (line), S (dash line) and the MP (dot line) models in a fed-batch culture with a constant substrate supply versus time (h). The integrated supply is equal to 0.387 g N l^{-1}. The S and SP models are superimposed. See Table 2 for parameters values. N is the substrate amount in the culture (g N l^{-1}), e the reserve density (g N g V^{-1}), V the structural biomass (g V l^{-1}) and *spec. maint. fluxes* represents j_E^M (g N g V^{-1} h^{-1}) and j_V^M (h^{-1}), *Cumul V* the cumulative structural biomass (g V l^{-1}) and *Input* = I_N (g N l^{-1} h^{-1}).

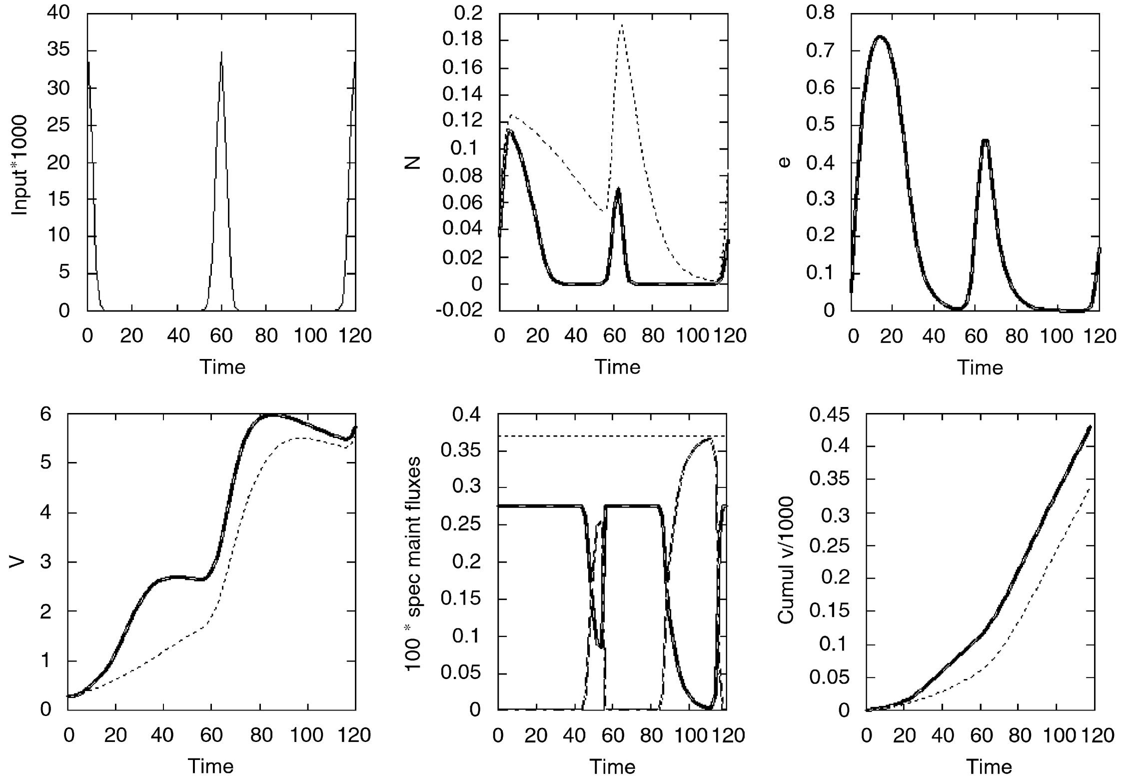

Fig. 7. Simulation of the SP (line), S (dash line) and the MP (dot line) models in a fed-batch culture with an oscillating substrate supply versus time (h). The integrated supply equals $0.387\,\mathrm{g\,N\,l^{-1}}$. The S and SP models are superimposed. See Table 2 for parameters values. N is the substrate amount in the culture ($\mathrm{g\,N\,l^{-1}}$), e the reserve density ($\mathrm{g\,N\,g\,V^{-1}}$), V the structural biomass ($\mathrm{g\,V\,l^{-1}}$) and *spec. maint. fluxes* j_E^M ($\mathrm{g\,N\,g\,V^{-1}\,h^{-1}}$) and j_V^M ($\mathrm{h^{-1}}$), *Cumul V* the cumulative structural biomass ($\mathrm{g\,V\,l^{-1}}$) and $Input = I_N$ ($\mathrm{g\,N\,l^{-1}\,h^{-1}}$).

substrate (in $\mathrm{g\,N\,l^{-1}\,h^{-1}}$):

$$I_N(t) = A\,\exp[D(\cos(Pt) - 1)], \tag{2}$$

where A is the amplitude of the oscillations, D the dispersion of the input peak and P its pulsation.

The total amount of supplied substrate in cases (ii) and (iii) is taken to be the same and such that the cumulative amount of supplied substrate during the "experiment" equals the initial amount in case (i): $N_{int} = \int_0^{t_{max}} I_N\,\mathrm{d}t = 0.387\,\mathrm{g\,N\,l^{-1}}$. Some amplitude variations of N_{int} will be compared.

The comparison of Figs. 4 and 5 shows that the distance between the MP and SP models increases with the amount of substrate. The biomass in the MP model tends to grow faster than in the SP model because of the absence of reserve (Fig. 5). Similarly, the structural biomass in the MP model decreases as soon as substrate is exhausted, while the decrease is delayed if reserve is present (as in the SP model).

Biomass in the MP model grows more slowly at low substrate levels (Figs. 6 and 7); this model underestimates the cumulative structure and overestimates the substrate. Indeed, for low substrate amounts ($N \ll K_N$), we have

$$j_{NAm}\frac{N}{N + K_N} \simeq \frac{j_{NAm}}{K_N}N. \tag{3}$$

For the MP model j_{NAm}/K_N equals $0.021\,\mathrm{g\,V^{-1}\,h^{-1}}$, while it equals $0.261\,\mathrm{g\,V^{-1}\,h^{-1}}$ for the SP model. This is the reason for the time delay of the structure dynamics of the MP model in varying environments (Fig. 7).

Figs. 5–7 also show when the cells make the switch of using structure for maintenance.

The MP and SP models can have a similar behaviour in constant environments at low substrate levels, with the same parameters set (results not shown), but they become different in varying environments.

Figs. 6 and 7 show that the amount of structure at the end of the experiment increases with the amplitude of the oscillations, while the cumulative amount of supplied substrate is the same.

4. Discussion and conclusion

The SP and MP models turned out to be rather different, especially in varying environments. When fitted to the same data, parameters that have the same interpretation can result in different values (see Fig. 4 and Table 2). Furthermore, models that give similar predictions under one set of conditions, can give different predictions under other conditions (Fig. 4 compared to Fig. 5). These fact arise a problem in the comparison of different models.

This study shows the importance of the choice of model to obtain realistic parameter values from fitting the model to experimental data and of the role of transients induced by a dynamic environment, where the reserve compartment dominates the behaviour. But we also put in evidence that the new preference model presents some advantages above the empirical switch model: (i) it better links up with processes at the molecular level, (ii) it can better handle variable environments and (iii) it has better numerical properties which can help to get more relevant biological properties.

Although the P model is the simplest way to reproduce the switch and has the best link up with the underlying processes at the molecular level, it is complex in terms of non-linearity and contains many variables and parameters. The S model is a particular case of the SP model, and the SP model a particular case of the P model. The SP and S models turned out to behave very similar, and with the "cost" of a single parameter we could remove a switch and still preserve the link with underlying processes. Indeed, the SP model should allow a better comprehension of the internal dynamics in variable environment. This makes it easier to link to models and phenomena at the molecular level, while still being applicable at the population and ecosystem levels. If we would be able to determine the switch time of the use of reserve and structure for the maintenance, we would be able to look deeper into cell's machinery with help of this preference model and have an improved access to cell's chemical composition.

Brandt et al. (2004) modelled the diauxic growth of microorganisms that live on two substitutable substrates. Diauxic growth patterns arise from the expression if one type of carrier for the uptake of substrate suppresses the expression of the other type. Although applied in a very different context, this module is, in retrospection, identical as the one that we developed here, but then applied to supply systems (substrate controlled), while we had to use demand systems (substrate controlled). Assimilation (the key process in the study of Brandt) is a supply process, while maintenance is a demand process. Although the concept is the same, the resulting equations look very different. Demand systems are much more complex to model, as is further demonstrated by the study of Kuijper et al. (2004) on the use of carbohydrate versus protein reserves for maintenance purposes in zooplankton, assuming that these reserves are partly substitutable.

Contrary to the Brandt formulation, the preferential model allows the absolute priority of a substrate on another because one substrate can drive out another one in the SU-substrate complex. This simple mechanism is can also be used more generally in any transformation that involves substitutable compounds with preferences for one substrate.

Not all organisms can use structure for maintenance, and die if shrinking is too fast or too far. Muller and Nisbet (2000) implemented death due to starvation when somatic maintenance requirements cannot be met from reserve energy. They showed that organisms grow bigger at varying food density, rather than constant density with similar average level. We also found that the biomass increases with the amplitude of the substrate supply rate, for reasons that are very similar to the hyperphagia in animals as reported by Gurney et al. (2003). Food fluctuations may lead to death by starvation, the likelihood of which increases with the strength and duration of the bleak periods. Like Kooijman (1993, pp. 132–134), Muller and Nisbet suggested that organisms become bigger with increasing latitude (Bermann's law) due to an increasing seasonal variability in food. The results of these different studies are consistent.

Measurements on perturbed (i.e. realistic) environments are not widely available. The models developed here can now suggest new experiments on variable environments: which parameters must be measured, what should be an optimal frequency for measurement, what would be a good time scale to obtain data at several organization levels (ex: biochemical and biological) with a minimum cost. Furthermore, the mechanistic formulations can help to understand the physiological state of the individual and can have a wide applicability in modelling metabolic processes.

Acknowledgments

We like to thank Ms T.A. Troost for fruitful discussions and helpful comments. The first and last authors thank also the French programme of Coastal Environment (PNEC—AMPLI—2004/1140716) for the financial support.

Appendix A. Specification of the P model

The change in the fractions of SU that are unbound and bound to one or two substrates in the P model (see Fig. 2(A)) is given by

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}\theta_{..}\\ \theta_{E.}\\ \theta_{.V}\\ \theta_{EV}\end{pmatrix} = \begin{pmatrix}-\rho_V j_V-\rho_E j_E & k_E & k_V & k_{EV}\\ \rho_E j_E & -k_E & 0 & 0\\ \rho_V j_V & 0 & -\rho_{EV} j_E-k_V & 0\\ 0 & 0 & \rho_{EV} j_E & -k_{EV}\end{pmatrix}\begin{pmatrix}\theta_{..}\\ \theta_{E.}\\ \theta_{.V}\\ \theta_{EV}\end{pmatrix}. \tag{A.1}$$

where j_V and j_E are the binding flux per unit of structure, respectively, for structure and reserve (t^{-1}). Table 1 describes the variables and parameters. The flux of mobilized structure j_V is taken to be constant, because the turnover of structure represents a substantial part of the maintenance costs and $j_E = (h_E - r)e$ with r the growth rate per unit of structure (Appendix C).

Here, j_*^{Π} is the flux per unit of structure, of the compound * (i.e. S, E, V and P) due to the process Π (A = assimilation, M = maintenance and G = growth).

Let $J_*^{\Pi} = j_*^{\Pi} V$ be the volumetric flux of compound *, associated to the process Π. As molecular dynamics are much faster than population dynamics, j_*^{Π} is obtained from the steady state fractions of SU. The volumetric reserve and structure fluxes allocated to maintenance are

$$\begin{cases} J_V^M = j_V^M V = -(\rho_V j_V \theta_{..}^* + k_{EV}\theta_{EV}^*)V, \\ J_E^M = j_E^M V = -y_{EV} j_E(\rho_E \theta_{..}^* + \rho_{EV}\theta_{.V}^*)V, \end{cases} \tag{A.2}$$

where θ_{ij}^* is the steady-state fraction of SU at the binding state i and j and y_{EV} the yield coefficient of reserve on structure (i.e. cost for structure in terms of reserve).

The release of product in association with maintenance amounts to

$$\frac{\mathrm{d}}{\mathrm{d}t} P = y_{PE}(k_E \theta_{E.}^* + k_{EV}\theta_{EV}^*)V + y_{PV} k_V \theta_{.V}^* V, \tag{A.3}$$

which can be rewritten as

$$\frac{\mathrm{d}}{\mathrm{d}t} P = J_P^M = j_P^M V \quad \text{with} \quad j_P^M = y_{PE} j_E^M + y_{PV} j_V^M. \tag{A.4}$$

The maintenance flux per unit of structure is taken to be constant (output controlled system), so we require that j_P^M is constant ($j_P^M = k_M$) by allowing k_E, k_{EV} and k_V to depend on θ_{ij}^*. We define unequal dissociation rates, $k_V = \alpha k_M/\theta$, $k_E = \beta k_M/\theta$ and $k_{EV} = \gamma k_M/\theta$ with $\theta = \alpha y_{PV}\theta_{.V}^* + y_{PE}(\beta\theta_{E.}^* + \gamma\theta_{EV}^*)$.

Appendix B. Specification of the SP model

We simplify the P model by defining $k_{E+} = k_E = k_{EV} = k_M/\theta$ and $\theta_{E+} = \theta_{E.} + \theta_{EV}$, where $\theta = \alpha y_{PV}\theta_{.V}^* + y_{PE}\theta_{E+}^*$ (Fig. 2(B)). Eq. (A.1) then reduces to

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} \theta_{..} \\ \theta_{.V} \\ \theta_{E+} \end{pmatrix} = \begin{pmatrix} -(\rho_V j_V + \rho_E j_E) & \alpha k_M/\theta & k_M/\theta \\ \rho_V j_V & -\alpha k_M/\theta - \rho_{EV} j_E & 0 \\ \rho_E j_E & \rho_{EV} j_E & -k_M/\theta \end{pmatrix}\begin{pmatrix} \theta_{..} \\ \theta_{.V} \\ \theta_{E+} \end{pmatrix}. \tag{B.1}$$

The fluxes, per unit of structure, of reserve and structure that are allocated to maintenance are

$$\begin{cases} j_V^M = \alpha\theta_{.V}^* k_M/\theta^*, \\ j_E^M = (k_M - y_{PV} j_V^M) y_{EP}. \end{cases} \tag{B.2}$$

Note that, since the maintenance costs per unit of structure are constant, and the turnover of structure comprises a substantial part of these costs, its natural to give at j_V, the mobilisation rate of structure per unit of structure, a value just enough to pay maintenance costs in the worst case. The worst case is without reserve ($j_E = 0$); all must be paid from structure. This gives $\rho_V j_V \geqslant k_M y_{VP}$. Indeed, if $\rho_V j_V < k_M y_{VP}$, it can only pay maintenance costs if there is some reserve left over; and if $e < e_S$, it should die because it can no longer pay maintenance costs. If we want to minimize payment of maintenance costs from structure, the particular case $\rho_V j_V = k_M y_{VP}$ corresponds to a structure flux just enough to pay maintenance costs. But, when $j_E = 0$, this equality fixes the steady-state solutions at $\theta_{..}^* = 1$ and $\theta_{.V}^* = 1 - \theta_{..}^* = 0$. Thus, the chosen value of $\rho_V j_V$ influences the evolution of the steady-state fractions of SU.

The steady-state solutions are explicit, but complicated. For $\rho_E = \rho_{EV}$, $\rho_E j_E = j'_E$ and $\rho_V j_V = j'_V$, the steady-state solutions simplify considerably and the structure flux per unit of structure allocated to maintenance is

$$j_V^M = \frac{2Ak_M/y_{PV}}{2A + y_{PE}\left(\sqrt{B^2 - 4AC} - B\right)} \tag{B.3}$$

with $A = \alpha j'_V k_M y_{PV}$, $B = y_{PE}C + ((1-\alpha)j'_E + j'_V)k_M$ and $C = -j'_E(j'_E + j'_V)$.

Appendix C. Implementation of the maintenance modules in the DEB model

We now implement the SP module in the DEB model for unicellulars, which accounts for assimilation and growth:

$$\begin{array}{lll} \frac{\mathrm{d}}{\mathrm{d}t} S = I_S - j_S^A V, & & j_S^A = j_{SAm} f(S) \\ \frac{\mathrm{d}}{\mathrm{d}t} e = j_E^A - h_E e & \text{with} & j_E^A = y_{ES} j_S^A \\ \frac{\mathrm{d}}{\mathrm{d}t} V = rV & & f(S) = S/(S + K_S). \end{array} \tag{C.1}$$

Substrate S is, in the example that we use here, the nitrogen compound represented by N ($\mathrm{g\,N\,l^{-1}}$). I_S is the input of substrate ($\mathrm{g\,N\,l^{-1}\,h^{-1}}$), $f(S)$ is a Michaelis–Menten function with the half saturation constant K_S ($\mathrm{g\,N\,l^{-1}}$), y_{ES} is the yield of reserve on substrate and r the growth rate per unit of structure. According to the DEB theory, the growth rate per unit of structure is

$$r = \left(\frac{h_E e - j_E^M}{e + y_{EV}}\right) - j_V^M\left(\frac{y_{EV}}{e + y_{EV}}\right). \tag{C.2}$$

The first part of the Eq. (C.2) corresponds to the sum of growth and maintenance processes from reserve; the second part corresponds to the maintenance costs that are covered by structure (shrinking). The maintenance costs are no longer constant, due the varying way the costs are covered:

$$j_P^M = y_{PE} j_E^M + y_{PV} j_V^M = k_M \Rightarrow j_E^M + y_{EP} y_{PV} j_V^M = y_{EP} k_M.$$

Thus, if $y_{EP} y_{PV} = y_{EV}$, the last equation is equivalent to the standard growth rate per unit of structure of the DEB theory:

$$r = \left(\frac{h_E e - y_{EP} k_M}{e + y_{EV}}\right). \tag{C.3}$$

In this growth rate per unit of structure (Eq. (C.2)), reserve kinetics does not change during shrinking with the implication that growth continues as long as there is some reserve.

We replace j_E^M and j_V^M by the expression of S (Eq. (1)) or SP (Appendix B) models. Note that j_V^M is a function of $j_E = (h_E - r)e$ (Eq. (B.3)), and so of r; we indicate this with $j_V^M(r)$. This implies that the growth rate per unit of structure is given implicitly only. This does not give much practical problems, however, since the sequence:

$$r_{i+1} = \frac{h_E e - k_M/y_{PE} + (y_{PV}/y_{PE} - y_{EV}) j_V^M(r_i)}{e + y_{EV}} \tag{C.4}$$

rapidly converges, $r_i \to r$, in a few steps, starting from $r_0 = 0$.

By replacing the S module (Eq. (1)) in the Eq. (C.2), as long as $j_E > k_M y_{EP}$, the growth rate per unit of structure amounts to Eq. (C.3).

Payment of maintenance from structure starts when $j_E = k_M y_{EP} = j_E^M; j_V^M = 0; r = 0$ and $e = y_{EP} k_M / h_E = e_S$ is at the threshold value. The growth rate after this moment switches to $r = -j_V^M$ with $j_V^M = (k_M - y_{PE} j_E^M) y_{VP}$ and $j_E^M = j_E = (h_E - r)e$ (all the stored resource are mobilized for maintenance). Thus, in the S model, the growth rate per unit of structure after the switch is

$$r = \frac{h_E e - y_{EP} k_M}{e + y_{EP}/y_{VP}}. \tag{C.5}$$

Since $\lim_{e \uparrow e_S} r = \lim_{e \downarrow e_S} r = 0$, the growth rate is continuous around the switch, but not differentiable for $y_{PV} \neq y_{PE} y_{EV}$. This also applies to $\mathrm{d}e/\mathrm{d}t$.

In MP model, we have (see Eq. (C.1) for j_S^A description):

$$r = y_{VS} j_S^A - y_{VP} k_M. \tag{C.6}$$

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