Mathematical Biology

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Optimal allocation of building blocks between nutrient uptake systems in a microbe

Abstract. A bacterial cell must distribute its molecular building blocks among various types of nutrient uptake systems. If the microbe is to maximize its average growth rate, this allocation of building blocks must be adjusted to the environmental availabilities of the various nutrients. The adjustments can be found from growth balancing considerations. We give a full proof of optimality and uniqueness of the optimal allocation regime for a simple model of microbial growth and internal stores kinetics. This proof suggests likely candidates for optimal control regimes in the case of a more realistic model. These candidate regimes differ with respect to the information that the cell's control system must have access to. We pay particular attention to one of the three candidates, a feedback regime based on a cellular control system that monitors only internal reserve densities. We show that allocation converges rapidly to balanced growth under this control regime.

1. Introduction

Microbes assimilate abiotic nutrients, and from these nutrients they derive building blocks which they use to grow more of themselves [15]. As they grow, the microbes distribute the building blocks among various types of macromolecular structures. They thus naturally face an allocation problem. We will consider a particular sub-problem, confining ourselves to the molecular machinery involved in the uptake and processing of the various nutrients. Elsewhere we have studied a different allocation subproblem, concerning the choice between growth machinery and uptake machinery [5].

While allocation determines the rates at which nutrients enter the microbial cell, nutrient accumulation may be used by the cell to determine the allocation regime, as we will show in this paper. There is thus an interesting reciprocal interaction between the uptake and the processing of nutrients.

To simplify the mathematical analysis as much as possible, we assume that there are only two chemical elements that make up a microbial cell: carbon and

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nitrogen. Furthermore, we assume that the cell can synthesise only two types of uptake machinery: one that assimilates a nutrient from which the cell derives carbon (and only carbon), and one that assimilates a nutrient that donates nitrogen only. In making these assumptions, we drastically simplify the actual situation. However, we are confident that we retain the essential features of the problem; see [4] for a treatment of more realistic cases. The allocation problem is to distribute building blocks between the carbon nutrient assimilating machinery and the nitrogen nutrient assimilating machinery.

We assume that fixed proportions of carbon and nitrogen are required to synthesize structural biomass, which includes catalytic machinery. ('Structural biomass' is biomass excluding reserves [8, 11, 13].) We also assume that the cell turns as much of its nutrient supply into structural biomass as it possibly can (in [3] this assumption is discussed and related to other classic growth models). The cell will then (i) use up all of its carbon and nitrogen to synthesize such machinery ('balanced case'); or (ii) have some carbon reserves left over ('nitrogen-limited case'); or (iii) have some nitrogen reserves left over ('carbon-limited case'). Our working hypothesis is that the cell strives to achieve the balanced case (i). It is intuitively obvious that the cell maximizes its growth rate when case (i) applies. It is less clear, however, how a cell should behave if it is either nitrogen- or carbon-limited, and acts so as to maximize its biomass over a given amount of time. The aim of this paper is to discover such optimal behaviour.

In section 2, we develop a simple model for microbial growth and surplus kinetics. Section 3 presents a full solution to the optimal control problem. The model is extended in section 4, and likely candidates for (near) optimal control are studied.

2. Formulating the model

A microbial cell incorporates its supply of amino acids into various proteins. A subset of these proteins is involved in the assimilation of nutrients. These proteins are transporters that translocate nutrient molecules from the cell's ambient into the cell's interior, as well as cytosolic enzymes that convert the nutrients into the metabolites that feed the catabolic and anabolic pathways of the cell's metabolism. We will refer to the assembly of proteins required to assimilate a nutrient as the nutrient's 'uptake system'. We enquire how a microbe should allocate its amino acids among the various uptake systems that correspond to the various essential nutrients required by the organism.

An intuitive answer to the question of optimal allocation is sketched in Figure 1. When the availability of one of the nutrients drops, the transport system for that nutrient is only able to sustain a reduced assimilation of the nutrient. This distorts the stoichiometry of uptake, and a surplus of the other nutrient arises. However, by having more transporters for the reduced-availability nutrient, the original stoichiometry of uptake can be restored. Since the *per capita* quantity of uptake systems is naturally constrained, the cell must have less of the transporters for the nutrient that is still in relative abundance. The combination of required uptake stoichiometry and environmental nutrient availabilities defines an allocation of molecular
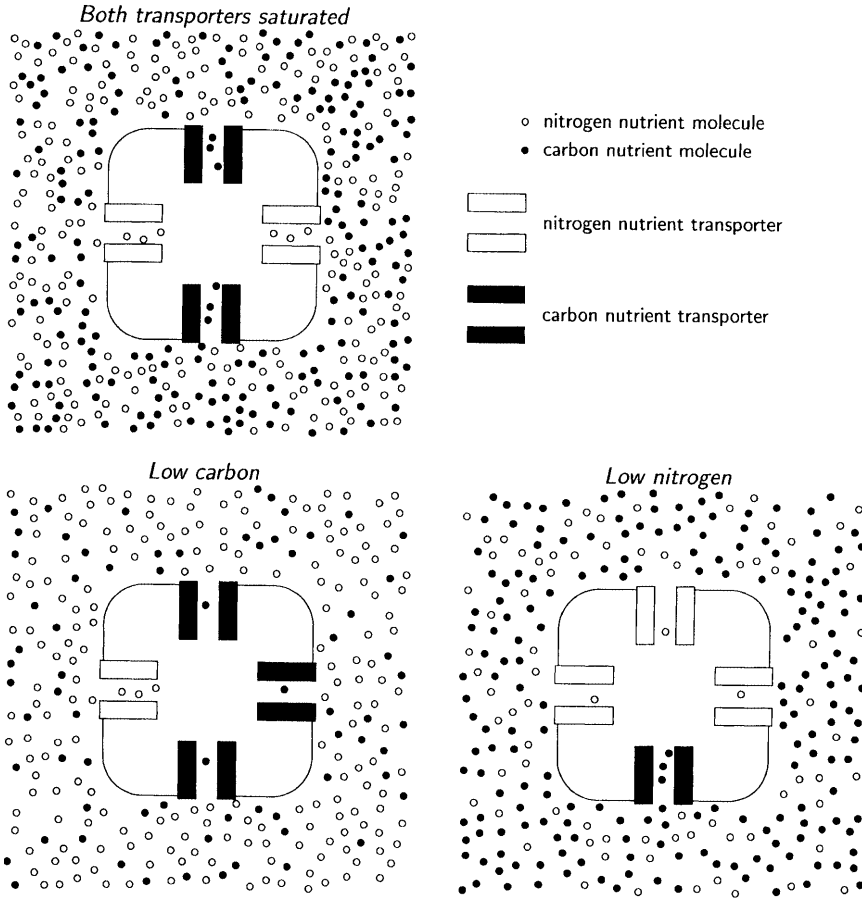
Fig. 1. The principle of adaptive re-allocation. To illustrate the principle, it is assumed that the cell requires the same numbers of nitrogen and carbon nutrient molecules per unit of time. Panels schematically depict a microbial cell surrounded by a medium containing the nitrogen and carbon nutrients. Equal numbers of transporters conduct nutrient fluxes at the required 1 : 1 ratio when both nutrients are present at saturating concentrations (top panel). The 1 : 1 ratio can also be attained under low carbon conditions (bottom left panel) and under low nitrogen conditions (bottom right panel) by investing more building blocks in carbon or nitrogen transporters, respectively.

building blocks among the uptake systems. We now cast these intuitions in a more precise form.

Let Q_C denote the cell's carbon quota, that is the total quantity of carbon atoms in the cell over all types of molecules, including constitutive as well as reserve structures, as defined by [8]. Likewise, let Q_N denote the nitrogen quota. A certain amount of catalytic proteins, proportional to Q_C, could be synthesised from the carbon quota if the nitrogen quota were infinitely large. Taking the number of carbon atoms per weight unit of protein to be a constant, we can express the quantity

of proteins which the carbon quota allows to be synthesized in terms of the number of carbon atoms present in these proteins (C-moles). The quota Q_C then directly represents a potential amount of catalytic machinery in C-moles. Similarly, if $\rho_{C:N}$ is the ratio of carbon to nitrogen atoms in protein, $\rho_{C:N} Q_N$ represents a C-molar amount of catalytic machinery allowed by the nitrogen quota. Since both carbon and nitrogen atoms are required to synthesize protein, the actual C-molar amount of catalytic machinery is the minimum of the two:

$$Q_{\text{cata}} = \min\{Q_C, \rho_{C:N} Q_N\} . \qquad (1)$$

We will assume that a fixed proportion $\alpha \in (0, 1)$ of the catalytic machinery is devoted to uptake systems, and that of these, a variable fraction $y \in (0, 1)$ is devoted to the uptake of carbon, and the remainder $(1 - y)$ to the uptake of nitrogen. Thus, the C-molar amount of carbon uptake machinery is $y\alpha Q_{\text{cata}}$. The variable y plays a central role in this paper: it represents the allocation between carbon and nitrogen uptake systems.

A fixed fraction $(1 - \alpha)$ is allocated to catalytic machinery other than that directly involved in assimilation. This machinery is involved in turn-over as well as growth. We assume that the allocation $(1 - \alpha)$ provides under all circumstances sufficient catalytic capability to convert building blocks into catalytic machinery at the rate permitted by the minimum consideration which underlies equation (1). This assumption simplifies matters: in reality, under low growth conditions, the organism might lower α and invest more building blocks in uptake and less in growth; we have considered the uptake/growth trade-off elsewhere [5].

On the above assumptions, the quotas obey the following kinetics:

$$\dot{Q}_C = f_C \beta_C y \alpha Q_{\text{cata}} \qquad (2)$$
$$\dot{Q}_N = f_N \beta_N (1 - y) \alpha Q_{\text{cata}} \qquad (3)$$

where f_C, f_N are dimensionless saturation factors, and β_C, β_N are uptake rate parameters.

The saturation factors express to what degree the uptake systems are saturated. A classic example of a model for the saturation factor is the Michaelis-Menten equation [14]:

$$f_C = \frac{S_C}{K_C + S_C}$$

where S_C is the environmental concentration of the carbon nutrient, and K_C is a saturation parameter. Here we need not concern ourselves with the choice of a good model to link environmental conditions to the saturation factors: we simply treat f_C, f_N as the effective representations of these conditions.

The rate parameters β_C, β_N translate the C-molar amounts of catalytic machinery into carbon (nitrogen) fluxes that would result if the uptake systems were fully saturated (that is, at $f_C = 1$, $f_N = 1$). These rate parameters subsume various conversion coefficients: the investment costs (amino acids per transporter), the nutrient flux rate that can be sustained by a transporter, and the yield of carbon (nitrogen) atoms from each nutrient molecule.

The following definitions help to simplify the kinetics:

$$\omega_1 \overset{\text{def}}{=} \frac{f_C \alpha \beta_C}{f_C \alpha \beta_C + f_N \alpha \beta_N \rho_{C:N}}$$

$$\omega_2 \overset{\text{def}}{=} \frac{f_N \alpha \beta_N \rho_{C:N}}{f_C \alpha \beta_C + f_N \alpha \beta_N \rho_{C:N}}$$

$$x_1 \overset{\text{def}}{=} \alpha Q_C \tag{4}$$

$$x_2 \overset{\text{def}}{=} \alpha \rho_{C:N} Q_N$$

$$\varphi \overset{\text{def}}{=} \min\{x_1, x_2\}$$

$$t' \overset{\text{def}}{=} (f_C \alpha \beta_C + f_N \alpha \beta_N \rho_{C:N}) t$$

The quantity φ is the amount of uptake systems in C-moles. Since α is fixed, φ is a valid *numéraire* of structural biomass, and the ratio $\dot{\varphi}/\varphi$ represents the relative growth rate of the microbial cell. The dimensionless coefficients ω_1, ω_2 are both contained in (0, 1) and add to 1. Environmental conditions are thus entirely represented by ω_1 (or, equivalently, ω_2). Scaled time is denoted by t' here; we will omit the prime in what follows. On this scaling equations (2) and (3) become:

$$\dot{x}_1 = \omega_1 y \varphi \tag{5}$$

$$\dot{x}_2 = \omega_2 (1 - y) \varphi \tag{6}$$

Our objective now is to find a control of allocation y that will maximize structural biomass $\varphi(t)$ for all $t > 0$. We can formulate this as a problem to maximize $\varphi(T)$ for some fixed value of T. This is equivalent to the problem of maximizing structural biomass for all t provided that the solutions are independent of T, which happens to be true of all results presented below.

The condition $x_1 = x_2$ implies that none of the carbon or nitrogen atoms are tied up in reserves (i.e. surplus structures that do not serve as catalytic machinery or structural elements such as the cell envelope). The situation where $dx_2/dx_1 = 1$ is known in microbiology as 'balanced growth' [15]. When $x_1 < x_2$, growth is limited by carbon, and when $x_1 > x_2$, growth is limited by nitrogen. It will be convenient to denote the corresponding partitioning of the x_1, x_2 phase plane as follows:

$$I_1 \overset{\text{def}}{=} \{x = (x_1, x_2) \in R^2 : 0 < x_1 < x_2\}$$

$$I_2 \overset{\text{def}}{=} \{x = (x_1, x_2) \in R^2 : 0 < x_2 < x_1\} \tag{7}$$

$$I_{12} \overset{\text{def}}{=} \{x = (x_1, x_2) \in R^2 : 0 < x_1 = x_2\} .$$

3. Instantaneous adaptation of allocation

In this section we assume that the cell is able to alter the allocation y instantaneously. Physiologically, this means that there are mechanisms to retract, say, a carbon nutrient transporter from the cell envelope, and replace it by a nitrogen nutrient transporter. Moreover, the turn-over of such machinery must be more rapid than

the time scale of our model (which is $\sim \varphi/\dot{\varphi}$). These assumptions may be warranted in the case of eukaryotic cells, but are not likely to be satisfied by bacteria [6, 15]. In section 4 we consider an alternative which is more realistic for prokaryotic cells. However, the results of the present section will still prove to be relevant.

The variable y is taken to be the control parameter, $y \equiv v$. We can state the optimal control problem as follows:

$$\begin{cases} \dot{x}_1 = \omega_1 v \varphi(x) \quad 0 < \omega_1 < 1 \\ \dot{x}_2 = \omega_2 (1 - v) \varphi(x) \quad 0 < \omega_2 < 1 \\ x_1(0) = x_{10} > 0 \\ x_2(0) = x_{20} > 0 \\ T > 0 \text{ is fixed} \\ x(T) \text{ is free} \\ v(t) \in [0, 1] \quad \forall t \in [0, T] \\ \varphi(x(T)) \to \max_{v(\cdot)} \end{cases} \quad (8)$$

If the system moves along the diagonal I_{12}, we have $x_1 = x_2$, and no carbon or nitrogen surpluses are built up. This suggests a control regime that moves the system as quickly as possible to the diagonal, and subsequently ensures that it remains on the diagonal, to be optimal. This intuition is confirmed by the following theorem.

Theorem 1. *The unique optimal control function $v_{op}(t)$ in problem (8) is defined by*

$$v_{op}(t) = \begin{cases} v_1(t) \text{ if } x_1(0) < x_2(0) \\ \omega_2 \quad \text{ if } x_1(0) = x_2(0) \\ v_2(t) \text{ if } x_1(0) > x_2(0) \end{cases} \quad (9)$$

or in feedback form

$$v_{op}(x) = \begin{cases} 1 \quad \text{if } x_1 < x_2 \\ \omega_2 \text{ if } x_1 = x_2 \\ 0 \quad \text{if } x_1 > x_2 \end{cases} \quad (10)$$

where

$$v_1(t) = \begin{cases} 1 \ 0 \le t \le T_\star \\ \omega_2 \ T_\star < t \le T \end{cases}$$

$$v_2(t) = \begin{cases} 0 \ 0 \le t \le T_\star \\ \omega_2 \ T_\star < t \le T \end{cases}$$

$$T_\star = \max \left\{ \frac{1}{\omega_1} \ln \frac{x_2(0)}{x_1(0)}; \ \frac{1}{\omega_2} \ln \frac{x_1(0)}{x_2(0)} \right\} .$$

Lemma 3 and Lemma 4 below imply the prescription of the control regime given by this theorem, as well as its uniqueness. Existence can be established as follows.

Lemma 1. *The optimal solution to problem (8) exists.*

Proof. We shall consider measurable functions as admissible control functions in problem (8) and establish the result for this case. Writing the above dynamics in the form $\dot{x} = F(x, v)$ we can see that the system has the following properties: (i) $||F(x, v)|| \leq \sqrt{2} \, ||x|| \;\; \forall v \in [0, 1]$; and (ii) the set $G(x) = \{g \in R^2 : g = F(x, v), v \in [0, 1]\}$ is convex for all $x \in R_+^2$. These properties imply that the controlled system satisfies all conditions of Filippov's Theorem [7,9] which tells us that the attainability set of (8) is a closed bounded set in two-dimensional space (i.e. it is a compact set). This fact, together with the continuity of the function $\varphi(x)$, implies the existence of an optimal solution to (8).   □

As a preamble to Lemma 4, the following result establishes that there are no 'short-cut trajectories' through either region I_1 or I_2 compared to a trajectory along the diagonal I_{12}.

Lemma 2. *Let $\tilde{x}(t)$, $\bar{x}(t)$ be the phase trajectories of system (8) responding to admissible controls $v(t) = \tilde{v}(t)$ and $v(t) = \omega_2$, respectively, where $t \in [\tau_1, \tau_2]$ and $\tilde{x}(\tau_1) = \bar{x}(\tau_1) \in I_{12}$. If either $\tilde{x}(t) \in I_1$ or $\tilde{x}(t) \in I_2$ for all $t \in (\tau_1, \tau_2)$ then*

$$\varphi(\tilde{x}(\tau_2)) < \varphi(\bar{x}(\tau_2)) \, .$$

Proof. Let

$$\tau_1 = 0, \;\; \tau_2 = \tau; \quad \tilde{x}_1(0) = \tilde{x}_2(0) = \bar{x}_1(0) = \bar{x}_2(0) = a$$

for the sake of simplicity and $\tilde{x}(t) \in I_1 \;\; \forall t \in (0, \tau)$ for definiteness. Clearly $\varphi(\bar{x}(\tau)) = ae^{\omega_1\omega_2\tau}$ since $\bar{x}_1(t) = \bar{x}_2(t) = ae^{\omega_1\omega_2 t}$, $t \in [0, \tau]$. If $\tilde{v} \equiv 1$ for $t \in [\tau_1, \tau_2]$, we immediately have $\varphi(\tilde{x}_2(\tau_2)) = a < \varphi(\bar{x}(\tau_2))$, which is the required result. Otherwise we have from $\tilde{x}(t) \in I_1 \Leftrightarrow \tilde{x}_1(t) < \tilde{x}_2(t)$

$$\dot{\tilde{x}}_2 = \omega_2(1 - \tilde{v})\tilde{x}_1 < \omega_2(1 - \tilde{v})\tilde{x}_2$$

for at least some $t \in (0, \tau)$, which implies, for $t \in (0, \tau]$,

$$a \exp\left\{\omega_1 \int_0^t \tilde{v}(s)ds\right\} = \tilde{x}_1(t) \leq \tilde{x}_2(t) < a \exp\left\{\omega_2 \int_0^t (1 - \tilde{v}(s))ds\right\} \, .$$

This means that

$$\omega_1 \int_0^t \tilde{v}(s)ds < \omega_2 \int_0^t (1 - \tilde{v}(s))ds = \omega_2 t - \omega_2 \int_0^t \tilde{v}(s)ds \, , \quad t \in (0, \tau]$$

or (as $\omega_1 + \omega_2 = 1$) $\int_0^t \tilde{v}(s)ds < \omega_2 t$. This inequality implies that $\varphi(\tilde{x}(\tau)) = a \exp\left\{\omega_1 \int_0^\tau \tilde{v}(s)ds\right\}$ is strictly smaller than $ae^{\omega_1\omega_2\tau} = \varphi(\bar{x}(\tau))$.   □

To prove the $t \leq T_\star$ part of Theorem 1, we need to show that optimality requires moving the system as quickly as possible to the diagonal I_{12}.

Lemma 3. *Let $x(0) \in I_1$ (or I_2) and $T \leq T_\star$ where*

$$T_\star = \max\left\{\frac{1}{\omega_1} \ln \frac{x_2(0)}{x_1(0)}; \frac{1}{\omega_2} \ln \frac{x_1(0)}{x_2(0)}\right\} \, .$$

Then the control function $v_*(t) = 1$ (or 0), $t \in [0, T]$ provides an optimal control regime for (8).

Proof. Assume $x(0) \in I_1$ for definiteness. Then $T_\star = \dfrac{1}{\omega_1} \ln \dfrac{x_{20}}{x_{10}}$ and for an arbitrary control function $v(t)$ the system is described by

$$\begin{cases} \dot{x}_1 = \omega_1 v x_1 & x_1(0) = x_{10} \\ \dot{x}_2 = \omega_2(1-v)x_1 & x_2(0) = x_{20} \end{cases}$$

and $x_1(t) \le x_2(t) \; \forall t \in [0, T]$ (equality is possible only at $t = T$). Thus $\varphi(x(t)) = x_1(t) \; \forall t \in [0, T]$. The choice $v(t) = 1 \; \forall t \in [0, T]$ maximizes the rate at which x_1 increases (x_2 remaining equal to x_{20}). Since, for this choice, $x_1(t)$ reaches x_{20} at $t = T_\star$ and is smaller than $x_2(t) \equiv x_{20}$ for $t < T_\star$, we see that for $T < T_\star$ the choice $v \equiv 1$ is optimal. □

Finally, for the $t > T_\star$ part of Theorem 1, we establish the optimality of balanced growth, that is, of staying on the diagonal I_{12} once the system is on it.

Lemma 4. *If $x(0) \in I_{12}$ the control $v_*(t) = \omega_2$, $t \in [0, T]$ is optimal in (8).*

Proof. Lemma 1 guarantees the existence of an optimal control for (8). Assume that $\tilde{v}(t)$, $t \in [0, T]$ is an optimal control. It defines an optimal trajectory $\tilde{x}(t)$, $t \in [0, T]$, $\tilde{x}(0) = x(0)$. There are two possibilities for the trajectory $\tilde{x}(t)$. We may have $\tilde{x}(t) \in I_{12}$ for all $t \in [0, T]$, in which case the result is trivial: from $\tilde{x}_1(t) \equiv \tilde{x}_2(t)$, $\dot{\tilde{x}}_1(t) \equiv \dot{\tilde{x}}_2(t)$, $t \in [0, T]$ it follows directly that $\tilde{v}(t) = \omega_2$ for all $t \in [0, T]$. Otherwise, we have $\tilde{x} \in I_{12}$ not for all $t \in [0, T]$. But this is impossible. Assume to the contrary that there is at least one time interval $(\tau_1, \tau_2) \in [0, T]$ such that $\tilde{x}(\tau_1) \in I_{12}$, $\tilde{x}(t) \notin I_{12}$ for all $t \in (\tau_1, \tau_2)$. There are two subcases to consider: either $\tau_2 = T$ or $\tau_2 < T$ with $\tilde{x}(\tau_2) \in I_{12}$. If $\tau_2 = T$ the required contradiction follows from the fact that we can find a control which does better than the postulated optimal $\tilde{v}$; this alternative control is prescribed by

$$v(t) = \begin{cases} \tilde{v}(t) & t \in [0, \tau_1) \\ \omega_2 & t \in [\tau_1, T] \end{cases}$$

and its superiority to $\tilde{v}$ follows from Lemma 2. When $\tau_2 < T$ we have the following control, which allows, again by Lemma 2, the system to reach the state $\tilde{x}(\tau_2)$ at a strictly earlier time, say $\tau_2 - \delta\tau$ (with $\tau_1 < \tau_2 - \delta\tau < \tau_2$):

$$v(t) = \begin{cases} \tilde{v}(t) & t \in [0, \tau_1) \\ \omega_2 & t \ge \tau_1 \end{cases} .$$

Using the autonomy of the system, we can now shift the behaviour of the system under $\tilde{v}(t)$ for $t \in [\tau_2, T)$ backwards in time by an amount $\delta\tau$, which means that the final state under $\tilde{v}$, which is $\tilde{x}(T)$, is reached at the earlier time $T - \delta\tau$. Choosing $v = \omega_2$ for the final segment $[T - \delta\tau, T]$, we can formulate a control regime which is superior to $\tilde{v}$:

$$v(t) = \begin{cases} \tilde{v}(t) & t \in [0, \tau_1) \\ \omega_2 & t \in [\tau_1, \tau_2 - \delta\tau) \\ \tilde{v}(t + \delta\tau) & t \in [\tau_2 - \delta\tau, T - \delta\tau) \\ \omega_2 & t \in [T - \delta\tau, T] \end{cases}$$

but as $\tilde{v}$ was already optimal by hypothesis we have the desired contradiction. □

Theorem 1 proposes a mixed feedforward/feedback control, as the control system requires knowledge of the internal state (x) as well as the external conditions (ω_2). One might suppose that a simpler set-up would perform nearly as well. For instance, a pure feedforward system would set $v \equiv \omega_2$ for all t. In that case, the trajectory $x(t)$ has a slope equal to 1 everywhere, and the cells would not attain the diagonal I_{12} unless $x(0) \in I_{12}$. However, $dx_2/dx_1 = 1$ does imply balanced growth in the sense that surplus densities, defined by

$$z_1(x) \overset{\text{def}}{=} \frac{x_1 - \varphi(x)}{\varphi(x)}, \quad z_2(x) \overset{\text{def}}{=} \frac{x_2 - \varphi(x)}{\varphi(x)} \tag{11}$$

converge to zero. For instance, if $x(0) \in I_1$, growth is carbon-limited ($\varphi \equiv x_1$, $z_1 \equiv 0$) and there is a nitrogen surplus. But since $x_2(t) = x_1(t) + (x_2(0) - x_1(0))$, the nitrogen surplus density z_2 goes to zero as $x_1 \to \infty$.

Alternatively, one might consider a pure feedback control, which relies only on knowledge of x. This requires a choice, say $v = 1$, to be made for v when $x_1 = x_2$ (we already have a feedback control for $x_1 \neq x_2$). In the present model this results in degenerate behaviour, a problem resolved in the case of the more realistic model set up in the next section.

4. Adaptation of allocation by growth dilution

In section 3 we treated y as the control variable, where $y \equiv v$ was the fraction of uptake machinery devoted to the uptake of carbon. This assumption is warranted only if the cell is able to convert one type of uptake machinery into another, and to do this sufficiently rapidly compared to the time-scale of growth. However, prokaryotic microbes are probably not able to change machinery that is already in place [6, 15]. When such a cell alters its allocation of amino acids between carbon and nitrogen nutrient transporters, this alteration can only affect the transporters that are presently being formed. The change in allocation relies on dilution by growth. We must therefore consider the kinetics of the allocation fraction y.

Let $u \in [0, 1]$ denote the fraction of newly formed uptake protein devoted to the uptake of carbon nutrient. We assume that cellular control mechanisms govern the value of u. The quantity $y\varphi$ represents the C-molar quantity of uptake systems devoted to carbon uptake. Its rate of change equals the rate at which uptake systems are synthesised, multiplied by the allocation variable u:

$$\frac{d}{dt}(y\varphi) = u\dot{\varphi}$$

or, since $\frac{d}{dt}(y\varphi) = \dot{y}\varphi + y\dot{\varphi}$,

$$\dot{y} = (u - y)\dot{\varphi}/\varphi \ . \tag{12}$$

The optimal control problem now becomes, with scaling as in (4):

$$
\begin{cases}
\dot{x}_1 = \omega_1 y \varphi(x) \quad \omega_1 > 0 \\
\dot{x}_2 = \omega_2 (1 - y) \varphi(x) \quad \omega_2 > 0 \\
\dot{y} = (u - y)\dot{\varphi}/\varphi \\
x_1(0) = x_{10} > 0 \\
x_2(0) = x_{20} > 0 \\
0 < y(0) = y_0 < 1 \\
T > 0 \text{ is fixed} \\
x(T) \text{ is free} \\
u(t) \in [0, 1] \quad \forall t \in [0, T] \\
\varphi(x(T)) \to \max_{u(\cdot)}
\end{cases}
\tag{13}
$$

Before we begin investigating optimal control in this system, let us establish that it makes sense to look for it.

Lemma 5. *The optimal solution to problem (13) exists.*

Proof. By Filippov's Theorem [7,9] as in Lemma 1.   □

One special case can be dealt with immediately, in virtue of the results of section 3. This is the case where $x(0) \in I_{12}$ ($x_{10} = x_{20}$) and $y = \omega_2$. Setting $u \equiv \omega_2$, we obtain $y(t) \equiv \omega_2$, and $x(t)$ remains on the diagonal I_{12} (growth is balanced). By Lemma 4, this control regime is optimal.

This non-generic case points to the difficulty engendered by the dilution kinetics (12) in the generic case, where generally $y(t') \neq \omega_2$ on a moment t' where $x \in I_{12}$, which means that the actual allocation y among carbon and nitrogen uptake forces the state x off the diagonal again (the trajectory traverses the diagonal). Moreover, since the instantaneous allocation u is naturally contained in [0, 1], there are limitations to the time-course that can be imposed on $y(t)$ (otherwise $y(t)$ could be forced to follow a prescribed time-course $f(t)$ by $u(t) = y(t) + f'(t)\varphi/\dot{\varphi}$, $\dot{\varphi} \neq 0$). For this reason, the results of section 3 do not carry over to problem (13). Nevertheless, we may use these results to identify likely candidates for a solution to problem (13). From a biological point of view, finding an optimal solution is not the overriding concern: a control regime must also be biologically plausible, in particular, physiologically feasible. For this reason, our focus in this section is on whether biologically plausible candidate regimes are well-behaved. We discuss three such candidates, all inspired by the foregoing results.

4.1. Rapid transference to balanced growth

Lemma 3 suggests that the system should move to the diagonal I_{12} as quickly as possible, and subsequently stay on it. This motivates an auxiliary time-optimal

transference problem:

$$
\begin{cases}
\dot{x}_1 = \omega_1 y \varphi(x) \quad 0 < \omega_1 < 1 \\
\dot{x}_2 = \omega_2(1 - y)\varphi(x) \quad 0 < \omega_2 < 1 \\
\dot{y} = (u - y)\dot{\varphi}/\varphi \\
x_1(0) = x_{10} > 0 \\
x_2(0) = x_{20} > 0 \\
0 < y(0) = y_0 < 1 \\
u(t) \in [0, 1] \ \forall t \in [0, T^*] \\
x_1(T^*) = x_2(T^*), \quad y(T^*) = \omega_2 \\
T^* \to \min_{u(\cdot)}
\end{cases}
\tag{14}
$$

where T^* is the moment when the system hits the diagonal I_{12} under the 'special' condition $y = \omega_2$. As we have just seen, the control $u \equiv \omega_2$ is then optimal from T^* onwards. There is a control regime which achieves this transference in minimal time:

Theorem 2. *Time-optimal feedback control for problem (14) is defined by*

$$
u(x, y, \omega_2) = \begin{cases} 0 & x_2 < K(y, \omega_2)x_1 \\ 1 & x_2 > K(y, \omega_2)x_1 \end{cases}
\tag{15}
$$

where

$$
K(y, \omega_2) = \begin{cases} (1 + \frac{(y-\omega_2)^2}{2(1-\omega_2)\omega_2(1-y)})^{-1} & 0 < y < \omega_2, \\ 1 + \frac{(y-\omega_2)^2}{2(1-\omega_2)\omega_2 y} & \omega_2 \le y < 1. \end{cases}
$$

We omit the proof of this non-trivial result (see [1, 12]). The diagonal I_{12} is crossed at most once by the trajectory under the control regime prescribed by this theorem. The control regime is of a mixed feedforward/feedback type: the allocation control system must integrate information from the outside (ambient nutrient concentrations, here represented by ω_2) and from the inside (the state (x_1, x_2, y)) in order to behave in the prescribed manner.

Optimal control results need not make sense from a biological point of view. In [5] we discuss several potential difficulties; we now consider two of those, concerning feasibility and plausibility, in more detail. Reflecting on these two objections, we are led to consider simpler prescriptions for allocation control.

One objection which naturally occurs to a biologist is to question the plausibility of the control proposed by Theorem 2 on the basis of the complicated algebraic formulas involved in the control regime. However, the structural robustness of the proposed input/output behaviour itself is what matters, rather than the complexity of the algebra used to describe it. Structural robustness is relevant here inasmuch as the proposed input/output behaviour of the control system is only a model idealization, which means that slight variations about that behaviour should not affect the overall behaviour of the model too much.

An essential requirement for physiological plausibility of any proposed control regime is that the cell's control systems be able to obtain the required information. Is there a signalling mechanism which acts so as to convey the information required as input to the proposed control system? Information about ω_2 involves the

ability to gauge the ambient concentrations of nutrients. Signalling systems which connect ambient concentrations to gene expression are well-documented [6, 15]. Similarly, it is quite plausible that signals about the cell's internal nutritional state serve as inputs to allocation control. Below we will consider control regimes based exclusively on ambient nutrient concentrations (ω_2, section 4.2) or on nutritional status (x, section 4.3). The control regime proposed in Theorem 2 also requires information about the realized allocation y. This would require the intracellular signalling/control pathways to somehow gauge the amounts of uptake machinery devoted to carbon and nitrogen, respectively. Although not impossible in principle, such signalling would seem to require simultaneous gauging of ambient concentrations and realized fluxes, as well as an exquisitely precise integration of these two information flows. For this reason, we do not regard the control proposed by Theorem 2 as a likely candidate from a physiological point of view.

4.2. Feedforward control

A simpler control regime would only monitor external conditions (i.e. ω_2). Only $u = \omega_2$ can satisfy the balanced growth requirement $dx_1/dx_2 \overset{!}{=} 1$. The following result affirms the well-behavedness of this control regime.

Theorem 3. *If $u(t) \equiv \omega_2$ and $y(0) \in (0, 1)$, the solution of (13) for any initial point $x_0 \in R_+^2$ possesses the following property:*

$$\frac{x_2(t)}{x_1(t)} \to 1, \quad t \to +\infty .$$

Proof. For $x \in I_1$, $\dot{\varphi}/\varphi = \omega_1 y$ and thus $\dot{y} = \omega_1 y(\omega_2 - y)$, which gives the unstable solution $y(t) \equiv 0$ for $y_0 = 0$. For $x \in I_2$, $\dot{\varphi}/\varphi = \omega_2(1 - y)$ and thus $\dot{y} = \omega_2(1 - y)(\omega_2 - y)$, which gives the unstable solution $y(t) \equiv 1$ for $y_0 = 1$. We therefore exclude the initial conditions $y_0 = 0, 1$, because these leave either x_1 or x_2 at the initial value. We can furthermore exclude the initial condition $y_0 = \omega_2$, for which $x_2(t) - x_1(t)$ is constant in time, which immediately implies the desired result. Either $\dot{y}$ or $\frac{d}{dt}(1 - y)$ is given by a logistic equation, whence

$$y(t) = \begin{cases} \omega_2 \dfrac{y(\vartheta)e^{\omega(t-\vartheta)}}{\omega_2 + y(\vartheta)(e^{\omega(t-\vartheta)} - 1)} & x(s) \in I_1, \ \vartheta \le s < t \\[4mm] 1 - \omega_1 \dfrac{(1 - y(\vartheta))e^{\omega(t-\vartheta)}}{\omega_1 + (1 - y(\vartheta))(e^{\omega(t-\vartheta)} - 1)} & x(s) \in I_2, \ \vartheta \le s < t \end{cases} \tag{16}$$

where $\omega = \omega_1\omega_2$. We will give the proof for $x(0) \in I_1$ (the cases $x(0) \in I_2$ and $x(0) \in I_{12}$ can be dealt with in a similar manner). Let $y_0 \in (0, \omega_2)$, and consider the ratio x_2/x_1. It is described by a linear differential equation with time-varying co-efficients containing y, for which we can substitute the first of the above solutions. The solution is

$$\frac{x_2(t)}{x_1(t)} = 1 + \frac{1 + C}{e^{\omega t} + C}\left[\frac{x_{20}}{x_{10}} - 1\right] + \frac{C\omega_2 t}{e^{\omega t} + C} \tag{17}$$

where $C = (\omega_2 - y_0)/y_0$. Since $C > 0$, we see that $x(t) \in I_1$ for all $t \geq 0$, and thus the use of the first solution for $y(t)$ is warranted. Also, it is clear that $x_2(t)/x_1(t) \to 1$ as $t \to \infty$. When $y_0 \in (\omega_2, 1)$, the trajectory crosses the diagonal I_{12} at some time $t^\star$. We can compute this time $t^\star$ by considering $g(t) \stackrel{\text{def}}{=} x_2(t) - x_1(t)$. It is easy to show that $\ddot{g}(t) \equiv 0$, and therefore $g(t) = g(0) + \dot{g}(0)t$. Since $g(t^\star) = 0$, we have

$$t^* = -\frac{g(0)}{\dot{g}(0)} \equiv \frac{x_{20} - x_{10}}{x_{10}(y_0 - \omega_2)} > 0 \ .$$

Furthermore, at $t = t^\star$ the slope of the trajectory dx_2/dx_1 is smaller than 1 since

$$\left. \frac{dx_2}{dx_1} \right|_{t=t^\star} = \frac{\omega_2(1 - y(t^*))}{\omega_1 y(t^*)} \quad \text{and} \quad y(t^*) \in (\omega_2, 1) \ .$$

Therefore the phase point enters I_2. For $t > t^\star$, the situation is symmetric to the previous case, under the exchanges $x_1 \leftrightarrow x_2$, $y \leftrightarrow (1 - y)$, and $\omega_1 \leftrightarrow \omega_2$. We thus find $x_1(t)/x_2(t) \to 1$ as $t \to \infty$. $\square$

Feedforward control $u \equiv \omega_2$ requires that the allocation control system can monitor the ambient conditions, in particular, can assess the degree to which the uptake apparatus is saturated. Molecular signalling pathways which are able to achieve this might exist, but even then it is difficult to see how such a system could be well-calibrated. Moreover, feedforward control systems are intrinsically more vulnerable to noise acting on internal dynamics, while feedback systems are more robust in this respect. We therefore turn to a simple feedback system.

4.3. Feedback control

The third candidate we will consider is the feedback regime proposed by Theorem 1. The special case $x \in I_{12}$ with $y = \omega_2$ is not generic, and, as we shall see, is never attained unless it happens to be the initial condition. We therefore disregard this nongeneric case and consider the following control

$$u(x) = \begin{cases} 0 & x_2 < x_1 \\ 1 & x_2 > x_1 \end{cases} \tag{18}$$

which nearly coincides with the time-optimal feedback control specified by Theorem 2. In particular, since $K(y, \omega_2) = 1 + \mathcal{O}((y - \omega_2)^2)$, the two controls are nearly equivalent when y is near ω_2, and regime (18) in fact brings y close to ω_2 (see Lemma 9 below for a more precise statement).

In physiological terms, the simple feedback control regime defined by (18) relies only on the system being able to gauge which nutrient is in surplus. Elsewhere [4] we discuss a plausible mechanism which is able to do this. Briefly, all that is required are small signalling molecules, a unique one for each type of internal store, such that the concentration of each is proportional to the density of its corresponding store. Appropriate interaction of such signalling molecules with the upstream activating sequences of the genes for uptake systems provides a molecular basis for the feedback control system described here.
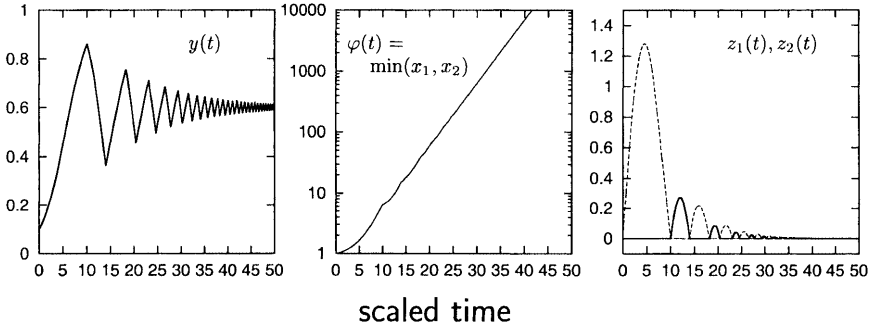
Fig. 2. Behaviour of the feedback model. Simulation showing the main features of the feed-back controlled model. $\omega_1 = 0.4$, $y(0) = 0.1$, $x_1(0) = x_2(0) = 1$. Left panel: allocation y converging to ω_2. Middle panel: structural biomass φ converging quickly to exponential growth with relative rate $\omega_1\omega_2$. A 'lag' phase is apparent. Right panel: alternating surpluse densities of carbon (solid line) and nitrogen (dashed line). The excursions of the densities tend to zero.

Under control regime (18) the allocation $y(t)$ oscillates around ω_2 with di-minishing amplitude (Figure 2, left panel). Structural biomass φ quickly settles on exponential growth (Figure 2, middle panel); it is interesting that only the first os-cillation is clearly visible on a semi-logarithmic plot such as this one, pointing to a possible mechanistic basis for the well-known 'lag phase' [15]. The trajectory $x(t)$ winds around the diagonal (Figure 3), with the points of crossing the diagonal mov-ing arbitrarily close together in time as $t \to \infty$. The following result allows us to focus on this winding behaviour.

Lemma 6. *A trajectory starting at an arbitrary point $x(\vartheta) \in I_1$ or $x(\vartheta) \in I_2$ moves toward the diagonal I_{12} and intersects it at a slope $dx_2/dx_1 \neq 1$.*

Proof. By symmetry, we need only consider the case $x(\vartheta) \in I_1$. The allocation fraction obeys a logistic equation $\dot{y} = \omega_1 y(1 - y)$ with solution

$$y(t) = \frac{y(\vartheta)e^{\omega_1(t-\vartheta)}}{1 + y(\vartheta)(e^{\omega_1(t-\vartheta)} - 1)} \quad x(s) \in I_1, \quad \vartheta \le s < t \quad (19)$$

which shows that y is strictly increasing from $y(\vartheta)$ to 1. As soon as y becomes larger than ω_2, the trajectory's slope

$$\frac{dx_2}{dx_1} = \frac{\omega_2}{\omega_1}\frac{1-y}{y} \quad x \in R_+^2 \quad (20)$$

becomes smaller than 1, and the trajectory moves toward the diagonal I_{12}. As $\dot{x}_1 > \omega_1 y(\vartheta)x(\vartheta) > 0$, the trajectory intersects I_{12} at a finite time, and as $y > \omega_2$, the trajectory's slope at that moment is smaller than 1. If $y(\vartheta) < \omega_2$, the trajectory will initially move away from I_{12}, until y reaches ω_2. The argument is analogous for $x(\vartheta) \in I_2$; here y is strictly decreasing. $\qquad\square$

By the above lemma, we can restrict ourselves to a trajectory starting on I_{12} with slope $dx_2/dx_1 \neq 1$ at $t = \tau_0$, winding itself around the diagonal, alternately
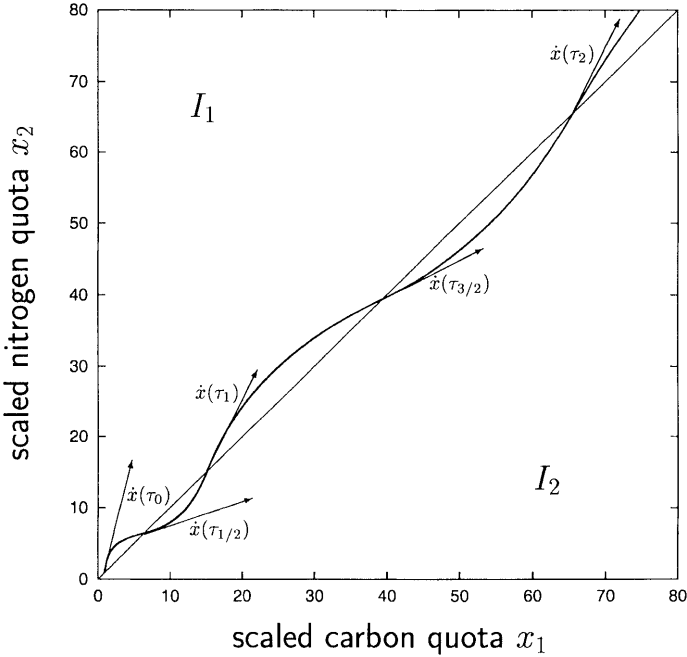
Fig. 3. Trajectory of the feedback model. The state x alternately traverses regions I_1 (carbon limitation) and I_2 (nitrogen limitation); the diagonal is region I_{12}. Indicated are the slopes at the points where the diagonal is crossed, as well as the labelling of the times of crossing.

traversing I_1 and I_2. Without loss of generality, we assume that the first arc traverses I_1. We label the times of crossing from I_2 to I_1 as $\tau_1, \tau_2, \ldots$ and the times of crossing from I_1 to I_2 as $\tau_{1/2}, \tau_{3/2}, \ldots$ (Figure 3).

From a biological point of view, the single most important aspect of the system's behaviour is that maximum excursions of the surplus 'densities' defined by equations (11) approach zero as $t \to \infty$. The behaviour of these surpluses is shown in Figure 2, right panel: surpluses of carbon and nitrogen alternate. Thus the system homes in on balanced growth.

Theorem 4. *Feedback control (18) for problem (13) achieves balanced growth. In particular, the following properties obtain:*

(i)
$$\lim_{k \to \infty} \tau_k = +\infty$$

(ii)
$$\lim_{k \to \infty} (\tau_k - \tau_{k-1}) = 0$$

(iii)
$$\lim_{k \to \infty} \max_{t \in [\tau_{k-1}, \tau_k]} z_i(x(t)) = 0, \quad i = 1, 2$$

(iv)

$$\lim_{k\to\infty} \max_{t\in[\tau_{k-1},\tau_k]} y(t) = \lim_{k\to\infty} \min_{t\in[\tau_{k-1},\tau_k]} y(t) = \omega_2 \ .$$

To establish these results, we begin by noting a key geometric property of the trajectory $x(t)$:

Lemma 7. *Let* $\gamma_k \overset{\text{def}}{=} \left.\dfrac{dx_2}{dx_1}\right|_{t=\tau_k}$. *The trajectory slopes* γ_k *at the points where the trajectory crosses the diagonal* I_{12} *tend toward 1:*

$$\gamma_0 > \frac{1}{\gamma_{1/2}} > \gamma_1 > \frac{1}{\gamma_{3/2}} > \cdots > \gamma_k > \frac{1}{\gamma_{k+1/2}} > \gamma_{k+1} > \cdots > 1$$

with

$$\lim_{k\to\infty} \gamma_k = \lim_{k\to\infty} \gamma_{k+1/2} = 1 \ .$$

Proof. By symmetry, we need only proof $\gamma_k > 1/\gamma_{k+1/2}$ to establish the chain of inequalities. Thus, we consider a trajectory arc through I_1 which starts on the diagonal I_{12} with slope $dx_2/dx_1 = \gamma_k > 1$. This arc is a segment of a curve described by

$$x_1(t) = x_1(\tau_k)\left[1 + y(\tau_k)\left(\exp\{\omega_1(t-\tau_k)\} - 1\right)\right] \tag{21}$$
$$x_2(t) = x_1(\tau_k)\left[1 + \omega_2(1 - y(\tau_k))(t-\tau_k)\right] \tag{22}$$

as is not difficult to derive from the dynamics equations (13), using the behaviour of $y(t)$, equation (19). From equations (19) and (20) we obtain a description of the slope of the curve:

$$\frac{d}{dt}\left(\frac{dx_2}{dx_1}\right) = -\omega_1 \frac{dx_2}{dx_1}$$

or

$$\frac{dx_2}{dx_1} = \gamma_k \exp\{-\omega_1(t-\tau_k)\} \ . \tag{23}$$

From this last equation, we find that the slope dx_2/dx_1 equals $1/\gamma_k$ at time $t^\star = \tau_k + (2/\omega_1)\ln\{\gamma_k\}$. Substituting $t^\star$ in equations (21) and (22), we find

$$\frac{x_2(t^\star)}{x_1(t^\star)} = \frac{1/y(\tau_k) + 2\gamma_k \ln\{\gamma_k\}}{1/y(\tau_k) + \gamma_k^2 - 1} \ .$$

It is an elementary exercise to show from this that $x_2(t^\star) < x_1(t^\star)$. But then we must have

$$\tau_{k+1/2} < t^\star \ .$$

(recall that $\tau_{k+1/2}$ denotes the time at which the arc reaches the diagonal I_{12}). We now use the monotony of the slope (equation (23)) to conclude that

$$\gamma_{k+1/2} > 1/\gamma_k$$

or $\gamma_k > 1/\gamma_{k+1/2}$, which establishes the chain of inequalities.

Let $\bar{\gamma} \stackrel{\text{def}}{=} \lim_{k\to\infty} \gamma_k$. This limit exists as the sequence $\{\gamma_k\}$ is monotonically decreasing and bounded from below by 1. By the chain of inequalities which we have just derived, $\bar{\gamma} \geq 1$. To show that $\bar{\gamma} = 1$, we consider the relationship between γ_k to γ_{k+1}. Using equations (21)–(23) and symmetry, it can be shown that, for all k, γ_k is carried into γ_{k+1} by the same continuous function $F : R^+ \mapsto R^+$; (i.e. $\gamma_{k+1} = F(\gamma_k)$, $k = 0, 1, 2, \dots$). By the continuity of F we have

$$\bar{\gamma} = \lim_{k\to\infty} \gamma_{k+1} = \lim_{k\to\infty} F(\gamma_k) = F(\lim_{k\to\infty} \gamma_k) = F(\bar{\gamma}),$$

which, together with the properties $F(1) = 1$ and $F(x) < x$ for all $x > 1$, implies $\bar{\gamma} = 1$. A similar argument establishes $\lim_{k\to\infty} \gamma_{k+1/2} = 1$. □

Lemma 7 helps to establish the next pair of results, which are biologically significant as they establish convergence to balanced growth.

Lemma 8. *The maximal excursions of the surplus densities z_1 and z_2 approach zero as $k \to \infty$:*

$$\lim_{k\to\infty} \max_{t\in[\tau_k,\tau_{k+1}]} z_i(x(t)) = 0, \quad i = 1, 2.$$

Proof. By symmetry, we can restrict ourselves to a trajectory arc through I_1. In this case, $z_1(t) \equiv 0$, $t \in [\tau_k, \tau_{k+1/2}]$, and the relevant surplus is $z_2(t)$. The maximal excursion of z_2 occurs when the ratio x_2/x_1 is maximal, since $z_2 = x_2/x_1 - 1$. Denote the maximal excursion of x_2/x_1 during the interval $[\tau_k, \tau_{k+1/2}]$ by ξ_k. When $x_2/x_1 = \xi_k$, the trajectory must have the same slope as the line $x_2 = \xi_k x_1$ through the origin. But by equation (23) the trajectory's slope monotonically decreases from γ_k to $\gamma_{k+1/2}$, and therefore $\xi_k \in [\gamma_{k+1/2}, \gamma_k]$. By Lemma 7, $\lim_{k\to\infty} \gamma_k = \lim_{k\to\infty} \gamma_{k+1/2} = 1$, and thus $\lim_{k\to\infty} \xi_k = 1$. The result follows since $\max_{t\in[\tau_k,\tau_{k+1}]} z_2(x(t)) = \xi_k - 1$. □

Lemma 9. *The maximal excursions of the allocation fraction y from ω_2 approach zero as $k \to \infty$.*

Proof. From equation (19) and symmetry we see that y deviates most from the 'singular' value ω_2 at the diagonal crossing times $t = \tau_{i/2}, i = 0, 1, 2, \dots$ (that is, extremata of $|\omega_2 - y(t)|$ occur at these times). Equation (20) implies

$$y(\tau_k) = \frac{\omega_2}{\omega_1 \gamma_k + \omega_2}$$

and since $\lim_{k\to\infty} \gamma_k = 1$ by Lemma 7, we have $\lim_{k\to\infty} y(\tau_k) = \omega_2$. By symmetry, the same obtains for $y(\tau_{k+1/2})$, $\tau = 0, 1, 2, \dots$. □

We have now proved statements (iii) and (iv) in Theorem 4. Statement (ii) is easy to establish:

Lemma 10. *The frequency of switching between $u \equiv 1$ and $u \equiv 0$ diverges, that is, $\lim_{k\to\infty}(\tau_k - \tau_{k-1}) = 0$.*

Proof. By symmetry, we need only consider a trajectory arc through region I_1. From equation (23) we find the time needed to transverse the arc:

$$\tau_{k+1/2} - \tau_k = \left(\ln\{\gamma_k\} - \ln\{\gamma_{k+1/2}\}\right)/\omega_1 .$$

Taking k to infinity, and making use of Lemma 7, we find that the time taken to traverse the arc goes to zero. □

We have not yet discussed $\bar{\tau} \overset{\text{def}}{=} \lim_{k\to\infty} \tau_k$. In principle, the situation where $\bar{\tau} < \infty$ might arise; in that case, for $t > \bar{\tau}$ $x_1(t) \equiv x_2(t)$, $y \equiv \omega_2$. However, such behaviour is not found for the present system.

Lemma 11. *The sum of times between switching diverges:* $\lim_{k\to\infty} \tau_k = +\infty$.

Proof. We need to show that the series

$$\sum_{k=0}^{\infty} (\tau_{k+1} - \tau_k)$$

diverges. Using equation (23), as well as its analogue in I_2, we can bound the summand in this series as follows:

$$\tau_{k+1} - \tau_k \geq \omega_2 \left(\tau_{k+1} - \tau_{k+1/2}\right) + \omega_1 \left(\tau_{k+1/2} - \tau_k\right)$$
$$= \ln\{\gamma_k\} - \ln\{\gamma_{k+1}\} > \ln\{\gamma_k\} . \tag{24}$$

It may be shown that $\ln\{\gamma_k\} = \mathcal{O}(1/k)$ from careful consideration of the properties of F, which establishes the divergence of the series. □

In fact, the question of $\bar{\tau}$ being finite or not is largely irrelevant from a biological point of view, because the idealizations implicit in the model break down in either case. After all, it is not plausible that the allocation regime u be 'all-or-none' when x_1 and x_2 are nearly identical, as the cell's control system can gauge $|x_2 - x_1|$ only within some margin of error (moreover, the dynamics of the cell's actual control system cannot support switching at an infinite rate). Thus, for states near I_{12}, we would expect a physiologically more realistic model to have no 'hard' switching but a gradual dependence on the surplus densities. Regime (18) represents essentially a limiting case of any model along these lines, for instance the one outlined in [4].

5. Discussion

We have established a rationale for allocating building blocks to uptake machinery of those nutrients that are in short supply. It is natural to ask whether microbes actually behave in this way. The allocation strategy proposed here generally concurs with known feedback mechanisms [6, 15], but to our knowledge there has been no systematic research into the relative levels of mRNA transcription for various uptake systems in relation to both environmental conditions and internal storages. Such a project is technically quite challenging, since a considerable number of quantities need to be measured at the same time in the same organism.

Even if individual organisms do not possess control systems that alter allocation according to environmental conditions, a similar process of adaptation might still

occur on an evolutionary time scale. Slight mutations in, for instance, the upstream regulatory sequences of uptake system genes will lead to variations in allocation, and the mutant that achieves a higher growth rate will be favoured.

Our analysis has been limited to time-constant ambient conditions, which allow exponential growth, as in Figure 2. An obvious further step would be to explore ambient conditions which vary with time. We expect that the basic result, Theorem 1, would still obtain. Our heuristic reason for expecting this is that the value of T is essentially immaterial to this result, since it allows a feedback formulation. Therefore the result holds good for time-varying conditions made up by piece-wise constant functions. Thus it seems reasonable to expect that the result will still hold for continuously time-varying f_*. The scaling $t \mapsto t'$ then becomes a continuous distortion of the time-axis, but this would not seem to pose a serious problem.

Extension of the results to time-varying conditions would allow us to motivate adaptive re-allocation not only under conditions conducive to exponential growth, but also under those of batch growth and transients in the chemostat. In a batch culture, the growing colony depletes the limited stock of available nutrients. During chemostat transients, ambient conditions and biomass density approach an equilibrium where the former allow the cells to grow exponentially at the dilution rate of the plant. In actual ecosystems, the kinetics of the ambient conditions not only depend on the activities of the biota under consideration (depletion of the medium) but on various external influences as well, such as changes in the supply to the ecosystem, and the activities (production as well as consumption) of other organisms present in the ecosystem. However, the precise mechanisms underlying the ambient fluctuations are typically irrelevant to the problem at hand, which is, in essence, whether striving toward the allocation $y = \omega_2$ allows the organism to outgrow its competitors. Thus the extension envisaged is to time-varying conditions *per se*, regardless of what dictates the kinetics of those conditions. From the point of view of the individual cells, the chemostat culture at steady state, is no different from the exponential growth phase in a batch culture, and thus it remains true that the relative growth rate is maximized for the allocation $y = \omega_2$.

In our treatment, we ignored a number of biological complications. For instance, nutrients may not be broken down to single elements, but rather to chemical groups (ranging from simple groups, such as $-NH_3$, to complex carbon bodies [6]). This makes no difference to the mathematical analysis. Also nutrients may yield more than one such group; this problem is taken up elsewhere [4]. Of course, there are more than two essential elements in reality. A generalization of the present results is straightforward, however. The 'singular' allocation for which all surpluses are zero can readily be derived (cf. [4]). As for the feedback control regime, observe that there is generically only one reserve type not in surplus (the 'limiting' reserve, see [3]). Allocation of the building blocks to the uptake system associated with the limiting nutrient generalizes the regime investigated in the present paper.

We have also ignored additional modulation of the activity of uptake systems. This is another, quick-acting, way in which organisms can limit the increase of surplus densities without re-allocation. For instance, a nitrogen surplus might induce the phosphorylation of the catalytic molecules in the nitrogen uptake system, rendering them inactive [15]. The effect of such a mechanism would be to

keep surpluses in check, and thus limit the variations in carbon:nitrogen ratios. As long as this modulation allows surplus to accumulate to some (moderate) extent, the feedback control proposed in section 4.3 can still have access to the information it requires. Thus the proposed feedback control can still effect re-allocation in the presence of such additional modulation.

Another important aspect of microbial physiology which has been ignored in this paper is the expenditure of reserves on turn-over and the maintenance of structural integrity. Taking this process into account, we would have to add a loss term to the quota kinetics (for instance that of carbon):

$$\dot{Q}_C = f_C \beta_C y \alpha Q_{\text{cata}} - m_C Q_{\text{cata}}$$

where m_C is a maintenance rate coefficient. Replacing the saturation factor f_C by an effective factor $\tilde{f}_C \overset{\text{def}}{=} f_C - m_C/(\beta_C y \alpha)$, we recover the model as analysed above. This move to $\tilde{f}_C$ is merely a technical maneuver. In particular, it does not mean that we commit ourselves to a 'demand-driven' rather than a 'supply-driven' model. (The demand/supply distinction is essentially moot in the present approach, which combines straightforward bookkeeping with specification of cellular control of the so-called 'active' fluxes. As shown in [2], depending on how this control is specified, one can recover models traditionally thought of as 'demand-driven' as well as 'supply-driven' models.)

Since $\tilde{f}_C$ depends on y, we need to establish the time-varying result, as discussed above. Moreover, as long as $\tilde{f}_C$ remains positive, this time-variability is the only difficulty to contend with. We do not know whether the optimality result still holds when $\tilde{f}_C$ becomes negative. Even then, it still makes intuitive sense to condition allocation on the lowest surplus, even when that surplus is negative. However, such re-allocation will not be able to restore a positive flux if the carbon nutrient concentration is too low (when $f_C \leq m_C/(\beta_C \alpha)$, to be precise). In such cases, we must discard our working hypothesis that the cell strives to achieve balanced growth. To allow for nitrogen wastage in endogenous metabolism, a maintenance term $-m_N Q_{\text{cata}}$ may be added to the kinetics of the nitrogen quota Q_N. The factor f_N is then replaced by an effective factor $\tilde{f}_N$; this does not introduce any additional difficulties.

Surpluses are essential in eco-physiological situations where organisms can only survive such periods of famine if they accumulate sufficient reserves in advance. In those cases, growth-balancing re-allocation is not to be expected. On the other hand, if periods of non-availability of carbon and nitrogen alternate on a time scale which is sufficiently short, the cell might still utilize the control regimes proposed in this paper. In that case, the transversals of I_1 and I_2 might follow the periodic environmental fluctuations. Paradoxically, such an organism would always be synthesising the uptake system for the nutrient that is presently unavailable. It is intriguing that such paradoxical behaviour is actually observed in some phototrophic bacteria [10] (in a phototroph the availability of the carbon nutrient is not only determined by the concentration of HCO_3^- but also by the level of irradiance). Whether the feedback control (18) would still be (near) optimal in such a situation remains an open question. The answer to this question might depend

on the relative magnitudes of the time scales of the environmental alternations and growth/re-allocation.

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